NOVA
University of Newcastle Research Online

nova.newcastle.edu.au

Dyson, Rebecca M.; Palliser, Hannah K.; Kelleher, Meredith A.; Hirst, Jonathan J.; Wright, Ian M. R "The guinea pig as an animal model for studying perinatal changes in microvascular function". Originally published in Pediatric Research Vol. 71, Issue 1, p. 20-24 (2012)

Available from: http://dx.doi.org/10.1038/pr.2011.9

Accessed from: http://hdl.handle.net/1959.13/1059114

Title: The guinea pig as an animal model for studying perinatal changes in microvascular function

Running Title: Modeling perinatal microvascular changes

Author List:

Rebecca M. Dyson, Hannah K. Palliser, Meredith A. Kelleher, Jonathan J. Hirst, Ian M. R. Wright *

Institutional Affiliations:

Mothers and Babies Research Centre [R.M.D., H.K.P., M.A.K., J.J.H., I.M.R.W.], Hunter Medical Research Institute, Newcastle, NSW, 2305, Australia; Discipline of Paediatrics and Child Health [R.M.D., I.M.R.W.], School of Medicine and Public Health, University of Newcastle, NSW, 2308, Australia; School of Biomedical Sciences and Pharmacy [H.K.P., M.A.K., J.J.H.], University of Newcastle, NSW, 2308, Australia; Kaleidoscope Neonatal Intensive Care Unit [I.M.R.W.], John Hunter Children's Hospital, Newcastle, NSW, 2305, Australia

Corresponding Author:

A/Prof Ian M R Wright, FRACP

Locked Bag 1, Hunter Regional Mail Centre, New South Wales, 2310, Australia

e-mail: ian.wright@hnehealth.nsw.gov.au

Telephone: (61) 2 49214362

Fax: (61) 2 49214408

Statement of Financial Support: This study was funded by a John Hunter Hospital Charitable Trust Project Grant awarded to IW and RD. RD was supported by a Hunter Children's Research Foundation Scholarship.

Category of Study: Basic Science

Word Count (Abstract): 183

Word Count (Manuscript): 4338

ABSTRACT

Microvascular dysfunction, characterised by inappropriate vasodilatation and high blood flow in the peripheral microcirculation, is linked to physiological instability and poor outcome in newborn neonates. Specifically, preterm neonates have significantly higher levels of baseline microvascular flow than term neonates at 24 hours postnatal age. Due to similarities between human and guinea pig endocrine profiles and maturity at birth, we hypothesised that the preterm guinea pig neonate would also demonstrate high microvascular flow compared to term controls, providing a suitable model for studying the mechanisms underlying transitional microvascular function. Guinea pigs that were delivered preterm show immaturity and had markedly reduced viability. Laser Doppler flowmetry was used to study microvascular blood flow at 23 hours postnatal age in surviving preterm and term animals. Baseline microvascular flow was significantly higher in preterm than term animals. No effect of intrauterine growth restriction or birth weight on baseline flow was observed in either preterm or term animals. These results are consistent with recent clinical findings and support the use of the guinea pig as a suitable model for future studies of the mechanisms underlying perinatal microvascular behaviour.

Key Words: neonate; microvascular

ABBREVIATIONS

LDF, laser Doppler flowmetry

PU, perfusion units

INTRODUCTION

In term infants the circulation undergoes rapid and extensive changes in the initial hours of extrauterine life to allow the infant to effectively deal with extrauterine systemic vascular resistance and hence provide normal and adequate perfusion of tissues (1-3). The transitional circulation of preterm neonates (especially those born at 30 weeks gestation or less) differs significantly from infants born at full term. In the preterm infant, a number of crucial physiological responses to extrauterine life are commonly delayed, allowing for persistence of atrial and ductal shunting and inappropriate blood flow throughout the periphery during the perinatal period (4-8).

Previous studies in preterm infants suggest that abnormal microvascular tone, characterised by inappropriate vasodilatation of the peripheral microvasculature may contribute to the development of circulatory compromise in preterm neonates (4, 8). These studies found that the functional integrity of the microvasculature (including appropriate control of vasodilatation) in preterm neonates is significantly altered compared to neonates born at later gestational ages (GA). Very preterm neonates (24-28 weeks GA) are known to have significantly higher microvascular blood flow at 24 hours postnatal age than preterm infants born at 29-34 weeks GA and neonates born at term. High baseline microvascular blood flow in premature infants is significantly correlated with clinical illness severity and poor outcome in the immediate postnatal period (4, 9). Such dysfunction in the microvasculature is also a well established observation associated with the onset of other causes of multisystem organ failure in neonates (10).

In addition to disorders relating to short gestation, intrauterine growth restriction (IUGR) and low birth weight are also associated with increased neonatal morbidity and mortality, particularly compromise associated with cardiovascular maladaptation (11, 12). Previous studies have demonstrated significant changes in peripheral microcirculation during the transitional period, however no effect of low birth weight or IUGR on baseline microvascular flow has been shown (13, 14).

Differences in microvascular blood flow dependant upon gestational age, postnatal age and sex have been observed in both physiological and biochemical studies. Physiological

studies have included the assessment of peripheral microcirculation using laser Doppler flowmetry (LDF). Recent studies have shown that such evaluation of the peripheral microcirculation is useful for the study of cardiovascular changes within the initial extrauterine period, allowing for changes in the microvascular bed of the infant to be assessed (15, 16). These studies also found that measurement of microvascular behaviour is useful for the detection of cardiovascular compromise as the regulatory mechanisms of the peripheral microcirculation both contribute to, and are involved in, the neuroendocrine response to cardiovascular compromise (12). Despite a number of such physiological studies, the mechanisms underlying these differences are poorly understood. In order to better understand these mechanisms an animal model is required to allow for investigation of the systems controlling microvascular blood flow, studies which are not possible in the human neonate. Very little evidence of the mechanisms controlling microvascular maturation exists. The normal ontogeny is not well described and the development of a suitable animal model for necessary invasive mechanistic investigations is essential to complement ongoing clinical assessments.

The guinea pig has previously been used as an animal model for studying IUGR and lung injury associated with prematurity (17-20). Unlike other laboratory rodents, the guinea pig has a long gestational period. It has placental endocrine control, and fetal and neonatal endocrine profiles that are comparable to that of the human, making it a particularly suitable model for studying human endocrine function and behaviour during pregnancy and early extrauterine life (21, 22). The guinea pig is also more precocious than other species of small laboratory animal, with the young born with a highly developed central nervous system, and is thus again more comparable to the human neonate than rats or mice in terms of perinatal adaptations, as the majority of brain development takes place in utero.

The aim of the present study was to establish an animal model of prematurity suitable for studying the mechanisms underlying control and dysregulation of microvascular function in the initial extrauterine period in preterm neonates. It was hypothesised that microvascular blood flow, as measured by LDF, would be dependent upon gestational age and sex, as seen in previous human clinical studies (4, 8).

METHODS

Animals. The Research Support Unit of the University of Newcastle supplied time-mated, pregnant outbred, tricolour guinea pigs. All procedures were approved by the University of Newcastle Animal Care and Ethics Committee and carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Male and female offspring were used for this study, with no dam represented by more than 2 pups of each sex in any group.

Spontaneous Term Delivery. In order for spontaneous vaginal delivery to occur, the pubic symphysis of the pregnant dam must relax and open, allowing for passage of the fetus. Detectable separation of this joint for two consecutive days indicates imminent onset of labour. Dams were monitored for delivery daily throughout late gestation, and then hourly via infrared camera following pubic symphysis separation. Pups were removed from the dam immediately following delivery.

Caesarean Section Delivery. Pregnant dams were allocated to either preterm or term delivery. For preterm delivery, caesarean section delivery was performed between 62 and 64 days GA (full term approximately 71 days (22)). As a percentage of completed gestation (87%), this is the equivalent of a human infant born in the late preterm period (approximately 34.5 weeks gestational age). This time point was chosen as it is the earliest gestation that guinea pig pups can be kept alive with non-invasive support (23). Guinea pigs have been shown to be markedly resistant to glucocorticoids such as betamethasone, with significantly decreased glucocorticoid receptor binding affinity (24). For this reason, 1mg/kg betamethasone (compared to an average dose of 0.2mg/kg used clinically in human pregnancies) was administered to pregnant dams via subcutaneous injection at 48 and 24 hours prior to delivery to stimulate fetal lung maturation. This dosage has been shown to exert effects in the guinea pig similar to those seen clinically in human pregnancies (25, 26). Term caesarean sections were performed at 69 days gestation, or earlier if the pubic symphysis had been open for 2 consecutive days. The dam was anaesthetised for surgical delivery by administration of 4% isoflurane in medical grade oxygen via chamber and mask inhalation for 15 minutes prior to delivery. At delivery, the uterus was exposed via an incision made down

the midline of the ventral abdomen and removed from the abdominal cavity maintaining blood supply. Pups were rapidly removed from the uterus to a warm bed for immediate resuscitation.

Neonatal Respiratory Support. Fluid was cleared by suction from the airways of pups. Preterm pups were then placed in a supine position and administered 50μl surfactant (80mg/ml Poractant Alfa, Douglas Pharmaceuticals, Australia) into the oropharynx with a gasp induced by nasal occlusion. Pups then received continuous positive air pressure with positive pressure ventilation as required, administered in a 1:1 ratio of oxygen and medical air at a rate of 8l/min. A Neopuff Infant T-Piece Resuscitator (Fisher & Paykel Healthcare, New Zealand) with positive end-expiratory pressures of 7cmH_2O was used to establish and maintain functional residual capacity, and, when required, mechanical ventilation with peak inflation pressures (PIP) of 20cmH_2O was administered. In pups with no spontaneous breathing present following birth, ventilation commenced with an initial sustained inflation with a PIP of 20cmH_2O for 20 seconds (27).

Neonatal Monitoring. Once stable, birth weight was recorded and pups were maintained in a humidified incubator at temperatures of 34-38ºC as required for maintenance of neonatal body temperature for the duration of the study period. Rectal temperature was measured two-hourly. As pups were delivered by non-recovery surgery of the pregnant dams, frequent feeding and monitoring of the neonates was required. Pups were monitored every two hours and clinical states recorded using a novel neonatal monitoring score based on three criteria – respiration, posture, and alertness/movement. A score out of 4 was assigned for each criterion, thus a maximum of 12. Scores of 3 and below were categorised as very poor, 4 to 6 poor, 7 to 9 good, and 10 to 12 very good. Preterm pups were also fed two-hourly with commercial guinea pig milk formula via a single lumen polyvinylchloride orogastric tube. For term pups, the milk formula was titrated intraorally.

Laser Doppler Flowmetry. Laser Doppler readings were conducted at 23 hours postnatal age, no less than 30 minutes after the last feed of pups. The PeriFlux System 5001 (Perimed AB, Jarfalla, Sweden) with a Small Straight Probe (Probe 407) and miniature probe holder (PH07-4) attached was used. The probe was applied gently to the rear of the pad of the hind foot to establish baseline microvascular flow. A baseline microvascular flow was

considered established when backscatter was stable and heart rate and rhythmical flow could be visualised within the PeriSoft (PSW2.0, Perimed) display for at least 30 seconds. The microvasculature was then subjected to a one-minute period of occlusion (achieved by applying pressure to occlude blood flow). Biological baseline (reported value) was determined by subtracting minimum value during occlusion ("biozero") from mean value for baseline reading. Microvascular blood flow data was recorded and analysed using custom PeriSoft software and is expressed in arbitrary perfusion units (PU) (28). Only recording sequences free from movement artefacts were analysed. All laser Doppler studies were performed by a single investigator (RD).

Post-Mortem Tissue Collection. Body, brain and liver weights were collected post-mortem at 24 hours postnatal age. IUGR was defined by brain-to-liver weight ratio >0.9. This has previously been defined as a marker of asymmetric growth restriction in the guinea pig (18). In order to compare with human data where liver weights are not known and small for gestational age infants with head sparing are diagnosed as IUGR via head circumference to body weight, brain-to-body weight ratio was also calculated for neonates.

Statistics. Prism 4 for MacOSX (GraphPad Software Inc., La Jolla, CA) was used for statistical analyses and generation of graphs. Unless otherwise stated, data for physical characteristics is presented as median (interquartile range) and analysed via Two-Way ANOVA. Incidence and mortality data were analysed using Fisher's Exact Test. LDF data were not normally distributed and were therefore log-transformed for further analysis (logPU). Differences between groups for microvascular blood flow logged data are presented as mean±SEM and were analysed by unpaired *t* test or Two-Way ANOVA as indicated. Significance level was set at $p<0.05$ for all data examined.

RESULTS

Physical Characteristics. Spontaneous vaginal delivery (n=3) occurred at 69 (67-70) days gestation (term caesarean section 69 (68-69) days gestation; n=5). No differences in physical characteristics or blood flow analysis were found between term pups delivered by caesarean section or spontaneous vaginal delivery and therefore term pups have been grouped (data not shown). Preterm delivery at 62 (62-63) days gestation (n=19) resulted in considerable immaturity and decreased viability. Preterm pups had significantly higher mortality rates than pups delivered at term, $p<0.001$ (Figure 1*A*). For preterm animals, males were found to have higher mortality rates than females (Figure 1*B*) but this did not reach statistical significance, $p>0.05$. Henceforth, data presented is for pups surviving to 24hours postnatal age only, with each dam represented by no more than two male and two female pups (median pups per litter = 2 (range 1-4)). Therefore, each group is represented by a minimum of 6 litters. Physical characteristics for surviving pups are presented in Table 1. Surviving preterm pups required respiratory support including surfactant administration and continuous positive air pressure with positive pressure ventilation, incubation and tube feeding. As a reflection of this, preterm pups returned significantly lower neonatal monitoring scores over the 24-hour study period (9.2 (8.0-9.9)) than term animals (12 (11.9-12)), $p<0.001$. Birth weight was significantly reduced in preterm pups (66.4g (53.9-70.4)) compared to term animals (76.3g (68.0-92.1)), $p<0.001$. There was no significant sex difference in body weight between males and females in either preterm or term animals.

Intrauterine Growth Restriction. Pups were classified as growth-restricted based upon calculation of brain-to-liver weight ratio. IUGR resulted in significantly reduced birth weights of both sexes of neonates in both term ($p=0.001$) and preterm ($p<0.001$) groups and asymmetrical growth restriction was also evidenced by significantly increased brain weight to body weight ratios in IUGR compared to non-IUGR pups in both term ($p<0.001$) and preterm ($p<0.001$) groups (data not presented). Incidence of IUGR was not different between term and preterm groups (32.3% and 37.5% respectively, $p=0.78$) and rates did not differ between sexes in either gestational age group (Table 1).

Laser Doppler Flowmetry. Baseline microvascular flow was significantly higher in preterm (1.22±0.04 logPU) than term (0.99±0.05 logPU) animals ($p<0.001$) at 23 hours postnatal age (Figure 2). Baseline flow did not differ significantly between sexes in either gestational group (data not shown). Baseline microvascular blood flow was not different between term IUGR (1.001±0.08 logPU) and non-IUGR (1.005±0.07 logPU) ($p=0.97$) or preterm IUGR (1.223±0.07 logPU) and non-IUGR (1.218±0.05 logPU) neonates ($p=0.95$). No significant relationship between birth weight and baseline microvascular flow was found to exist for either term ($r=0.165$) or preterm ($r=0.148$) animals (Figure 3).

DISCUSSION

The key finding of this study was that preterm guinea pig neonates have significantly higher levels of baseline microvascular flow than those delivered at term, however IUGR status and birth weight show no relationship with microvascular blood flow at 23 hours postnatal age. This finding is consistent with observations made in human neonates born at earlier gestational ages (24-28 weeks gestation) compared to neonates born at later gestational ages (4, 9).

One of the major difficulties with establishing an animal model to represent the preterm human is determining developmental age equivalency of the species and humans. Based on physical characteristics and mortality in our preterm animals, we suggest that the neonatal guinea pig delivered at 62 days gestational age is an appropriate model for studying microvascular adaptations in preterm humans during the immediate postnatal period. Normal gestation in our guinea pig population is approximately 71 days (22). Preterm neonates were delivered at 62 days gestational age, the approximate equivalent of a human infant born at 34.5 weeks completed gestation. However, the demonstrated decreased viability and significant physiological immaturity of these preterm neonates suggests an earlier age equivalency. This is supported by previous studies in the guinea pig that have assessed lung development in the fetal guinea pig and suggest that pups born prior to 66 days show considerable lack of development, demonstrated by lung growth and development, and that guinea pig neonates born at <88% completed gestation cannot survive with non-invasive interventions (23). These findings are consistent with the significant mortality of preterm guinea pig neonates observed in the present study. Previous studies have concluded that lung growth and development parallels the overall development of a species at birth (29). In a previous study of lung development, fetal guinea pigs at 61 days gestation had lung morphology indicative of the saccular stage of lung development, seen in the human neonate from approximately 24 weeks of age through to term (23). Viability and support levels in our neonatal guinea pigs suggest they are more physiologically equivalent to a human infant born at approximately 29 weeks completed gestation; as approximately half of the infants born at this gestation could be expected to survive with only our limited interventions.

It has long been established that male infants born prematurely are at in increased risk of poor outcome compared to females of the same gestational age (8, 30-32). This increased death rate appears to be associated with cardiorespiratory compromise (33). The mechanisms underlying this increased risk are not yet understood, although sex differences in physiological responses to the fetal-to-neonatal transition are well described (34-37). Our mortality rates for males and females in the preterm cohort are in line with such clinical observations. Although the difference in mortality between male and female neonates did not reach statistical significance (Figure 1*B*), it suggests that the preterm guinea pig delivered at this gestational age may be comparable to the human preterm neonate in terms of sexual dimorphisms seen in viability at earlier gestational ages. Significant loss of viability in male compared to female preterm neonates has been reported in large cohort human studies (38) and power analyses of our data suggests that a much larger number of litters (n=100) would have to be included for this result to reach significance at the same effect size as in the human population in our animal cohort. This is neither practical nor ethical for animal studies of this nature.

In humans, preterm male infants have been shown to have significantly higher baseline levels of microvascular flow than females. This sexual dimorphism is evident at 24 hours of postnatal age but no longer observed when the infant reaches 72 hours postnatal age (8). While we did not observe a significant sex difference or effect of IUGR in baseline microvascular blood flow, we propose that this may be a survival effect or a postnatal age effect. Further studies will need to investigate these factors in order to determine the usefulness of this model for studying sexual dimorphism in the control of extrauterine microvascular blood flow. Such studies will need to investigate microvascular function at earlier time points to assess if neonates with higher microvascular flow, which is known to correlate with adverse outcome are not represented by the group of neonates surviving to 24 hours. Earlier time points also need to be considered as the transition from fetal-to-neonatal circulatory systems is a rapid and extensive process, and it may be that 24 hours postnatal age in the neonatal guinea pig is outside the window of adaptation when maximal

dysregulation resulting in circulatory compromise and adverse outcome could be expected to occur.

Our results are consistent with previous clinical studies, which show that preterm birth, but not IUGR or low birth weight, is associated with high baseline microvascular blood flow at 24 hours postnatal age. Further studies at earlier postnatal ages have great potential to further our understanding of the relationship between dysregulation of the peripheral microvasculature and illness severity or mortality. Taken together, results from the current study support the preterm guinea pig as an animal model for studying perinatal microvascular changes in the initial extrauterine period. This will allow mechanistic studies in order to better understand the control of microvascular flow in the preterm neonate during this period of circulatory compromise.

REFERENCES

1. Bhutani VK 1997 Extrauterine adaptations in the newborn. Semin Neonatol 2:1-12
2. Sinha SK, Donn SM 2006 Fetal-to-neonatal maladaptation. Semin Fetal Neonatal Med 11:166-173
3. Rudolph AM 1971 Circulatory adjustments after birth: effects on ventricular septal defect. Br Heart J 33:Suppl:32-34
4. Stark MJ, Clifton VL, Wright IM 2008 Microvascular flow, clinical illness severity and cardiovascular function in the preterm infant. Arch Dis Child Fetal Neonatal Ed 93:F271-F274
5. Evans N 2006 Assessment and support of the preterm circulation. Early Hum Dev 82:803-810
6. Evans NJ, Archer LN 1990 Postnatal circulatory adaptation in healthy term and preterm neonates. Arch Dis Child 65:24-26
7. Kluckow M 2005 Low systemic blood flow and pathophysiology of the preterm transitional circulation. Early Hum Dev 81:429-437
8. Stark MJ, Clifton VL, Wright IM 2008 Sex-specific differences in peripheral microvascular blood flow in preterm infants. Pediatr Res 63:415-419
9. Stark MJ, Clifton VL, Wright IM 2009 Neonates born to mothers with preeclampsia exhibit sex-specific alterations in microvascular function. Pediatr Res 65:292-295
10. Smith SD, Tagge EP, Hannakan C, Rowe MI 1991 Characterization of neonatal multisystem organ failure in the surgical newborn. J Pediatr Surg 26:494-497; discussion 497-499
11. Gilbert WM, Danielsen B 2003 Pregnancy outcomes associated with intrauterine growth restriction. Am J Obstet Gynecol 188:1596-1599; discussion 1599-1601
12. Ishiguro A, Sekine T, Kakiuchi S, Nishimura R, Goishi K, Tsuchida S, Ohtsu H, Igarashi T 2010 Skin and subcutaneous blood flows of very low birth weight infants during the first 3 postnatal days. J Matern Fetal Neonatal Med 23:522-528

13. Martin H, Lindblad B, Norman M 2007 Endothelial function in newborn infants is related to folate levels and birth weight. Pediatrics 119:1152-1158
14. Norman M, Martin H 2003 Preterm birth attenuates association between low birth weight and endothelial dysfunction. Circulation 108:996-1001
15. Hanssler L, Roll C, Breukmann H 1992 Laser Doppler flowmetry in newborn infants with low birth weight. The effect of differences in humidity on peripheral circulation. Klin Padiatr 204:359-361
16. Martin H, Norman M 1997 Skin microcirculation before and after local warming in infants delivered vaginally or by caesarean section. Acta Paediatr 86:261-267
17. Lafeber HN, Rolph TP, Jones CT 1984 Studies on the growth of the fetal guinea pig. The effects of ligation of the uterine artery on organ growth and development. J Dev Physiol 6:441-459
18. Carter AM, Kingston MJ, Han KK, Mazzuca DM, Nygard K, Han VK 2005 Altered expression of IGFs and IGF-binding proteins during intrauterine growth restriction in guinea pigs. J Endocrinol 184:179-189
19. Langley SC, Kelly FJ 1994 Depletion of pulmonary glutathione using diethylmaleic acid accelerates the development of oxygen-induced lung injury in term and preterm guinea-pig neonates. J Pharm Pharmacol 46:98-102
20. McKendry AA, Palliser HK, Yates DM, Walker DW, Hirst JJ 2010 The effect of betamethasone treatment on neuroactive steroid synthesis in a foetal Guinea pig model of growth restriction. J Neuroendocrinol 22:166-174
21. Kaufmann P, Davidoff M 1977 The guinea-pig placenta. Adv Anat Embryol Cell Biol 53:5-91
22. Palliser HK, Zakar T, Symonds IM, Hirst JJ 2010 Progesterone receptor isoform expression in the guinea pig myometrium from normal and growth restricted pregnancies. Reprod Sci 17:776-782
23. Sosenko IR, Frank L 1987 Lung development in the fetal guinea pig: surfactant, morphology, and premature viability. Pediatr Res 21:427-431

24. Fuller PJ, Smith BJ, Rogerson FM 2004 Cortisol resistance in the New World revisited. Trends Endocrinol Metab 15:296-299
25. Owen D, Matthews SG 2007 Repeated maternal glucocorticoid treatment affects activity and hippocampal NMDA receptor expression in juvenile guinea pigs. J Physiol 578:249-257
26. Li T, Koshy S, Folkesson HG 2009 IL-1beta-induced cortisol stimulates lung fluid absorption in fetal guinea pigs via SGK-mediated Nedd4-2 inhibition. Am J Physiol Lung Cell Mol Physiol 296:L527-533
27. te Pas AB, Siew M, Wallace MJ, Kitchen MJ, Fouras A, Lewis RA, Yagi N, Uesugi K, Donath S, Davis PG, Morley CJ, Hooper SB 2009 Effect of sustained inflation length on establishing functional residual capacity at birth in ventilated premature rabbits. Pediatr Res 66:295-300
28. Leahy MJ, de Mul FF, Nilsson GE, Maniewski R 1999 Principles and practice of the laser-Doppler perfusion technique. Technol Health Care 7:143-162
29. Engel S 1953 The structure of the respiratory tissue in the newly-born. Acta Anat (Basel) 19:353-365
30. Naeye RL, Burt LS, Wright DL, Blanc WA, Tatter D 1971 Neonatal mortality, the male disadvantage. Pediatrics 48:902-906
31. Stephens BE, Vohr BR 2009 Neurodevelopmental outcome of the premature infant. Pediatr Clin North Am 56:631-646
32. Fanaroff AA, Stoll BJ, Wright LL, Carlo WA, Ehrenkranz RA, Stark AR, Bauer CR, Donovan EF, Korones SB, Laptook AR, Lemons JA, Oh W, Papile LA, Shankaran S, Stevenson DK, Tyson JE, Poole WK 2007 Trends in neonatal morbidity and mortality for very low birthweight infants. Am J Obstet Gynecol 196:147 e141-e148
33. Henderson-Smart DJ, Hutchinson JL, Donoghue DA, Evans NJ, Simpson JM, Wright I 2006 Prenatal predictors of chronic lung disease in very preterm infants. Arch Dis Child Fetal Neonatal Ed 91:F40-F45

34. Baenziger O, Jaggi JL, Mueller AC, Morales CG, Lipp HP, Lipp AE, Duc G, Bucher HU 1994 Cerebral blood flow in preterm infants affected by sex, mechanical ventilation, and intrauterine growth. Pediatr Neurol 11:319-324

35. Chen SJ, Vohr BR, Oh W 1993 Effects of birth order, gender, and intrauterine growth retardation on the outcome of very low birth weight in twins. J Pediatr 123:132-136

36. Davis M, Emory E 1995 Sex differences in neonatal stress reactivity. Child Dev 66:14-27

37. Hoffman EL, Bennett FC 1990 Birth weight less than 800 grams: changing outcomes and influences of gender and gestation number. Pediatrics 86:27-34

38. Ingemarsson I 2003 Gender aspects of preterm birth. BJOG 110 Suppl 20:34-38

FIGURE LEGENDS

Figure 1. Mortality rates at 24 hours postnatal age in term and preterm animals. *A)* Compared to animals delivered at full term, preterm delivery resulted in significantly decreased viability despite ongoing respiratory, thermal and nutritional support. * denotes significant difference $p<0.001$. *B)* Mortality was greater in preterm males than females. Although not statistically significant, the increased male mortality is comparable to that seen in large cohorts of human preterm neonates. Data is expressed as % mortality ± 95% confidence intervals.

Figure 2. Baseline microvascular blood flow in guinea pig neonates. Baseline microvascular blood flow, expressed as log of arbitrary perfusion units (logPU), was measured via laser Doppler flowmetry in term (●, n=26) and preterm (○, n=31) male and female animals at 23hours postnatal age. Baseline microvascular blood flow was significantly higher in preterm animals than term controls. Data is expressed as mean±SEM. * denotes significant difference $p<0.001$.