11 Outcomes of children at two years after multiple courses of antenatal corticosteroids for threatened preterm birth: the multiple antenatal corticosteroids study (MACS)

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OBJECTIVE: A single course of antenatal corticosteroids (ACS) is associated with a reduction in respiratory distress syndrome and neonatal death. Our placebo controlled trial of multiple courses of ACS, every 14 days, found no benefit. Infants exposed to multiple courses of ACS weighed less and had smaller head circumferences. (Lancet 2008; 372: 2143-51). The secondary outcome of this study was to determine if there was a difference in the risk of death or neurological impairment at 18-24 months of age.

STUDY DESIGN: 1858 women were randomized to receive multiple courses of ACS vs. placebo, every 14 days, until 33 6/7 weeks or delivery whichever came first. The primary outcome was a neonatal composite outcome. The secondary outcome was death or neurodevelopmental impairment defined as cerebral palsy or abnormal cognitive development. Abnormal cognitive development was defined as a score of < 70 on the Bayley Scale of Infant Development-II or comparable neurocognitive assessment or a delayed mental developmental age in the absence of a standardized assessment. Biometry was also assessed.

RESULTS: Of the original 2305 infants/foetuses, a total of 2104 (91.3%) were assessed at 18-24 months of age. The risk of death or neurodevelopmental impairment was similar between groups (148 [13.8%] ACS vs. 142 [13.7%] placebo group; OR 1.001; 95% CI 0.75-1.31, p=0.95). Toddlers exposed to multiple courses of ACS weighed less than those exposed to placebo (11.94g ACS vs. 12.14g placebo; risk difference −0.2; 95% CI −0.38,-0.006; p=0.04).

CONCLUSION: Administration of multiple courses of ACS, every 14 days, did not lead to a difference in death or neurologic impairment at 18-24 months of age. However, children exposed to multiple courses of ACS weighed less. Longer term follow-up studies are required (ClinicalTrials.gov number, NCT 00187382).

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12 Pharmacological actions of progesterone (P4) or 17-alpha-hydroxyprogesterone caproate (17P) to inhibit cervical ripening or prevent term delivery depend upon the route of administration and effects on the myometrium

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OBJECTIVE: Recent clinical reports indicate that progestins, P4 or 17P applied vaginally or parenterally, might be useful to treat recurrent preterm labor. Our objectives were to evaluate the cervix throughout pregnancy and estimate the effects of P4 (vaginal application and injection), 17P and the P4 antagonist RU-486 on cervical ripening and delivery in pregnant rats.

STUDY DESIGN: The cervices of timed-pregnant rats (normal delivery on day 22) were followed with LIF (collagen light-induced fluroescence). Daily treatments consisted of: (1) P4 (4mg s.c.) (2) P4 (2mg to 15 mg, vaginally bid) (3) 17P (10 mg s.c) or (4) vehicle (n=6 for each group) from day 13 of pregnancy until delivery. Some rats were also treated with a single injection of RU-486 (3 mg s.c.) on day 16 .Cervical ripening was assessed every second day in vivo by LIF using a collascope instrument by placing the probe on the cervix. Statistics were assessed by ANOVA and the Student's t-test ($P<.05$).

RESULTS: LIF is significantly higher in the P4-injection group and in the 17P treated group (until day 19 only for the 17P) compared with vehicle controls. There is no significant difference between the vaginal P4 group and vehicle controls at any time in gestation. Only injection of P4 delays delivery. LIF is significantly lower in the RU-486 treated rats during preterm delivery compared to controls.

CONCLUSION: The cervix progressively softens during pregnancy and reverts to a rigid state postpartum. Parenteral treatment with P4 delays and reduces softening and completely blocks delivery. Vaginal P4, even at very high dose, has no effect on cervical softening or delivery. Injections of 17P also delays and reduces cervical softening until term but it does not inhibit delivery. Parenteral P4 treatment may be the preferred treatment to prevent preterm cervical ripening and prevent delivery as it also inhibits uterine contractility.

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13 Development of a noninvasive test for intraamniotic infection using proteomic analysis of cervicovaginal fluid in women with preterm labor

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OBJECTIVE: To develop a noninvasive test for intraamniotic infection (IAI) in women with preterm labor, intact membranes (PTL-I).

STUDY DESIGN: Among 105 cases of PTL-I before 37 wks, IAI was defined by amniocentesis results: positive amniotic fluid culture and/or 16S ribosomal DNA PCR (16S-rDNA), a highly sensitive & specific test for presence of eubacteria, *Mycoplasma,* and *Ureaplasma sp.* Immunoassays were used to quantitate proteins in cervicovaginal fluid (CVF). Candidate CVF proteins were entered individually and in combination into logistic regression models.

RESULTS: IAI was present in 13% (14/105), 11 by both 16S-rDNA & culture, 3 by 16S-rDNA alone. From 75 candidate CVF proteins associated with IAI, 5 were included in a final model: 1 plasma protein, 2 cytokine/chemokines, 1 cell adhesion protein, 1 peroxidase. Final model had area under ROC 0.983. A dichotomous classification rule based on these 5 proteins had sensitivity 93%, specificity 93%, PPV 72%, NPV 99% for prediction of IAI, and screen positive rate 19%. Test performance was not impaired by blood in the CVF specimen (present in 12 cases without IAI, none with IAI.)

CONCLUSION: In PTL-I, the CVF proteome is measurably altered by IAI. A prototype multiple-marker test based on the concentrations of 5 distinct proteins in CVF appears to discriminate between IAI and non-infected cases, potentially reducing need for amniocentesis. Such a test could be very useful in the management of PTL-I. Research regarding the role of various proteins in CVF may yield insight into the pathophysiology of PTL.

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14 Identification of fetal and maternal single nucleotide polymorphisms in candidate genes that predispose to spontaneous preterm labor with intact membranes

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OBJECTIVE: Preterm labor/delivery can be caused by the activation of several pathologic pathways. A genetic association study was undertaken to determine whether the maternal and/or fetal carriage of single nucleotide polymorphisms (SNPs) in candidate genes predisposes to spontaneous preterm labor/delivery.

STUDY DESIGN: A case-control study was conducted. Cases were patients with preterm labor who delivered <37 weeks. Controls consisted of women who delivered a normal AGA neonate at term; 190 candidate genes and 775 SNPs were studied. Cases were 223 mothers and 179 fetuses while controls consisted of 599 mothers and 628 fetuses. SNP discovery was performed by DNA sequencing, and genotyping was carried out using the MassARRAY(TM) System. Single locus and haplotype association analyses were performed separately on maternal and fetal DNA variants. Single locus tests of association were performed using logistic regression (additive model). We used a linkage disequilibrium based SNP pruning approach in the Plink software with a maximum r^2 and a false discovery rate to correct for multiple testing ($q^*=0.15$).

RESULTS: 1) The strongest fetal single locus association with spontaneous preterm birth was observed in the interleukin 6 receptor (*IL6R*) (OR=2.07 95% CI [1.42-3.02], p=0.000148); 2) The strongest maternal single locus association with spontaneous preterm labor/delivery was observed in a SNP in tissue inhibitor of metalloproteinase 2 (*TIMP2*) (OR=1.98 95% CI [1.38-2.83], p=0.000197); 3) These associations remain statistically significant after correction for multiple comparisons; 4) Global haplotype analysis indicated an association between a fetal DNA variant in insulin-like growth factor 2 (global p=0.004) and maternal alpha 3 type IV collagen isoform 1 (*COL4A3*, global p=0.007).

CONCLUSION: 1) A SNP involved in the control of fetal inflammation (*IL6R*) doubled the risk of preterm birth; and 2) DNA variants in maternal genes encoding for proteins involved in extracellular matrix biology also increased the risk of preterm birth.

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15 17P vehicle, castor oil, exerts a uterotonic effect in human myometrium in pregnancy

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OBJECTIVE: There is evidence that the clinical use of 17 Hydroxyprogesterone caproate (17P) in women at high risk of preterm delivery (PTD), results in a reduction in the rate of PTD, and a reduced risk of serious neonatal morbidity. The mechanism by which 17P exerts this effect is unknown. The possibility that the vehicle for 17HP, castor oil, exerts an independent effect on human uterine contractility, has previously been questioned, and is hitherto unknown. The aim of this study was to evaluate the potential effects on contractility of exposure of isolated human myometrial preparations, obtained during pregnancy, to castor oil.

STUDY DESIGN: Biopsies of human myometrium were obtained at elective cesarean section (n=8). Dissected myometrial strips suspended under isometric conditions, had contractility induced, for a 30 minute period using oxytocin (0.5nM). Strips were removed from the tissue bath and inserted in Castor Oil or physiological salt solution (PSS), for a 30 minute period. Strips were then re-suspended under isometric conditions and exposed to further oxytocin challenge. Contractile integrals were expressed in relation to the first challenge, and compared between study and control groups.

RESULTS: Strips exposed to castor oil demonstrated increased contractile activity elicited by oxytocin after the castor oil exposure (165.53%± 17.03%, n=8, P=0.004), in comparison to that measured prior to its exposure. For strips exposed to PSS only, the second oxytocin challenge reduced contractile activity (72.57%±7.48%, n=8, P=0.003). Comparison of the contractile activity, between castor oil and PSS exposed strips, revealed a significant increase in contractile activity for those exposed to castor oil (p<0.001).

CONCLUSION: These findings indicate that exposure of human myometrial preparations to castor oil results in an enhanced state of contractility in this in vitro model, in comparison to control experiments. The use of castor oil as placebo for 17P studies may be linked to increased contractility, and higher preterm delivery rates, among the placebo arm.

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16 Does mid-trimester cervical length 25 mm predict preterm birth in high risk women?

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OBJECTIVE: To estimate whether mid-trimester cervical length (CL) ≥ 25 mm in high-risk women is predictive of preterm birth.

STUDY DESIGN: Planned secondary analysis of the NICHD-sponsored cerclage trial. Women with documented prior spontaneous preterm birth (SPTB) at 17-$33^{6/7}$ weeks' gestational age (GA) underwent serial cervical ultrasound scans initiated between 16 and $21^{6/7}$ weeks. Fundal pressure-induced and spontaneous dynamic shortening were assessed to determine the shortest CL. The final scan was scheduled before 23 weeks. Women whose CL was < 25 mm were invited to consent for the intervention trial.

RESULTS: Of 1014 eligible women who began ultrasound screening, 318 had CL < 25 mm. Delivery dates were unavailable in an additional 24 (3.4%), leaving a study population of 672 for this analysis. The median (interdecile range) GA at the qualifying scan was 21.7 (19.1, 22.7) weeks, with a median CL of 34 (27, 42) mm. For comparison, the incidence of preterm birth < 35 wks was 42% in the 153 women who had CL < 25 mm and were randomized to no cerclage, as compared to 16% in this study population of women whose CL was ≥ 25 mm (p<0.0001). In a linear regression model, CL did not predict birth GA (p=0.15). Only in a Cox survival model was there a significant rela-