The TOCC Study: Timing of Cord Clamping

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Background: Heart rate (HR) and peripheral oxygen saturation (SpO₂) are important clinical indicators of health at birth. Understanding normal values for HR and SpO₂ during transition from intra-uterine to extra-uterine life enables clinicians to identify infants who need assistance at birth. The timing of umbilical cord clamping may influence these two parameters. A previous study defined ‘normal’ reference values for HR and SpO₂ in infants who underwent early cord clamping (ECC), but little is known about the normal changes in the first minutes of life with delayed cord clamping (DCC).

Objectives: To define the normal values for HR and SpO₂ in the first ten minutes after birth for infants who receive DCC >90 seconds. We will produce new reference centile charts for these parameters, compared with previous values defined during ECC.

Methods: Eligible infants were ≥32 weeks’ gestation and received no resuscitation. Multiple births, birth via Caesarean section, lotus or water birth, and those with congenital anomalies were excluded. Following verbal parental consent, immediately after birth, a pulse oximeter sensor was applied to the infant’s right wrist which recorded HR and SpO₂ every two seconds.

Results: This pilot cohort studied 67 infants, recording >13,000 HR and SpO₂ data points. Infants had a mean (standard deviation, SD) gestation of 39 (1) weeks and birth weight 3453 (434) grams. Thirty-seven infants (55%) were male. Twenty-eight (42%) were instrumental births. The median (inter quartile range, IQR) time to cord clamping was 166 seconds (128, 271). The median time to obtain pulse oximetry data was 99 seconds. HR measurements for the 10th, 25th, 50th, 75th, and 90th centiles at two minutes were 60, 144, 163, 178, 187 beats per min (bpm). We found no statistically significant differences between HR measurements following DCC, compared with infants undergoing ECC, as reported in previous studies. However, we observed a faster rise in HR to ≥100 bpm following DCC. The SpO₂ for the 10th, 25th, 50th, 75th, and 90th centiles at two minutes were 60%, 71%, 77%, 81%, 92%. Following DCC, infants had higher SpO₂ at each minute than with ECC, and this was statistically significant at 60 seconds. We found no difference in HR or SpO₂ for gender or birth mode.

Discussion: This study indicates that DCC may improve cardiorespiratory physiology during transition. However, the full study cohort (n=470) is required to enable a full comparison with the previous cohort of infants receiving early cord clamping.
Understanding the Role of Mesenchymal Stem Cells in Ageing of the Human Placenta

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Background: Ageing is a complicated process which causes gradual loss of function in tissues and organs. As ageing proceeds, the incidence of chronic diseases rises in individuals. Recent evidence shows placental tissues undergo significant molecular age-related changes in a short period, between early-term (37 0/7 – 38 6/7 wks gestation) to late/post-term (41 0/7 wks gestation and beyond), after the due date of the pregnancy has been reached. Moreover, there is a dramatic increase in the risk of unexplained antepartum stillbirths in pregnancies that progress into the late/post-term period, which may be linked to increased ageing of placenta. Mesenchymal stem cells (MSCs) play an important role in embryogenesis and in the formation of the placenta. However, the role and contribution of MSCs to the ageing processes in the feto-maternal interface during the period from early-term to late/post-term is unknown.

Objectives: The objective of this study was to compare stem cell properties, and ageing/senescence functions of early-term and late/post-term MSCs isolated from the maternal decidua.

Methods:
1. Early-term and late/post-term MSCs were isolated from the maternal decidua that remains attached to the placenta following delivery. These decidual MSCs (DMSCs) were characterised by morphological features, stem cell marker expression and differentiation potential.
2. The potency of DMSCs from early-term and late/post-term was assessed by in vitro growth kinetics, and the ability to form cell colonies was determined.
3. Ageing/senescence related characteristics of early-term and late/post-term DMSCs were evaluated by detecting expression of the ageing marker, High mobility group box 1 protein (HMGB1), using flow cytometry. Cell death was measured by quantitative Annexin V apoptosis assay and resistance to oxidative stress was determined using the quantitative Aldeflour assay.

Results: DMSCs isolated from the placenta showed the expected characteristics and properties of this cell type. There was a significant increase in HMGB1 expression in late/post-term DMSCs compared with early-term DMSCs (n=6 each group, p-value <0.05). The level of cell apoptosis was significantly higher in late/post-term DMSCs compared with early-term DMSCs (n=6 each group, p-value <0.01). Using a quantitative Aldeflour assay, early-term DMSCs showed a significantly higher resistance to oxidative stress compared with late/post-term DMSCs (n=6 each group, p-value<0.01).

Discussion: These data provide the first evidence of advanced ageing and loss of important stem cell functions in late/post-term DMSCs. Future studies will employ whole genome array comparisons to determine the specific biological pathways, and regulatory molecules, involved in the advanced ageing of late/post-term DMSCs.

Skin-to-skin care in preterm infants receiving respiratory support does not lead to physiological instability

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Background: Skin-to-skin care (SSC) in very preterm infants is a standard practice in most neonatal intensive care units. However, there are conflicting results on the stability of oxygen saturation. Both excessive and insufficient oxygen supply to the brain contribute to morbidity and mortality and regional cerebral oxygenation (rcO2) measured by near-infrared spectroscopy (NIRS) can serve as a surrogate parameter. If there was no deterioration in rcO2 during SSC this would provide reassurance that SSC is “safe” in very preterm infants.

Objectives: To compare rcO2 and physiological parameters during SSC with those during incubator care in preterm infants <33 weeks gestation receiving respiratory support.

Methods: Prospective, single centre, non-inferiority trial including preterm infants (gestational age (GA) < 33 weeks) receiving respiratory support. The primary outcome was each infant’s difference in rcO2 (Foresight, Casmed) between SSC and incubator care. In this way, every infant served as his/her own control. Non-inferiority was determined by calculating the mean difference (95% CI) in the primary outcome; the chosen margin of non-inferiority was -1.5 %. Secondary outcomes were heart rate (HR), oxygen saturation (SpO2), fraction of inspired oxygen (FiO2), and temperature. All parameters were average per infants and compared using a paired t-test. Parents and caregivers were blinded to the measurements.

Results: 40 preterm infants median (IQR) GA of 27.6 (26.0-28.9) were studied at day 8 (5-18) of life. There was no deterioration in the primary outcome (rcO2) during SSC compared with incubator care. With regards to rcO2 SSC is non-inferior to incubator care. Moreover, no clinically important differences in HR, SpO2, FiO2, and temperature were observed. There was little evidence of a difference in rcO2 by mode of respiratory support (ETT mechanical ventilation (mean difference (95% CI) -0.2 (-3.1 to 2.7), n=10), CPAP (-0.1 (-1.3 to 1.6), n=15) and HFNC (-0.5 (-1.9 to 1), n=15, p=0.9).

Discussion: Cerebral oxygenation and other physiological measurements in ventilated preterm infants did not differ between SSC and incubator care. These findings were independent of the mode of respiratory support or oxygen requirement. Using these markers of physiologic stability, providing SSC to preterm infants receiving respiratory support is feasible and should be encouraged.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Incubator (baseline)</th>
<th>SSC (intervention)</th>
<th>Mean difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rcO2 (%)</td>
<td>74.7 (6.1)</td>
<td>74.9 (6.5)</td>
<td>0.2 (-0.8 to 1.1)</td>
</tr>
<tr>
<td>SpO2 (%)</td>
<td>94.1 (3.0)</td>
<td>93.6 (2.8)</td>
<td>-0.6 (-1.1 to -0.1)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>160 (11)</td>
<td>161 (12)</td>
<td>0.3 (-1.6 to 2.3)</td>
</tr>
<tr>
<td>FiO2 (%)</td>
<td>25.5 (1.0)</td>
<td>26.0 (6.8)</td>
<td>0.4 (-0.6 to 1.4)</td>
</tr>
<tr>
<td>Temp(°)</td>
<td>36.8 (0.3)</td>
<td>36.8 (0.4)</td>
<td>0.04 (-0.1 to 0.2)</td>
</tr>
</tbody>
</table>
Differential response of Diffuse Intrinsic Pontine Glioma cell lines to microbeam versus conventional radiotherapy

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Background: Diffuse Intrinsic Pontine Glioma (DIPG) is a devastating paediatric brainstem tumour with extremely poor prognosis and limited treatment options. Radiotherapy is the mainstay treatment but is limited to palliative use. Microbeam Radiotherapy (MRT) is a promising pre-clinical synchrotron radiotherapy modality which could improve the therapeutic ratio between normal tissue toxicity and tumour control through radiobiological mechanisms that are a radical departure from those of conventional radiotherapy (CRT).

Objectives: The aim of this study was to compare the cellular response of two human DIPG cell lines to MRT and conventional broad-beam radiotherapy (CRT). We hypothesised that MRT would elicit a different cellular response to CRT, and that different DIPG cell lines would have different intrinsic radio-sensitivities.

Methods: Two human DIPG cell lines, SF7761 and JHH-1, were exposed to MRT (112 to 560 Gy) or CRT (2 to 8 Gy) in vitro to produce clonogenic cell-survival curves. Equivalent CRT doses were interpolated for each MRT dose. Apoptosis induction and cell-cycle response assays were performed five days after irradiation via flow cytometry to assess differences in cellular response between the cell lines and radiotherapy modalities at equivalent doses.

Results: The SF7761 cell line, which originated from a patient with no prior history of radiation treatment, was significantly more radio sensitive to both CRT and MRT compared to the JHH-1 cell line, which originated from a six year old male who had previously undergone combined chemotherapy and radiotherapy (Figure 1). JHH-1 formed polyploid cells and exhibited delayed G2/M arrest following both CRT and MRT. Furthermore, apoptosis and cell cycle assays demonstrated that at equivalent doses, MRT induced more unrepaired DNA damage that was detrimental to the cell-cycle and cell viability for both cell lines five days following irradiation.

Discussion: This is the first study to compare the response of DIPG cell lines to MRT and CRT. Although MRT caused more DNA damage that was detrimental to the cell cycle compared to CRT, the JHH-1 cell demonstrated radio-resistance regardless of the radiation modality used. The findings of this study support the use of MRT as a potential alternative to CRT for patients with radiosensitive tumours and also contribute to our understanding of the differential response of cancer cells to MRT and CRT.

The virtues and vices of moral distress – healthcare professionals’ perspectives

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Background: Moral distress refers to anguish that arise when an individual makes a clear moral judgment about what they think is in a patient’s best interests but feels unable to act accordingly due to constraints including institutional or societal. Theoretical constructs of moral distress largely portray a negative phenomenon that leads to physiological and emotional burnout, poor retention rates and ultimately poor patient care.

Objectives: To explore how clinical experiences and perceptions of moral distress within two neonatal intensive care units (NICUs) compare with theoretical constructs of moral distress.

Methods: All medical and permanent nursing professionals at two tertiary level NICUs – one surgical, one perinatal – were invited to participate by undertaking a brief questionnaire on their perceptions and experience of moral distress.

Results: 345 healthcare providers from two NICUs participated: 286 nurses (77% response rate) and 59 medical professionals (98% responses rate). Moral distress (variably defined by the respondents) was experienced by >70% of medical and nursing professionals at least once a month.

Discussion: Overwhelmingly, (96% of respondents) viewed moral distress as a normal component of the NICU environment, reflecting the challenging nature of caring for very sick neonates amidst uncertainty and diverse viewpoints. Respondents desired more support during distressing periods. Yet despite the traditional negative connotations surrounding moral distress, few (8% of medical and 22% of nursing professionals) advocated removing all moral distress. Moral distress was not only viewed as inevitable but also as a necessity to promote discussion, advocacy and a progressive medical environment. Even more positively, moral distress was viewed as a marker of compassion and professionalism. Whilst interventions to reduce the clear negative impacts must still be sought, healthcare professionals’ perspectives of moral distress challenge traditional literature and suggest it has a virtuous role to play in progressive thought and practice within NICUs.

The challenge for NICUs remains to create an ethical climate where moral distress can be discussed, its negative impacts mitigated, and its beneficial effects on advocacy and empathy explored.
Investigation of Genes Associated with Increased Endometriosis Risk

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Background: Endometriosis is a common, oestrogen-dependent gynaecological disorder that affects 10% of women. Symptoms include dysmenorrhea, chronic pelvic pain and sub-fertility. The cause of endometriosis remains unknown; however, both genetics and environmental factors contribute to the disorder. Genome-wide association studies (GWAS) have revealed that complex combinations of single nucleotide polymorphisms (SNPs) culminate in increased disease risk. Identification of SNPs with significant effects on downstream gene expression (expression quantitative trait loci [eQTLs]) is key to determining biological pathways involved in the pathophysiology of endometriosis.

Objectives: 1) To determine if our new human telomerase reverse transcriptase (hTERT) immortalised endometrial stromal cell (ESCs) lines share key characteristics with primary cultured ESCs and a commercial cell line (tHESC), and thus may be potential tools for future endometriosis studies. 2) To determine if the known eQTL ‘long intergenic non-coding RNA00339’ (LINC00339) is hormonally regulated in ESCs. 3) To identify other potential endometriosis eQTLs from existing endometrial gene array data.

Methods: ESC purity in our new cell lines was determined using immunocytochemistry (ICC) for vimentin and cytokeratin. hTERT, oestrogen receptor (ERα/β) and progesterone receptor (PRA/B) expression was examined using RT-PCR and ICC. LINC00339 and new eQTLs examined by by ICC and RT-PCR before and after cells were treated with estradiol-17β (E2, 0.01M) and medroxyprogesterone acetate (MPA, 0.1mM) for 4hrs and 24hrs.

Results: 1) We confirmed that our new cell lines were pure stromal cell cultures (vimentin positive, cytokeratin negative) that expressed ERα, ERβ and PR, with no significant reduction in expression compared to primary ESCs and tHESCs. Of note, there was considerable inter-patient variability in expression among cell lines. 2) LINC00339 mRNA was expressed in all ESCs, but was not affected by hormone treatment. 3) SOX15, HSD17B12, CASC2 and EPL were identified as potential endometriosis eQTLs. These genes were expressed in primary ESCs and cell lines, with the exception of EPL. In addition, CASC2 was lowly expressed in primary ESCs and absent in the cell lines. mRNA expression of these eQTLs was not altered by hormone treatment.

Discussion: Preliminary results demonstrate that our new stromal cell lines maintain steroid hormone receptor expression, making them useful tools for future endometriosis studies; the variability among cell lines in terms of expression levels will be of added benefit when they are used to examine the regulation and function of eQTLs including LINC00339, SOX15, HSD17B12, CASC2 and EPL in endometriosis pathophysiology.

Interventions to improve rates of successful extubation in preterm infants: A systematic review and meta-analysis of the evidence

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Background: The introduction of mechanical ventilation for the treatment of respiratory failure in preterm infants has led to significant improvements in survival. However, prolonged mechanical ventilation can result in unintended harm. Mechanically ventilated preterm infants are at increased risk of developing bronchopulmonary dysplasia, sepsis, neurological injury, and retinopathy of prematurity. To minimize these risks, clinicians aim to extubate preterm infants as early as possible. In the most preterm infants, extubation is often unsuccessful due to lung disease or inadequate respiratory drive. Given that approximately two-thirds of infants born before 29 weeks’ gestation require intubation, it is important that clinicians are aware of strategies to improve rates of extubation success.

Objectives: To conduct a systematic review and meta-analysis of interventions to improve rates of successful extubation.

Methods: The review was conducted using the methods of the Cochrane Collaboration, with searches undertaken of PubMed and The Cochrane Library. Primary outcomes were (1) treatment failure or (2) re-intubation within 7 days of extubation. Studies were included if they were RCTs published in English, enrolling intubated preterm infants and reported at least one of our primary outcomes.

Results: The search was completed on 23 December 2015, yielding 1379 articles. 50 studies were eligible for inclusion, with results summarised in the table below.

Discussion: NCPAP and NIPPV improved extubation success, with NIPPV the superior intervention. HFNC and NCPAP had similar efficacy. Methykythamines reduced extubation failure. Corticosteroids and chest physiotherapy improved extubation rates, but carry significant side effects and warrant cautious use. The evidence for chest physiotherapy may have limited applicability to current practice. Doxapram did not aid successful extubation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trials</th>
<th>Subjects</th>
<th>Pooled RR (95% CI)</th>
<th>Pooled RD (95% CI)</th>
<th>NNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCPAP vs. headbox oxygen</td>
<td>8</td>
<td>667</td>
<td>0.59 (0.48, 0.72)</td>
<td>-0.19 (-0.26, -0.12)</td>
<td>6 (3, 9)</td>
</tr>
<tr>
<td>NIPPV vs. NCPAP</td>
<td>9</td>
<td>1368</td>
<td>0.70 (0.60, 0.81)</td>
<td>-0.13 (-0.18, -0.08)</td>
<td>8 (5, 13)</td>
</tr>
<tr>
<td>HPNC vs. NCPAP</td>
<td>3</td>
<td>658</td>
<td>1.11 (0.84, 1.47)</td>
<td>0.02 (-0.04, 0.09)</td>
<td>N/A</td>
</tr>
<tr>
<td>Methykythamines vs. placebo</td>
<td>6</td>
<td>197</td>
<td>0.48 (0.32, 0.71)</td>
<td>-0.27 (-0.39, -0.15)</td>
<td>4 (2, 7)</td>
</tr>
<tr>
<td>Corticosteroids vs. placebo</td>
<td>3</td>
<td>160</td>
<td>0.18 (0.04, 0.97)</td>
<td>-0.09 (-0.16, -0.01)</td>
<td>12 (6,100)</td>
</tr>
<tr>
<td>Doxapram vs. placebo</td>
<td>1</td>
<td>29</td>
<td>0.80 (0.22, 2.97)</td>
<td>-0.05 (-0.36, 0.26)</td>
<td>N/A</td>
</tr>
<tr>
<td>Chest physio vs. standard</td>
<td>4</td>
<td>315</td>
<td>0.32 (0.13, 0.82)</td>
<td>-0.07 (-0.13, -0.02)</td>
<td>15 (7,50)</td>
</tr>
</tbody>
</table>
Cytokine expression profiling and function of decidual mesenchymal stem cells from ageing term placentae

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Background: Premature ageing of the placenta is associated with important placental pathologies including preeclampsia and fetal growth restriction. Placenta from uncomplicated, term pregnancies also show signs of ageing. A recent study revealed signs of advanced ageing in placental tissue in the short period between early-term (37 0/7 – 38 6/7 wks gestation) and late/post-term (41 0/7 wks gestation and beyond). The late/post-term period is potentially important because it is associated with an increased number of unexplained antepartum stillbirths. Stem cell exhaustion and dysfunction contribute to the decline of function in ageing organs but their contribution to ageing of the feto-maternal interface during human pregnancy is poorly understood.

Objectives: This study focused on mesenchymal stem cells from the maternal decidua (DMSCs), with the aim of understanding the changes to the secretory phenotype and functional ability of these stem cells between the early-term and late/post-term period.

Methods: Cells were isolated from the maternal decidua (basalis) that remains attached to the placenta following delivery. A variety of standard assays confirmed the mesenchymal stem cell properties of the cells and their maternal origin. A qualitative cytokine array was used to screen for cytokines secreted into the growth medium by early-term and late/post-term DMSCs. Quantitative enzyme-linked immunosorbent assays (ELISA) determined the concentration of selected cytokines. One of the cytokines, MCP-1, is a regulator of cell migration. Scratch assays and transwell assays assessed whether MCP-1 acts as a stimulator or chemoattractant for DMSC migration, respectively.

Results: Cells met the standard criteria for DMSCs. The cytokine array showed 16 cytokines were secreted into the growth medium by early-term and late/post-term DMSCs. All cytokines levels were reduced in the late/post-term DMSCs compared with early-term DMSCs, except PAI-1, which was increased. ELISA assays verified cytokines MCP-1 and IL-6 were significantly decreased in the growth medium of late/post-term DMSCs compared to early-term DMSCs (n=7 each; p<0.01 and p<0.05, respectively). Scratch and transwell assays showed MCP-1 did not act as a stimulator or chemoattractant for early-term or late/post-term DMSC migration (n=3 each).

Discussion: Ageing in the decidua and placenta in the early-term to late/post-term period is poorly understood but potentially important given the increase in stillbirths in the late/post-term period. This study provides the first evidence that cytokine secretion in ageing DMSCs is significantly altered in the late/post-term period. Further studies are required to better understand the functional consequences of these changes in cytokine expression levels.

Physiologic based cord clamping improves hemodynamic stability vs umbilical cord milking in preterm newborn lambs

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Background: Human studies suggest that umbilical cord milking (UCM) may be beneficial to preterm infants but the physiologic effects have not been studied. An alternative is physiologic based cord clamping (PBCC) where the cord is clamped after lung inflation.

Objectives: To compare physiologic changes at birth using PBCC vs. UCM.

Methods: At gestational age=126 days (=26wks in humans), pregnant ewes were anaesthetized and 11 fetal lambs were surgically exteriorized, intubated, and instrumented (umbilical vein, umbilical and carotid artery blood flow probes). In lambs receiving PBCC, ventilation was initiated for 3min prior to clamping the umbilical cord (UC). In lambs receiving UCM, 10cm of the UC was milked×8, pushing blood in the UC towards the lamb. Between passes, the UC was released, allowing blood to refill from the fetal or placental end. After the 8th pass, the UC was clamped and ventilation was initiated 30s later. Net transfer of blood was calculated from the difference between umbilical venous and arterial blood flow.

Results: We studied 12 lambs. During UCM, there was a 32% increase in carotid artery blood flow (CABF=+5±2.4ml/kg/min, p=0.01) and a 15% increase in mean BP (+6.6±3mmHg, p=0.004). During the 1st 30s ventilation in PBCC, there was a 13% decrease in CABF (-2.4±2.8ml/kg/min, p=0.09) and an 11% decrease in mean BP (-5.2±3.5mmHg, p=0.007). There were no significant changes UC blood flow during either intervention (UCM=+2.5 ±4.6ml/min/kg, p=0.18 and PBCC=-2.4 ±19ml/min/kg, p=0.75).

Discussion: UCM significantly increased mean BP and CABF flow without a significant transfer of UC blood. Further review of UCM is warranted before it is adopted into routine clinical practice.
Development and evaluation of a novel, electronic fertility preservation decision aid for parents of children with cancer at The Royal Children’s Hospital, Melbourne.

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1 University of Melbourne, 2 Department of Obstetrics & Gynaecology, The Royal Women’s Hospital, University of Melbourne, 3Melbourne School of Population and Global Health, University of Melbourne, 4 Children’s Bioethics Centre, The Royal Children’s Hospital, 5Department of Paediatric and Adolescent Psychology *Shared senior author

Background: Infertility resulting from cancer treatment is a major survivorship concern, particularly in paediatric and adolescent cancer patients, of whom more than 80% survive into adulthood. Recent diagnosis, parental surrogate decision making, and the experimental nature of fertility preservation (FP) procedures make the decision complex. Decision aids (DA) help patients and families make informed decisions about complex healthcare problems, such as FP, minimising decisional conflict and possible later decisional regret (DR).

Objectives: To develop an online FP prototype DA for parents’ (an international first), and to assess usability, improvements in FP knowledge, and acceptance of the DA in parents at The Royal Children’s Hospital, Melbourne.

Methods: A targeted literature review informed DA requirements. Draft content was reviewed for acceptability and clinical appropriateness by a consumer, clinicians and senior researchers. The DA was revised as required, built using Content Management System software Wordpress and is hosted on an RCH domain. The DA is divided into male and female sections, containing information on fertility preservation, fertility and cancer, and includes an interactive values clarification exercise. Parents’ of paediatric cancer patients (RCH) who have already made an FP decision, and whose child is not receiving active treatment, were invited to participate. Parents of palliative and deceased patients were excluded. Prior to reviewing the DA, initial surveys captured basic demographic information, a brief social and medical history, purposefully designed FP knowledge scale and Decision Regret Scale. Post-DA completion, surveys evaluated user-friendliness, usefulness for decision making, improvements in FP knowledge, and changes in DR.

Results: Thirty-four parents returned the baseline survey, subsequently 19 parents withdrew and 15 parents returned their follow-up survey. The overwhelming majority of participants reported satisfaction with the DA. One-hundred per cent (n=15) reported that they were ‘satisfied’ or ‘very satisfied’ with the DA, and that it ‘met’ or ‘exceeded’ their expectations. Eighty-six percent (86.6%, n=13) reported that they would recommend the DA to parents considering FP. At follow-up there was a significant difference in FP knowledge at baseline (M=5.21, SD=1.72) and at follow-up (M=6.71, SD=2.02); t(14)=2.27, p=0.04. There was no significant difference between DR at baseline (M=16.92,SD=18.66), and at follow-up (M=19.62, SD=20-05); t(12)=0.31, p=0.76.

Discussion: This study resulted in an international first: a pilot, web-based decision aid. Data from this study will contribute to further revision of the DA and supports prospective evaluation of tool. It is expected that this DA will be a significant addition to fertility counselling at RCH.

Cerebral oxygenation during skin-to-skin care in preterm infants not receiving respiratory support

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Background: Preterm infant physiological stability during skin-to-skin care (SSC) remains a concern due to conflicting published reports. Too much and too little oxygen supply to the brain are known to contribute to adverse outcomes in preterm infants. Regional cerebral oxygenation (rcO2) measured by near-infrared spectroscopy (NIRS) is a measure of cerebral oxygen consumption and delivery. There is paucity of data on what happens to rcO2 during SSC. If rcO2 did not deteriorate during SSC, compared with incubator care, this would provide evidence and reassurance of physiological stability during SSC.

Objectives: To compare rcO2 and physiological parameters during SSC with those during incubator care in preterm infants <33 weeks gestation who are not receiving respiratory support.

Methods: This study was a prospective, observational, non-inferiority study. Primary outcome was rcO2 during SSC compared with incubator care. Secondary outcomes were oxygen saturation (SpO2), heart rate (HR), number of bradycardic (HR <100 bpm) and oxygen desaturations (SpO2 < 80 %), temperature, and the proportion of time spent in quite sleep. The non-inferiority margin was set at 1.5%. Outcomes were compared using a paired t-test. Each study comprised three 90 minute periods (control/SSC/post-SSC) including 30 minute washout post-handling. Infants acted as their own control. Parents and caregivers were blinded to NIRS measurements.

Results: Forty preterm infants (median (IQR) 30.6 (29.1-31.7) weeks’ gestation) were studied on day 14 (8-38). There was a statistically significant decrease in rcO2 and the lower end of the 95% CI crossed the pre-defined non-inferiority margin. Therefore, this is an inconclusive finding.

There was a decrease SpO2 and an increase HR and proportion of time spent in quite sleep. There was no difference in number of bradycardic and desaturation events and temperature. rcO2 during SSC [mean (SD) 73.6 (6.0) %] compared with control [74.8 (4.5) %, mean difference (95% CI) -1.3 (-2.2 to -0.4), p<0.01].

Discussion: During SSC, there was a statistically significant reduction in rcO2 and according to our pre-defined non-inferiority margin we can’t conclude that SSC is non-inferior to incubator care. However, changes in rcO2 and other physiological parameters, although statistically significant are of clinical negligible importance. These results provide reassurance that SSC can be further encouraged in the NICU given physiological stability is maintained. Future research should focus on possible long-term benefits of SSC.

<table>
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<tr>
<th>Variable</th>
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<tr>
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<td>Number of desaturation events</td>
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<td>0 (0-1)</td>
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<tr>
<td>Temperature</td>
<td>36.7 (0.3)</td>
<td>36.8 (0.4)</td>
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<td>Proportion of time in quiet sleep (%)</td>
<td>34.6 (24.3)</td>
<td>58.6 (21.2)</td>
<td>p=0.0001</td>
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Targeting BCL-2 in treatment-refractory MYCN-driven high-grade serous ovarian cancer; utilizing patient-derived xenograft (PDX) models

Ho G1,2, Heong V1, Lieschke E1, Kondrashova O1, Kyran E1, Holian R1, Phuong T1, McNally O2, Haluska P1, Australian Ovarian Cancer Study (AOCs) 4, Huang RY3, Bowtell D3, Wakefield MJ3 and Scott CL1

1Walter and Eliza Hall Institute of Medical Research, Melbourne; 2Royal Women’s Hospital, Melbourne; 3Mayo Clinic, USA, 4Peter MacCallum Cancer Centre, Melbourne, 5National University of Singapore.

Background: Over-expression of pro-survival family protein BCL-2 has been identified as a marker of poor prognosis and predictor of treatment failure in cancer5. We hypothesised that inhibition of BCL-2 via BH3-mimetic therapy could improve treatment efficacy in high-grade serous ovarian cancer (HG-SOC), particularly in the proliferative C5 subgroup defined by MYCN-pathway activation2.

Objectives: We aimed to explore the in vivo efficacy of BH-3 mimetic (ABT-199), a potent selective BCL-2 inhibitor, in combination with the anti-microtubule chemotherapeutic, vinorelbine3, or the BRD4 bromodomain inhibitor (I-BET726)4,5, both reported to have efficacy in C5 HG-SOC. Secondly, we aimed to investigate whether the in vivo efficacy was related to pre-treatment BCL-2 expression levels in C5 HG-SOC Patient-derived xenografts (PDX).

Methods: BCL-2 protein expression was assessed in four C5 PDX by IHC and WB. Single-agent in vivo response of each PDX to vinorelbine and to I-BET726 was established. Combination therapy of ABT-199 with either vinorelbine or I-BET726 was also explored.

Results: One of four C5 PDX had high BCL-2 expression and was refractory to both vinorelbine and I-BET726. The other three PDX had lower levels of BCL-2 expression, of which two were responsive to vinorelbine but refractory to I-BET726 and one responded both to vinorelbine and I-BET726. Preliminary findings indicate that the refractory BCL-2 high PDX was responsive to the combination of ABT-199/vinorelbine but not to the combination of ABT-199/I-BET726. Interestingly, response was seen to the combination of ABT-199/I-BET726 in one BCL-2 low C5 PDX, which was not responsive to I-BET726 alone.

Discussion: In select C5 PDX, BH3-mimetic therapy can be used to overcome treatment resistance when used in combination with either chemotherapeutic agent or BRD4 inhibitor. The mechanism of action is currently under investigation.

5. Scott et al, JCO (Meeting abstract), 2015

Monitors Enhancing the Education of Resuscitation skills, A Trial (MEERKAT)

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Newborn Research Centre, Royal Women’s Hospital

Background: Approximately 8% of newborn Australian infants fail to breath at birth and require respiratory ventilation. Establishing effective ventilation in the first minutes after birth is the first critical intervention in newborn resuscitation. This is an opportunity to make a difference by saving newborn lives and reducing poor neurodevelopmental outcomes but the skills are difficult to master and maintain. Mask leak is common and impedes effective ventilation. In this context there is a surprising lack of evidence on how best to train critical newborn resuscitation skills. Respiratory Function Monitors (RFMs) are a new technology that can be used to monitor the effectiveness of newborn ventilation by giving leak, volume and flow feedback. Their role in training has not yet been explored in a randomised controlled trial. This study aims to improve health outcomes for newborns by investigating a new method of teaching neonatal mask ventilation with the addition of a respiratory monitor.

Objectives: The primary objective is to compare the leak from mask ventilation performed by a healthcare professional after learning mask ventilation using a respiratory function monitor and after learning mask ventilation without using a respiratory function monitor.

Methods: This is a multi centre randomised controlled trial in level 1, 2 and 3 hospitals in Victoria. The outcome assessor is blinded. The population are adult health care professionals attending the Victorian Neonatal Resuscitation training program (a structured simulation training program, NeoResus) in Victoria during the study period. Doctors from any specialty, midwives, nurses, physiotherapists, occupational therapists, paramedics, medical students, midwifery and nursing students will be included.

The participant will be randomised to either the intervention group (neonatal mask ventilation taught with a respiratory function monitor) or control (standard teaching of neonatal mask ventilation). The primary outcome is the difference in facemask leak measured after training between the groups. Sample size is estimated at 382 (191 in each arm) to achieve a clinically significant reduction in leak (10%) (90% power).

Results: 66 participants have been recruited to date and recruitment is ongoing at Victorian sites.

Discussion: Improvements in teaching resuscitation skills allows us to translate research advances into real improvements in the care of sick newborns. It is a critical health intervention in particular in inexperienced or less frequent resuscitators.
Decision regret in families being offered Fertility Preservation at the Royal Children’s Hospital

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1Monash University, Melbourne, 2Department of Obstetrics & Gynaecology, Royal Women’s Hospital, University of Melbourne, 3Melbourne School of Population and Global Health, University of Melbourne, 4Children’s Bioethics Centre, Royal Children’s Hospital, 5Department of Paediatric & Adolescent Gynaecology, Royal Children’s Hospital, 6Children’s Cancer Centre, Royal Children’s Hospital, 7Children’s Cancer Centre, Monash Medical Centre, 8Department of Endocrinology Royal Children’s Hospital, 9Melbourne IVF, 10Reproductive Services Royal Women’s Hospital

Background: Paediatric oncofertility is a burgeoning field. With over 80% of paediatric and adolescent patients surviving into adulthood, quality of life issues such as fertility are increasingly important. However, little is known about potential regret around a decision to pursue or forgo fertility preservation (FP). In this population, decision-making is not a straightforward process due to the experimental nature of available techniques and the involvement of parents as surrogate decision-makers. Decision regret (DR) surrounding FP can adversely impact long-term wellbeing in these patients.

Objectives: We aimed to examine DR around FP decisions in families involved in making a FP decision at the Royal Children’s Hospital (RCH) since 1987, and to explore factors that contribute to it.

Methods: This is a single site, cross-sectional study conducted at an established paediatric oncofertility centre. Parents and patients ≥15 years enrolled in the FP program at the RCH were invited to participate. Participants were asked to complete a 10-item survey based on the validated decision regret scale (Brehaut et al.) to obtain quantitative and qualitative data regarding satisfaction with the decision to pursue or forego FP. A score ≥30 indicates high regret.

Results: Of 144 eligible families, 74.3% were recruited including 104 parents and 25 patients. Most participants (83.5%) reported low regret (mean score 13.7, SD 18.8; range 0-95). Referral to an oncofertility specialist and pre-treatment discussions were significantly associated with low regret on univariate analysis (p<0.05), with having an FP procedure being the independent predictor of low regret on multivariate analysis (p<0.0001, OR=109, CI=0.32 - 367).

Most participants believed that FP offers hope for future fertility, however those that are still satisfied with their decision still raise issues regarding the process of decision-making highlighting dissatisfaction with current clinical pathways. Namely, that consultation felt “rushed,” and that they had to initiate the discussion on FP. Reasons for regretting the decision made related to a lack of adequate information provision, and inadequate time for consultation. Furthermore, many participants that had samples preserved before the introduction of the program, requested contact to enquire about storage and sample quality.

Discussion: Overall levels of regret in the study population were low, with having a procedure and quality discussion influencing regret, similar to what is known regarding the adult FP population. However, dissatisfaction with the decision-making process itself reveals that refinements to the program are required to meet families’ information needs.

The ProNose Study- Use of a nasal barrier dressing for preterm infants receiving non-invasive respiratory support: a randomised clinical trial

Dilini Imbulana, Dr Brett Manley, Dr Louise Owen, Professor Peter Davis

Newborn Research Department, Royal Women’s Hospital

Background: Preterm infants often require respiratory support for the treatment of Respiratory Distress Syndrome. Use of non-invasive support such as Continuous Positive Airway Pressure (CPAP), or Nasal Intermittent Positive Pressure Ventilation (NIPPV), in very preterm or extremely low birth infants is associated with nasal trauma related to the use of nasal prongs. Delivery systems for CPAP/NIPPV are effective in generating constant airway pressure, but the force applied to the nose can compromise skin integrity and cause injury. Nasal injury causes pain and discomfort, may necessitate change in respiratory support therapy, and may ultimately require surgical intervention. A nasal barrier dressing may reduce the incidence of nasal trauma and improve the delivery of CPAP/NIPPV by reducing leak at the nose, leading to improved clinical outcomes such as shorter duration of support, oxygen supplementation and hospitalisation.

Objectives: To compare the efficacy of a nasal barrier dressing “Neo-Guard” with standard care to reduce nasal trauma during CPAP/NIPPV.

Methods: In this single centre, randomised trial at the RWH, we will enrol 206 very preterm infants (born <30 weeks’ gestational age, GA) or extremely low birth weight infants (birth weight <1250g) who require at least 4 hours of CPAP/NIPPV support, and who have received <48 hours of CPAP/NIPPV support prior to enrolment. Prospective, written parental consent will be obtained. Infants are randomised to either ‘No Barrier’ (control) group or ‘Barrier’ (intervention) group. Treatment allocation is contained in sealed, opaque envelopes, stratified by GA: <28 weeks’ GA or ≥28 weeks’ GA. In the Barrier Group, the nasal barrier dressing is applied as soon as possible, and within one hour of randomisation. The primary outcome is the incidence of nasal trauma during CPAP/NIPPV support, defined by the RWH Nasal Integrity and Pressure Chart. Secondary outcomes include blinded assessments of nasal trauma, made by a blinded trial investigator assessing de-identified photographs of the noses of study participants, as well as other important neonatal outcomes.

Results: Recruitment commenced in April 2016. To date, 58 babies have been eligible and 38 babies have been recruited. A total sample size of 206 infants is required to show a 40% reduction in the baseline nasal trauma rate of 50%. Recruitment is due to continue until August 2017. Analysis will be by intention to treat.

Discussion: This study will evaluate whether nasal barrier dressing use during CPAP/NIPPV support reduces the rate of nasal trauma in extremely preterm and very low birth weight infants.
The effect of mesenchymal stem cell-derived exosomes on ageing of placental stem cells

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Department of Maternal-Fetal Medicine, Pregnancy Research Centre and University of Melbourne Department of Obstetrics and Gynaecology, Royal Women’s Hospital

Background: Premature ageing is a feature of important placental pathologies. Placental tissues from uncomplicated, early-term (37 0/7 to 38 6/7 wks) deliveries show signs of early ageing. Advanced ageing of placental tissues is detected in the late/post-term period (41 0/7 wks and beyond), where there is an increased risk of stillbirth. Stem cells play a critical role in ageing of placental tissue. We study stem cells isolated from the maternal component of the placenta; the decidua (DMSCs). Late/post-term DMSCs, when compared to early-term DMSCs, show increased levels of cell death and higher expression of the ageing markers, consistent with advanced ageing and poor function. The functions of MSCs are mediated by secreted vesicles called exosomes. Here, we determine whether younger, early-term DMSC exosomes can reduce or reverse the effects of advanced ageing in late/post-term DMSCs.

Objectives: 1) Isolate exosomes from early-term DMSCs using three different methods (i.e. ultracentrifugation, precipitation and magnetic separation) and compare their size, morphological characteristics, yield and purity.
2) Add early-term DMSC exosomes to late/post-term DMSCs and measure the effect on attachment of late/post-term DMSCs to surfaces and their proliferation; two important properties of stem cells.

Methods: DMSCs were isolated from the maternal decidua that remains attached to the placenta following delivery. DMSCs were cultured in the laboratory and their growth medium, which contains their secreted exosomes, was obtained. Exosomes were isolated from the growth medium using ultracentrifugation, precipitation and magnetic separation methods. Electron microscopy methods were used to visualize the exosomes. Yield and purity were determined using protein assays and Nanosight analysis respectively. The effect of early-term DMSCs exosomes on late/post-term DMSCs attachment to surfaces and proliferation was determined by a real-time functional assay.

Results: 1) Highest yield of exosomes were obtained using precipitation method (26.09±3.46µg/ml) when compared to ultracentrifugation (1.39±0.34µg/ml) and magnetic separation (11.77±1.15µg/ml)(n=5 each method). However, ultracentrifugation gave the highest purity of exosomes (4.6±1.90 x 10^10 exosome-sized particles/ml) compared with precipitation (2.7±0.89 x 10^10 exosome-sized particles/ml) and magnetic separation (2.8±1.19 x 10^10 exosome-sized particles/ml). 2) Preliminary results show that term DMSC exosomes aid significantly in attachment of DMSCs (n=6, p<0.001) but not proliferation.

Discussion: This study provided the first evidence that exosomes from early-term DMSCs can reduce or reverse the decline in the ability of advanced ageing late/post-term DMSCs to attach to surfaces. Future work will examine the effect of early-term DMSC exosomes on other advanced ageing-related properties of late/post term DMSCs.

Synchrotron MRT radiation induces DNA damage and inflammatory response in normal mouse tissues distant from the irradiated volume

Jessica Ventura1,2, Jason Palazzolo2, Helen Forrester3, Carl N. Sprung1, Nicole Haynes4, Andrea Smith2, Andrew W. Stevenson5,6, Christopher J. Hall6, Jeffrey Crosbie7, John Hamilton8, Pavel Lobachevsky2,9, Olga A. Martin2,9,10
1 The Department of Obstetrics and Gynaecology, The Royal Women’s Hospital, Melbourne, VIC; 2 Molecular Radiation Biology Laboratory, Peter MacCallum Cancer Centre, Melbourne, VIC; 3 Hudson institute of medical research, Centre for Inmate Immunity and Infectious Diseases, Monash University, Clayton, VIC; 4 Gene Regulation Laboratory, Peter MacCallum Cancer Centre, Melbourne, VIC; 5 CSIRO, Clayton, VIC; 6 Australian Synchrotron, Clayton, VIC; 7 Applied Science Department, RMIT, Melbourne, VIC; 8 Clinical Sciences, Royal Melbourne Hospital; 9 Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, VIC; 10 Division of Radiation Oncology and Cancer Imaging, Peter MacCallum Cancer Centre, Melbourne, VIC.

Discovery of the radiation-induced bystander effect (RIBE) demonstrates that cell death and genomic instability are not restricted to cells that are directly exposed to ionising radiation. The RIBE refers to a situation where cells that have not been directly exposed to ionising radiation behave as though they have been exposed. This phenomenon presents real clinical consequences such as increased risk of secondary malignancies and inflammatory diseases after localised radiotherapy. Past reports indicate pronounced increase of DNA damage in bystander cells, especially in those of highly proliferative tissues. The fluctuations of the host’s immunological response elicited by localised radiation exposure are a proposed mechanism of the bystander effect. Our aim was to establish the contributions of DNA damage response and the immunological components in the propagation of the RIBE, by using synchrotron-generated irradiation of immune-compromised mice. The Imaging and Medical beamline (IMBL) at the Australian Synchrotron made it possible to investigate a new pre-clinical modality, microbeam radiation therapy (MRT), which yields superior therapeutic benefit while also preserving neighbouring healthy tissues in animal models, contrary to the broad beam modality currently used in hospitals. The MRT beam is generated when a single X-ray beam is split by a collimator, producing a lattice of planar microbeams. Wild-type C56BL/6 and Balb/c mice and immune-compromised mice (macrophage-depleted, CCL2 K/O and NSG) were irradiated with 10 Gy peak dose of MRT in an 8x8 mm^2 area on the right hind leg, with a dose rate of 49 Gy/sec. At 3 and 6 days post-irradiation, irradiated skin and unirradiated tissue samples were collected and probed for DNA damage using the COMET assay, apoptotic cell death and local immune response. Pronounced and robust DNA damage, apoptotic cells and immunological response were discovered in intestinal crypt cells of wild-type mice; these events were compromised in immune-deficient mice. The role of immune system components in propagation and persistence of systemic genome destabilisation after localised irradiation will be discussed.
A new suction mask to reduce leak during neonatal resuscitation: a randomised controlled trial

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1 Newborn Research Centre, The Royal Women’s Hospital, Melbourne, Australia
2 Department of Neonatology, University Children’s Hospital of Tübingen, Germany
3 Murdoch Childrens Research Institute, Melbourne, Australia
4 University of Melbourne, Melbourne, Australia

Background: Leak around the face mask is a common problem during neonatal resuscitation. A newly designed face mask using a suction system to enhance contact between the mask and the infant’s face may reduce leak and improve outcomes of neonatal resuscitation. The mask is designed to form a vacuum chamber between the inner and the outer rim. This is achieved by connecting the port of the mask to a negative pressure device via standard suction tubing found on most resuscitation trolleys.

Objectives: The aim of the study is to determine whether leak is reduced using the suction mask (Resusi-sure mask = A) compared with a conventional mask (Laerdal Silicone mask = B) during intermittent positive pressure ventilation of term and near term infants in the delivery room.

Methods: Single centre randomised controlled trial. Newborn infants ≥ 34 weeks gestation will be randomized to the intervention (A) or control (B) if they need intermittent positive pressure ventilation in the delivery room. Primary outcome is the difference in leak between intervention and control. Secondary outcomes are physiological outcomes such as oxygen saturation and heart rate, as well as clinical outcomes (e.g. difference in duration of mask ventilation, rates of endotracheal intubation, admission to intensive care unit, and pneumothoraces). The operators are blinded to the measurements obtained. A sample size of 44 infants (22 in each group) is needed to achieve a 50% reduction in leak and reduce the leak from 30% (using the conventional mask) to 15% (using the suction mask) [80% power and a two-tailed alpha error of 0.05].

Results: The study is still in progress. We attended 143 deliveries, 128 of these infants did not need any positive pressure ventilation and we were able to recruit 15 infants with a median gestational age of 36.7 (36.0-39.4) and a birth weight of 3060 (2849-3414) grams. Nine infants were randomised to the suction mask and six were randomised to the laerdal mask.

Serial lung ultrasound in healthy newborns from the first breaths to complete fluid clearance, an observational study

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1 Hudson Institute, Monash University, Melbourne, 2 Royal Women’s Hospital, Melbourne

Background: Lung ultrasound (LUS) has shown promise for evaluation of newborns with trouble breathing. However, no study has described the appearance of LUS in healthy newborns.

Objectives: To characterize the appearance of serial LUS in healthy infants >34 weeks from the first breath until full aeration and fluid absorption of the lungs is achieved.

Methods: This was a single-centre observational study enrolling neonates born at ≥35 weeks. We obtained serial LUS video recordings (2-D and M-mode) via the right and left axillae at 1-10 minutes, 11-20 minutes, 1, 2, 4, and 24 hours of life, using a L8-18i linear transducer at an initial depth of 2.5 cm. LUS videos were graded according to a previously validated system.

Results: We studied 115 patients, mean GA = 386/7 weeks ±11 days, mean wt = 3380 g ±555 g, 51 were delivered vaginally, 14 were delivered via caesarean section after a trial of labor, and 50 infants were delivered via elective caesarean section. We obtained and analyzed 1168 video recordings for this study. As assessed by lung ultrasound, lung aeration and fluid clearance occurred quickly. All infants had an established pleural line at the first examination (median time of examination = 2 (IQR 1-4) minutes). Only 14% of infants had substantial fluid retention by 10 minutes of life and all infants showed at least partial fluid clearance by 20 minutes of life. Forty-nine, 78%, and 100% of infants had complete fluid clearance 2, 4, and 24 hours respectively.

Discussion: In health transitioning newborn infants, lung aeration and partial fluid clearance is achieved in the first minutes of life with complete fluid clearance typically achieved within the first 4 hours after birth.

* Not assessed for student prize
A Comparison of Different Round Masks for Intermittent Positive Pressure Ventilation in Preterm Infants Prior to Intubation: The Mask study.


Newborn Research Centre, Royal Women’s Hospital

Background:
Establishing effective ventilation in the first minutes of life is a critical intervention in newborn resuscitation. This study aims to find the optimal mask size for achieving this when resuscitating premature newborns, which could improve outcomes for premature infants. Mask leak is common and is an impediment to effective resuscitation. An observational study recently performed at the RWH which measured preterm babies faces, concluded that smaller mask sizes are a better fit. We hypothesise that a smaller sized mask, which better fits the infants face, will reduce leak and lead to more successful mask ventilation.

Objectives: In infants ≤32 weeks gestation receiving positive pressure ventilation we aim to compare the facemask leak using a Fisher & Paykel (35mm or 42mm) facemask (intervention) to a Lærdal 0/0 (50mm diameter) facemask (control).

Methods: This is a single centred RCT currently recruiting in the RWH NICU. The participants will be randomised to the intervention (F&P masks) or the control (laerdal masks). A respiratory function monitor will be used to measure inflating pressures and gas flow via a flow sensor between the mask and the T-piece device with expiratory leak being calculated from the amount of gas that does not return back through the flow sensor on expiration. The primary outcome will be difference in leak measured between the mask and the infants face between the control and intervention groups.

Sample size: Assuming a leak of 50% in the control group, a sample size 128 (64 in each arm) would give a power of 80% to detect a 15% absolute difference in leak between the groups (50% versus 35%).

Results: Recruitment commenced for this study in RWH in September 2014 and is ongoing. 71 patients have been recruited to data. An interim data analysis by an external data monitoring committee is underway.

Discussion: Facemask leak is a significant barrier to effective preterm resuscitation and finding the optimal facemask in this group may lead to improved face mask ventilation.

Abstracts:

Early Career Researchers
Does body mass index impact on endometriosis?

Sarah Holdsworth-Carson1, Uri Dior1, Jenny Fung2, Grant Montgomery2, Martin Healey1, Peter Rogers1 and Jane Girling1.

1University of Melbourne, Department of Obstetrics and Gynaecology, Gynaecology Research Centre, Royal Women’s Hospital and 2University of Queensland, Institute for Molecular Bioscience.

Background: Endometriosis has been associated with low body mass index (BMI). The pathophysiology behind this observation has not been well explored, nor has the effect of high BMI on endometriosis.

Objectives: This study aimed to determine if BMI impacts on endometriosis; specifically, on disease severity and on regulation of endometrial gene expression.

Methods: Endometrial tissue, medical, surgical and histopathological information was collected from women (n=510) undergoing laparoscopy at the Royal Women’s Hospital. Endometriosis was diagnosed following surgical and histopathological confirmation and graded according to rAFS. Women with and without endometriosis were analysed based on BMI (underweight [<18.50kg/m²], normal [18.50-24.99kg/m²], pre-obese [25.00-29.99kg/m²] and obese [30.00->40.0kg/m²]). Total RNA from endometrial tissue was extracted and Illumina Human HT-12 Beadchips were performed to examine gene expression. Endometrium was examined by gene array to identify genes influenced by BMI and endometriosis.

Results: The proportion of women with endometriosis increased with decreasing BMI, whereas the proportion of women without endometriosis increased with increasing BMI (P=0.0001). This BMI-related pattern remained significant following incorporation of disease stage (Stage I+II and Stage III+IV, P=0.0004). The proportion of Stage I+II was greater in normal (55.1%) and pre-obese (53.1%) women versus obese women (29.9%). The proportion of Stage III+IV was unchanged across BMIs (17.6% normal, 16.4% pre-obese and 21.6% obese), but was decreased in normal and pre-obese women versus Stage I+II. Gene array studies showed that in Stage I+II disease, NEDD9 gene expression was increased in obese versus normal women (P=0.0037), NEDD9 was increased (P=0.0097) and SMARCAL1 was reduced (P=0.0353) in obese versus pre-obese women. XRN2 was reduced in endometrium from obese women versus underweight cases (P=0.0016). Gene array did not identify differently expressed genes between women with/without endometriosis relative to BMI.

Discussion: We observed a high proportion of endometriosis in women with lower BMIs, particularly for Stage I+II disease. For obese women attending the clinic for pain, only half had endometriosis. Obesity and Stage I+II disease were associated with differential gene expression in the endometrium. Therefore, BMI impacts on an individual’s likelihood of having endometriosis and disease severity.

Right from the start: Improving transition and support from the first breath of life

Louise Owen. Newborn Research Centre, RWH

Background: Every year >1,000,000 babies die from the complications of prematurity. Almost as many term babies also die annually following birth asphyxia. My research program focuses on newborn stabilisation, for term and preterm infants, from their first breath. My program includes randomised trials (RCTs), crossover, observational and manikin studies. Themes in the delivery room include the use of sustained inflations, effects of gas conditioning and evaluation of resuscitation equipment.

Objectives: To improve survival and outcomes for newborn infants. In this presentation I will focus on a recently completed RCT and a manikin study.

Methods 1: Preterm infants are at risk of hypothermia after birth, which adversely affects their outcome. Maintaining adequate temperature during resuscitation is difficult. Heating and humidifying the gases used for respiratory support after birth may improve their thermoregulation. This RCT determined whether use of heated-humidified gases at birth resulted in lower rates of hypothermia on admission to Neonatal Intensive Care (NICU). This multi-site trial randomised infants born <30 weeks’ gestation to receive either heated-humidified gases, or standard unconditioned gases during stabilisation. The primary outcome was hypothermia rate on NICU admission.

Results 1: Of the 273 infants enrolled, fewer infants in the heated-humidified group were hypothermic on admission to NICU (27%) compared with controls (43%, p<0.01). There were no differences in rates of hyperthermia, mortality or respiratory outcomes.

Conclusions 1: The use of heated-humidified gases during stabilisation significantly reduced admission hypothermia.

Methods 2: Supporting breathing during transition using a facemask and self-inflating resuscitation bag (mask ventilation) is a difficult skill to master. The resuscitator must minimise mask leak and nasal obstruction to achieve appropriate lung pressures and volumes. This manikin study investigated whether new equipment (new facemask and self-inflating bag), designed for resource-limited settings, improved mask ventilation delivery. Forty operators were recorded performing mask ventilation on a manikin using both standard and new equipment.

Results 2: This study showed that the new resuscitation bag was no different from standard equipment but that the new facemask significantly reduced mask leak (15% vs. 30% leak, p<0.001). Additionally, the new facemask was preferred by >80% of participants.

Conclusions 2: The new facemask was easy to use and improved the effectiveness of mask ventilation in a manikin. Clinical studies are now required.

Discussion: Both studies provide evidence to support simple changes in the delivery room that could improve neonatal outcomes. Other studies within my research program are also working towards this goal.
Identification of genes differentially expressed during menstrual breakdown and repair.

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Background: Menstruation is induced by progesterone withdrawal at the end of the menstrual cycle and involves endometrial tissue breakdown, regeneration and repair. Perturbations in the regulation of the menstrual cycle may result in menstrual disorders including abnormal uterine bleeding.

Objectives: We aimed to elucidate the changing molecular profile of human endometrium on days 2, 3 and 4 of menstruation and identify genes and pathways that play a role in the menstrual process.

Methods: Endometrial samples were collected by Pipelle biopsy on days 2 (n=9), 3 (n=9) or 4 (n=6) of menstruation. RNA was extracted and analysed by genome wide expression Illumina Sentrix Human HT12 arrays. Data were analysed using “Remove unwanted variation-inverse (RUV-inv). Ingenuity pathway analysis and the Database for Annotation, Visualisation and Integrated Discovery v6.7 were used to identify canonical pathways and functional gene clusters enriched between days 2, 3 and 4 of menstruation. Individual genes were validated by quantitative PCR.

Results: Significant canonical pathways and gene clusters enriched during menstrual bleeding included those associated with immune cell trafficking, inflammation, cell cycle regulation, extracellular remodelling and the complement and coagulation cascade (p<0.01). The largest number of differentially expressed genes (1176) was between days 2 and 4 of menstruation (p<0.05, fold change >2). We identified several novel genes in the context of menstruation including lipopolysaccharide binding protein (LBP), glutathione-S-transferase mu 1 and -2 (GSTM1/2), V-set domain containing T cell activation inhibitor 1 (VTCN1) and trefoil factor 3 (TFF3). Genes related to processes associated with inflammation were predominantly up-regulated on day 2 of menstruation (early-menstruation) whereas those associated with endometrial repair and regeneration were predominantly up-regulated on day 4 of menstruation (late-menstruation).

Discussion: The changing molecular profile during menstruation identifies a number of genes not previously associated with menstruation. Our findings provide new insights into the menstrual process and may present novel targets for therapeutic intervention in cases of endometrial dysfunction.

Vaccination has led to a dramatic decline in cervical human papillomavirus infections among Australian women under 36 years of age

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Background: In Australia, all adult women up to the age of 35 years have now been offered free vaccination with the quadrivalent human papillomavirus (HPV) vaccine (4vHPV: 6/11/16/18), as part of the publicly-funded vaccination program. This has led to rapid and substantial reduction in the prevalence of 4vHPV genotypes in Australian women up to the age of 24 years. The programs impact on HPV infections among 25–35 year old women has not been studied.

Objectives: We evaluated the impact of the national HPV vaccination program on prevalence of 4vHPV-targeted genotypes among 18–35 year old women attending family planning clinics in Victoria and NSW in 2015.

Methods: Repeat cross-sectional study compared prevalence of 4vHPV genotypes among two groups: (i) women aged 18–24 and 25–35 years recruited in 2015 (N=389) as part of an ongoing National HPV Monitoring Program, and (ii) women in the same age groups recruited in a pre-vaccine (2005–07) study (N=275). For women aged 18–24, we also compared prevalence with that in a post-vaccine study conducted in 2010–12 (N=688), to assess trends over time. Odds ratios for changes over time were estimated using logistic regression, adjusted for age, smoking and socioeconomic status (aOR).

Results: For the 2015 sample, the three-dose National HPV Vaccination Program Register-confirmed vaccine coverage was 53% (67%) and 39% among those 18–24 and 25–35 years, respectively. Among women aged 18–24 years, prevalence of 4vHPV types decreased from 22.7% in 2005–07 and 7.3% in 2010–12, to 1.5% in 2015 (aOR 0.05; 95% CI: 0.01–0.18; p-trend<0.001). A decline in 4vHPV genotypes was also observed among women 25–35 years (11.7% in 2005–07 to 1.6% in 2015 (aOR 0.34; 95% CI: 0.18–0.64; p=0.001)).

Discussion: Prevalence of 4vHPV genotypes has continued to decline among young women nine years after the vaccination program commenced. A substantial fall in 4vHPV genotypes was also evident in women aged 25–35 years, even though vaccination coverage was far lower in this group. The study finding provide the first evidence for the protective effect of HPV vaccination in women up to the age of 35 years in a real-world setting. This is likely to result in an equally dramatic reduction in HPV-related cervical disease.
Title: High-flow research at The Women’s: HIPERSPACE, HIPSTER and HUNTER.

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Background: Breathing difficulty (respiratory distress) is the main problem affecting preterm babies soon after birth. Neonatal clinicians aim to avoid mechanical ventilation in these babies by using ‘non-invasive’ breathing supports. Nasal high-flow (HF) is an easier to use, more comfortable non-invasive support than the standard therapy, nasal continuous positive airway pressure (CPAP). HF use in preterm infants has increased around the world, with The Women’s at the forefront of producing evidence from randomized clinical trials (RCTs) for this practice.

Objectives: To generate evidence from RCTs comparing HF with CPAP as:
1. Post-extubation support for very preterm infants in neonatal intensive care units (NICUs) (The HIPERSPACE trial, N Eng J Med 2013)
2. Primary respiratory support for preterm infants in NICUs (The HIPSTER trial, N Eng J Med 2016)
3. Primary respiratory support for newborn infants in Australian non-tertiary special care nurseries (SCNs) (The HUNTER trial, ongoing)

Methods: Newborn Research at The Women’s has led three multicentred, non-inferiority, RCTs, comparing HF with CPAP as respiratory support for newborn infants. HIPERSPACE compared HF with CPAP as post-extubation support in very preterm infants born <32 weeks’ gestation in three Australian NICUs. The primary outcome was treatment failure within 7 days, with a sample size of 300 infants. HIPSTER compared HF with CPAP as primary support in preterm infants born 28-36 completed weeks’ gestation, in nine NICUs in Australia and Norway. HUNTER is an ongoing trial comparing HF to CPAP as primary support for preterm and term infants born in nine Australian non-tertiary SCNs. In HIPSTER and HUNTER, the primary outcome is treatment failure within 72 hours of randomisation, with a sample size of 750 infants for each trial.

Results: HIPERSPACE (N=303) found HF to be non-inferior to CPAP as post-extubation support for very preterm infants. The NHMRC-funded HIPSTER trial (N=564) ceased early, as HF was found to have a significantly higher treatment failure rate than CPAP, although with ‘rescue’ CPAP there was no difference in intubation rates between the groups. The NHMRC-funded HUNTER trial is ongoing and has randomized 349 infants to date.

Discussion: The Women’s has led HF research that has changed clinical practice around the world. HF is an alternative to CPAP as post-extubation support for very preterm infants in NICUs, and can be used as primary support for preterm infants if CPAP is also available. If HF is non-inferior to CPAP in non-tertiary SCNs, its use will be rapidly adopted.

Controlling stem cell behaviour with bioengineered human placenta as a biological scaffold

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Background: Mesenchymal stem cells (MSC) are excellent candidates for therapeutic applications in a range of conditions relevant for maternal-fetal medicine and perinatology. There is constant need to expand MSC to obtain clinically useful numbers of cells without losing their regenerative properties. MSC are usually grown on plastic surfaces; however MSCs can grow more efficiently on a complex substance secreted by cells called the extracellular matrix (ECM). ECM can be prepared by chemically stripping away the bulk of the cells, which leaves behind the decellularised ECM (dECM). Our focus is on producing dECM from placenta cells and tissues. We created two placenta cell lines for dECM preparation. In addition, we prepared dECM from whole placental tissue and then used chemical/ enzymatic methods to form a hydrogel. Hydrogels can be seeded with MSC and injected, or they can be used to coat bioscaffolds, which can be seeded with MSC and used for regenerative medicine applications.

Objectives: Utilise dECM from placental cell lines and from placenta tissues to define a bioscaffold that promotes MSC expansion and more efficient osteogenesis.

Methods: Placental cell lines (DMSC23 and CMSC29) were decellularised to produce the deposited dECM. Cell proliferation was measured and osteogenic differentiation was quantified. Placenta tissue-derived dECM was produced from the human placenta, decellularised using 0.5% SDS, and lyophilised. To fabricate a hydrogel, placenta tissue-derived dECM was solubilised with pepsin and gelation was induced by neutralising the salt concentration and followed by warming at 37°C.

Results: Decellularisation produced both placenta cells and placenta tissue dECM (Fig 1A-B). MSC were cultured on these dECM substrates and different cellular behaviours were observed. A two-fold increase in MSC proliferation on dECM-DMSC23 was detected (Fig 1C) and 2.5 fold increase in osteogenesis was detected by staining of bone-like nodules deposited by the cells.

Discussion: Placental cells and tissue represent a useful tool in the study of ECM as they produce generous amounts of ECM when cultured in vitro. Further, the dECM-DMSC23 coating has the capacity to maintain the phenotype of subsequently seeded MSC populations as well as promoting osteogenic differentiation. The use of placenta as ECM source can further extend the clinical utility of ECM bioscaffolds by allowing the delivery of MSC via minimally invasive methods to injury sites. This project will provide new insights into cell-matrix interaction, and may lead to novel tissue engineering applications using placenta cells dECM, and placenta tissue dECM in the form of a hydrogel.