Progesterone receptor isoform expression in response to in utero growth restriction in the fetal guinea pig brain

HK Palliser, DM Yates, JJ Hirst

Mothers & Babies Research Centre and School of Biomedical Sciences, University of Newcastle, NSW, Australia.

Short title: Growth restriction and brain PR isoforms

Correspondence: Hannah K Palliser, PhD
Mothers & Babies Research Centre
Level 3 John Hunter Hospital
Locked Bag 1
Hunter Region Mail Centre
NSW 2310, Australia
Email: Hannah.Palliser@newcastle.edu.au
Phone: +61 2 4985 5642
Fax: +61 2 4921 4394

Keywords: Intrauterine growth restriction
Progesterone receptor
Compromised pregnancy
Fetal development
Perinatal brain injury
Neurosteroids

ABSTRACT

Intrauterine growth restriction (IUGR) is a significant in utero complication that can have profound effects on brain development including reduced myelination and deficits that can continue into adulthood. Progesterone increases oligodendroctye proliferation and myelin expression, an action that may depend on the expression of progesterone receptor (PR) isoforms A and B. The objective of this study was to determine the effect of IUGR on PR isoform expression in the brain of male and female fetuses and if effects were associated with a reduction in myelination.

We used a guinea pig model that involves selective reduction in maternal perfusion to the placenta at midgestation (35 days, term 70d). This resulted in significant reduction in body weight with marked sparing of brain weight. PRA, PRB and myelin basic protein (MBP) expression were measured in the brains of male and female growth restricted and control fetuses at late gestation.

MBP, as a measure of myelination, was found to decrease in association with IUGR in the CA1 hippocampal region with no change observed in the cortical white matter. There was a marked increase in PRA, PRB and total PR expression in the IUGR fetal brain. Control female fetuses demonstrated significantly higher PRA:PRB ratio than males, however this sex difference was abolished with IUGR.

These data suggest the central nervous system effects of clinical use of progesterone augmentation therapy in late pregnancy should be carefully evaluated. The overall upregulation of PR isoforms in association with IUGR suggests increased progesterone action and a possible neuroprotective mechanism.

INTRODUCTION

Intrauterine growth restriction (IUGR) is the failure of an infant to reach its full growth potential and is estimated to occur in up to 5% of all pregnancies (1). IUGR commonly results from placental insufficiency and a reduction in the supply of oxygen and nutrients to the developing fetus. Whilst IUGR is a major stressor that increases the risk of perinatal mortality (2), a large proportion of these infants are also delivered without any apparent trauma or brain injury. However, clinical evidence suggests that many IUGR babies have impaired neurodevelopment that may not be identified until school age or beyond (3-5).

Progesterone has been implicated in a variety of functions in the brain including maternal behaviour, learning and memory, mood, sexual differentiation and stimulation of myelin growth and neuroprotection (6-11). Recent studies have focused on progesterones role in neuroprotection as a precursor of the 5α-reduced metabolite allopregnanolone, which exerts its neuroprotective effects via $GABA_A$ receptors (12, 13). We have recently shown that inhibition of allopregnanolone production due to in utero steroid exposure or pharmacologic inhibition results in reduced myelination in the fetal guinea pig brain (14, 15). However, growing evidence suggests that progesterone itself has direct neuroprotective actions on the brain after traumatic brain injury, stroke and motorneuron degeneration particularly as a promyelinating agent (7-10). Progesterone stimulates the myelination of axons during development and the regeneration of myelin after injury (16-18) by inducing the proliferation and differentiation of oligodendrocytes and the stimulation of myelin production. This involves increased survival of oligodendrocytes, increased myelin basic protein (MBP) positive oligodendrocytes, stimulation of proliferation of oligodendrocyte

precursor cells (OPCs) and subsequent acceleration of their maturation into myelinating oligodendrocytes (18-20). These effects are reportedly mediated by the direct action of progesterone on the progesterone receptor (PR) as they are not seen in PR knockout mice (21). Furthermore, in support of PR mediated regulation of myelination, MBP expression in rat and mouse brain slices is increased by the selective PR agonist R5020 and abolished with RU486, a PR antagonist (21). IUGR can have many adverse effects in the fetal brain including reduced myelination which may occur due to a reduction in the number of myelinating oligodendrocytes or the reduced capacity of these oligodendroctyes to produce myelin (22, 23). Complications during pregnancy, such as IUGR, may disrupt expression of PR isoforms and hence affect progesterone action in the developing brain.

Progesterone action is regulated by two PR isoforms derived from a single gene: the N-terminally truncated PRA (80-90kDa) and the full length PRB (100-120kDa). Whilst structurally related and holding similar steroid hormone and DNA binding activities, the isoforms exhibit divergent transactivational properties and are functionally distinct (24, 25). The expression and functional role of the PR isoforms have been explored in a number of species with conflicting findings. In vitro studies suggest PRB to be functionally and transcriptionally active whilst PRA acts to inhibit the actions of PRB (25), however in vivo data is more equivocal. Whilst recent studies have centered on the individual role of these isoforms in normal physiological regulation of reproductive function and behaviour, the specific role of each isoform in neuroprotection and myelination has not been explored. The expression of PR isoforms are developmentally regulated in many regions of the male and female

rat and chick brain (26-30) suggesting a critical role in neural development. Specifically, the transient expression of PR in the hippocampus and cortex of the developing rat suggests a fundamental role in cognition, memory and learning.

Like humans, guinea pigs give birth to neuroanatomically mature offspring (31) and maintain high circulating progesterone levels throughout gestation (32) offering a suitable model in which to examine the role of PR isoform interactions in brain development and injury in fetal and neonatal life. Lafeber and colleagues were the first to apply the model of bilateral uterine artery ligation in mid-pregnancy to restrict growth of the placenta and fetuses in the guinea pig (33). An adaptation of this model that reduces the in utero death rate but maintains growth restriction of fetuses has recently been developed (15, 34). The restriction of maternal blood flow to the placenta leads to a decrease in oxygen and substrate supply and subsequently results in a hypoglycaemic, hypoxic, IUGR fetus (35, 36). There is considerable evidence that immature oligodendrocytes are highly susceptible to hypoxic-ischemic injury. This may have profound effects on neuronal development and may be regulated by disrupted PR expression (37-40).

In this study we examined the effect of IUGR on PR isoform expression and MBP immunostaining, as an indicator of myelination, in an area of the brain containing the hippocampus and cortex in the late gestation male and female fetal guinea pig. We hypothesise that IUGR alters PR isoform expression and reduces myelination.

MATERIALS AND METHODS

Animals

Outbred tri-color guinea pigs were time mated at the Research Support Unit of the University of Newcastle, Australia. All animal work was carried out in accordance with the University of Newcastle Animal Care and Ethics Committee. Surgery was performed at day 32-35 of gestation (term 70 days) under 1-3% isoflurane in medical grade E.P. oxygen. In order to establish placental insufficiency and subsequent IUGR in guinea pig fetuses (IUGR, n=7 male, n=8 female), a modification of the method of Turner and Trudinger was used (34). Briefly, the uterine horns were exposed and the fat pad manipulated to identify the uterine artery and the branches (spiral arteries) feeding each placental site. Diathermy was used to ablate approximately half the arteries supplying each placenta. Sham surgeries were performed in order to obtain control fetuses (control, n=7 male, n= 5 female). Dams were monitored daily until euthanasia at day 65 of gestation by CO_2 inhalation. Fetuses were removed from the uterus, sexed, body and organ weights recorded and brains collected. The brains were hemisected and coronal cuts made to divide each hemisphere into rostral, middle and caudal blocks. The middle block was used in the current study and contained cerebral cortex, subcortical white matter, corpus callosum, thalamus and the hippocampus. The right hemisphere was processed for subsequent immunoblotting (stored at -80°C) and the left hemisphere processed for immunohistochemistry (postfixed in 4% paraformaldehyde). There were 5-7 dams per group, with only one dam (litter) being represented by two fetuses per sex.

156

157 **Western blotting**

158 Frozen brain samples (middle blocks as described above) were pulverized on
159 dry ice and protein extracted. Briefly, samples (0.1mg) were homogenized in
160 1ml ice cold buffer (50mM Tris-HCl (pH7.5), 150mM NaCl, 1% NP-40, 0.5% Na
161 Deoxycholate, 0.1% SDS) containing Complete Protease Inhibitor Cocktail and
162 PhosphoSTOP Phosphatase Inhibitor Cocktail (Roche Diagnostics, Castle Hill,
163 Australia). After centrifugation, the supernatant was removed and protein
164 content determined using colorimetric detection and quantitation (Pierce Protein
165 Assay kit, ThermoFisher Scientific, Rockford, USA). Protein (110μg) was
166 separated using 10% Bis-Tris polyacrylamide pre cast gels (Invitrogen, Mt
167 Waverley, Australia) and transferred to PVDF (Hybond-P, GE Healthcare,
168 Sydney, Australia) by electroblotting. Membranes were then blocked in 5%
169 skim milk in TBST (25mM Tris-HCl, 15mM NaCl, 0.1% v/v Tween-20) at room
170 temperature for 1 hour. Membranes were incubated overnight at 4°C in a 1:100
171 dilution of PR antibody (MAI-410, Affinity Bioreagents, Thermo Fisher Scientific)
172 in TBST containing 5% skim milk. After washing in TBST, the membranes were
173 incubated in a 1:1000 dilution in 5% skim milk in TBST of anti-mouse IgG (HRP-
174 conjugated, Zymed, Invitrogen) for 2 hours at room temperature. The immune
175 complexes were visualized using SuperSignal West Pico chemiluminescent
176 substrate (Pierce, Thermo Fisher Scientific) detection system and captured
177 using the LAS-3000 Imaging System (Fuji Photo Film, Tokyo, Japan). Pre-
178 adsorbed antibody-peptide (MA1-410 neutralizing peptide, Affinity Bioreagents)
179 controls were run to determine specificity of PR isoform detection in the guinea
180 pig brain (Figure 1A). Relative amounts of PRA (80kDa) and PRB (100kDa)
181 were quantified by optical density analysis using Multi Gauge v2.4 software

(Fuji, Photo Film) after stripping and reprobing for β-actin (ab8227, Abcam, Cambridge, USA). The densities of the bands were determined and normalized with respect to corresponding β-actin background corrected values and subsequently to an internal/positive control (myometrium) to allow for comparison between blots. The PR ratio (PRA:B) is an arbitrary unit calculated on an individual animal basis using the paired PRA and PRB protein expression values.

Immunohistochemical detection of Myelin Basic Protein

Regions of brain containing the hippocampus and cortex were embedded in paraffin wax and 8μM thick coronal sections were cut (Leica RM2145 Microtome, Leica Microsystems, North Ryde, Australia). Sections were treated with 0.3% H_2O_2 to quench endogenous peroxidase activity and incubated in Revealit-AG Antigen Recovery solution (ImmunoSolution, Newcastle, Australia). Sections were blocked (0.5% BSA, 0.05% Saponin, 0.1M PBS pH7.2) for 1 hour at room temperature prior to overnight incubation in primary antibody (M9434, Sigma Aldrich, St Louis, USA) used at a dilution of 1:2000. After extensive washing in PBS, sections were incubated in biotinylated anti-rat secondary antibody (B7139, Sigma Aldrich; 1:300) for 5 hours followed by overnight incubation with streptavidin-biotin-HRP complex (GE Healthcare) at a dilution of 1:300. Labelling was revealed using DAB (3,3'-diaminobenzidine; Sigma, St Louis, USA) as a chromogen. Sections were coverslipped using Microscopy DPX (Merck, VIC, Australia). Control sections in which an equal amount of rat IgG was substituted for primary antibody were included and were routinely negative. Immunolabelled sections were viewed with a microscope (Zeiss Axioskop, Germany) and images acquired with a digital camera (Spot 2.20RT,

Diagnostic Instruments, USA). Digital images were imported into densitometric analyses software (ImageJ 1.40, National Institutes of Health, Bethesda, USA), binarized by threshold adjustment and percent area coverage measured in four fields of view from two sections per animal in each of the two regions of interest: the hippocampal CA1 and the subcortical white matter.

Statistical analyses

Data are shown as mean ± SEM. All data were analyzed using SPSS statistical software (Version 18, SPSS Inc., Chicago, IL, USA). Two way ANOVAs were performed to determine the effect of IUGR and sex. When an interaction was identified, bonferroni post hoc analysis was performed. Spearman correlations were performed to assess the relationship between growth parameters, PR and MBP expression. $P<0.05$ was considered to be statistically significant.

RESULTS

Induction of IUGR

IUGR fetuses had significantly lower body weight ($P<0.001$), nose-rump length ($P<0.001$), placental weight ($P<0.001$), liver weight ($P<0.001$), heart weight ($P<0.001$) and BLR ($P=0.003$) compared to the control fetuses (Table 1). No effect of sex, nor interaction of sex and IUGR, was identified in any measurements. Brain weight was not found to significantly change with either IUGR or sex.

PR isoform and ratio expression in the fetal IUGR brain

PRA expression demonstrated a significant effect of IUGR ($P=0.032$; Figure 1B) in the guinea pig brain. This affect was most evident in the male fetuses who demonstrated increased PRA expression with IUGR. PRB protein expression also demonstrated a significant effect of IUGR ($P=0.022$; Figure 1C) with a greater rise in expression seen in female fetuses. A significant effect of sex ($P=0.004$; Figure 1D) was identified in PRA:B expression, whereby female brains demonstrate a greater PRA:B ratio than the male fetuses. There was also a significant interaction of sex and IUGR ($P=0.003$) with respect to PRA:B. Post hoc analysis revealed PRA:B was greater in control female than male brains whilst this affect was not seen in IUGR brains where PRA:B ratios were not different between sexes. Total PR expression was significantly affected by IUGR ($P=0.032$; Figure 1E), whereby expression levels increased in IUGR fetuses. Whilst no sex effect was identified, this increase was most evident in male fetuses.

MBP expression

MBP expression in the CA1 region of the hippocampus was significantly decreased with IUGR (P=0.033; Figure 2A (region represented by thick dashed boxes) 2C & 2E) with no effect of sex. Quantification of MBP expression in the cortical white matter showed no significant effect of IUGR or sex despite a trend for less expression in females with IUGR that did not reach significance (P=0.08; Figure 2A (region represented by solid boxes), 2D & 2F). Qualitative assessment of the organization of MBP positive (MBP+) stained axons in the cortical white matter indicated fewer MBP+ axons (Figure 2A (region represented in outset image by fine lined dashed boxes) and 2B).

Correlations between body weight, BLR, nose-rump length, MBP and PR expression revealed a significant positive correlation between MBP expression in the CA1 region and fetal body weight ($r = 0.446$, $P= 0.043$) and fetal nose-rump length ($r=0.46$, $P=0.036$)(data not presented).

DISCUSSION

The key finding of this study was the significant effect of IUGR on PR isoform expression in the late gestation fetal brain, whereby IUGR increased expression of both PRA and PRB. This increase in PR expression coincided with decreased myelination in the hippocampal CA1 region of IUGR fetuses. Additionally, the potentially functionally relevant, basal PRA:B was found to differ between sexes and this difference was abolished by IUGR.

Induction of growth restriction by placental insufficiency is a common intervention in animal studies and the observed effects of decreased body weight with brain sparing is similar to the asymmetrical growth pattern often seen in human pregnancies affected by intrauterine growth restriction. These findings suggest perturbation in fetal growth affects PR isoform expression in the late gestation brain. We identified increased PR expression and decreased MBP expression in the fetal brain following IUGR, however whilst we found a positive correlation between body weight (and length) with hippocampal MBP expression, we did not find a significant correlation between MBP and PR expression in the regions studied. This suggests that the decrease in MBP specific myelination in the fetal brain due to poor in utero growth may be PR independent. As progesterone, via its receptors, has previously been shown to have a functional role in myelination (18, 19, 21) and the current finding that PR expression increased in IUGR fetal brains, we speculate that this increase in PR expression in the brain following in utero stress may be a mechanism in which to attempt to increase transcriptional activity of myelination-associated target

genes. This may represent an effort to protect the brain although the mechanisms involved remain to be elucidated.

Whilst both PRA and PRB increased with IUGR, the increase in PRA expression with IUGR was more evident in the male brain, whilst increased PRB expression following IUGR appeared more evident in the female brain. This was reflected by the observed differences in the PR isoform ratio. Basal PRA:B ratios were significantly higher in female brains than male, whilst there was no difference in the ratio following IUGR due to low ratios seen in both males and females. In tissues such as the myometrium, the PRA:B ratio is suggested to play an important functional role at labour. The usefulness of the PR isoform ratio in determining functional role of each progesterone receptor isoform remains controversial. Despite some support in the in vitro literature, there is little evidence in vivo for PRA inhibiting the transcriptional activity of PRB (41, 42). Secondly, it has been suggested that each isoform regulates individual sets of target genes with very few overlapping (43). In vivo studies in null mutant mice have suggested that the two isoforms work cooperatively to mediate progesterone-dependent gene expression and complex physiological responses or activities, such as in lordosis and that PRA appears to be the more transcriptionally dominant isoform in reproductive behaviour (42). In the current study, no significant sex specific differences were apparent in fetal guinea pig PRA or B expression. However, PRA:B revealed sex specific differences with greater PRA:B in females than males. Sex differences were considered in this study as infants male infants born preterm or with morbidities requiring NICU stays are twice more likely to die than female infants. This suggests some sexual dimorphism in their adaptation to adverse conditions.

315 Whilst the respective functions of PRA and PRB remain to be fully elucidated in
316 the nervous system, and the potential functional relevance of their ratio in the
317 brain is unknown, this study demonstrates sexually dimorphic expression whose
318 function may be of key importance.
319
320 This study aimed to investigate the effect of in utero growth restriction on the
321 expression of PR isoforms in the brain of the late gestation guinea pig fetus. As
322 progesterone readily passes through the blood brain barrier, fetuses are
323 exposed to high levels in utero, despite considerable metabolism by the
324 placenta. Due to high placental progesterone production throughout gestation,
325 the fetal brain is exposed to levels higher than those needed to saturate PR
326 (32). This suggests that changes in PR expression will have a major role in
327 progesterone action regardless of any sequestration or local synthesis in the
328 brain itself.
329
330 The hippocampal region, specifically the CA1, is known to be especially
331 sensitive to changes in oxygen delivery (36, 44-46) as may occur with placental
332 insufficiency. Our finding of reduced MBP expression in the hippocampus and
333 a positive relationship between fetal growth and MBP expression in the CA1
334 region indicates a deficiency in myelination following IUGR which may be due to
335 an inhibition or delay in MBP synthesis or increased degradation of MBP.
336 Previous studies have also reported decreased myelination in these areas
337 following IUGR in the guinea pig (22). In the guinea pig, the time of peak
338 myelination is in the last week of pregnancy (31). MBP is expressed by mature
339 oligodendrocytes and not OPCs hence the potential effect of IUGR on these
340 pre-oligodendrocytes in the current study was not defined. The examination of

postnatal guinea pig myelination is necessary to determine the permanency of the myelin deficits induced by in utero stress.

Based on the reported role of progesterone in myelination (7-10, 18), we hypothesized a positive association between PR expression and myelination, as represented by MBP immunostaining, and that IUGR would result in a reduction of both PR and MBP in the fetal brain. However, the current findings showed that IUGR resulted in increased PR expression and reduced myelination in the fetal brain. These observations therefore do not support the proposal that progesterone positively regulates myelination via expression of its receptor. Additionally, care must also be taken when considering the association of these factors, negative or positive, as it suggests but does not demonstrate cause or effect in the relationship of PR and MBP expression.

There is a large body of evidence, particularly in adult disease and trauma states, supporting the therapeutic role of progesterone in oligodendroctye precursor differentiation and myelin synthesis (47, 48). Comparatively little work has focused on the developing brain. Despite a number of animal studies and clinical trials currently evaluating progesterone to prevent or treat preterm labour, little examination and follow up of the effect of this treatment on the neonatal brain has been reported (49-51). One follow up study of postnatal progesterone and estrogen replacement therapy has revealed treated premature infants achieved normal psychomotor development earlier than untreated premature infants (52) supporting progesterones neuroprotective role ex utero in a clinical setting. In light of the present results demonstrating that in utero stress markedly influences brain PR isoform expression, the increased

exposure of the fetus to exogenous progesterone requires further evaluation. The administration of progesterone to prevent preterm birth and potential treatment of preterm infants with progesterone requires the consideration of the implications on neural development and myelination. Importantly, the influence of these treatments may be different following IUGR pregnancies and therefore the potential effects of such treatments on CNS development require careful consideration.

REFERENCES

1. Bryan SM and Hindmarsh PC*:* Normal and Abnormal Fetal Growth. Horm Res 2006; 65: 19-27.

2. Bernstein IM, Horbar JD, Badger GJ, Ohlsson A, and Golan A*:* Morbidity and mortality among very-low-birth-weight neonates with intrauterine growth restriction. Am J Obset Gynecol 2000; 182: 198-206.

3. Zubrick SR, Kurinczuk JJ, McDermott BMC, McKelvey RS, Silburn SR, and Davies LC*:* Fetal growth and subsequent mental health problems in children aged 4 to 13 years. Developmental Medicine & Child Neurology 2000; 42: 14-20.

4. Strauss RS*:* Adult functional outcome of those born small for gestational age: twenty-six year follow-up of the 1970 british birth cohort. JAMA 2000; 283: 625-632.

5. Low JA, Handley-Derry MH, Burke SO, Peters RD, Pater EA, Killen HL, and Derrick EJ*:* Association of intrauterine fetal growth retardation and learning deficits at age 9 to 11 years. Am J Obstet Gynecol 1992; 167: 1499-1505.

6. Bridges RS*:* A Quantitative Analysis of the Roles of Dosage, Sequence, and Duration of Estradiol and Progesterone Exposure in the Regulation of Maternal Behavior in the Rat. Endocrinology 1984; 114: 930-940.

7. O'Connor CA, Cemak I, Johnson F, and Vink R*:* Effects of progesterone on neurologic and morphologic outcome following diffuse traumatic brain injury in rats. Exp Neurol 2007; 205: 145-153.

8. Sayeed I, Wali B, and Stein DG*:* Progesterone inhibits ischemic brain injury in a rat model of permanent middle cerebral artery occlusion. Restorative Neurol and Neurosci 2007; 25: 151-159.

9. Schumacher M, Sitruk-Ware R, and De Nicola AF*:* Progesterone and progestins: neuroprotection and myelin repair. Current Opinion in Pharmacology 2008; 8: 740-746.

10. Gonzalez Deniselle MC, Garay L, Gonzalez S, Saravia F, Labombarda F, Guennoun R, Schumacher M, and De Nicola AF*:* Progesterone modulates brain-derived neurotrophic factor and choline acetyltransferase in degenerating Wobbler motoneurons. Exp Neurol 2007; 203: 406-414.

11. Frye CA and Walf AA*:* Progesterone enhances learning and memory of aged wildtype and progestin receptor knockout mice. Neuroscience Letters 2010; 472: 38-42.

12. Djebaili M, Guo Q, Pettus EH, Hoffman SW, and Stein DG*:* The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis and functional deficits after traumatic brain injury in rats. J Neurotrauma 2005; 22: 106-118.

13. Yawno T, Yan EB, Walker DW, and Hirst JJ*:* Inhibition of neurosteroid synthesis increases asphyxia-induced brain injury in the late gestation fetal sheep. Neuroscience 2007; 146: 1726-1733.

14. McKendry AA, Palliser HK, Yates DM, Walker DW, and Hirst JJ*:* The Effect of Betamethasone Treatment on Neuroactive Steroid Synthesis in a Foetal Guinea Pig Model of Growth Restriction. J Neuroendocrinol 2010; 22: 166-174.

15. Kelleher MA, Palliser HK, Walker DW, and Hirst JJ*:* Sex-dependent effect of a low neurosteroid environment and intrauterine growth restriction on fetal guinea pig brain development. J Endocrinol 2011; 208: 1-9.

16. Schumacher M, Guennoun R, Stein DG, and De Nicola AF*:* Progesterone: Therapeutic opportunities for neuroprotection and myelin repair. Pharmacology & Therapeutics 2007; 116: 77-106.

17. Brinton RD, Thompson RF, Foy MR, Baudry M, Wang J, Finch CE, Morgan TE, Pike CJ, Mack WJ, Stanczyk FZ, and Nilsen J*:* Progesterone receptors: Form and function in brain. Frontiers in Neuroendocrinology 2008; 29: 313-339.

18. Schumacher M, Guennoun R, Robert F, Carelli C, Gago N, Ghoumari A, Gonzalez Deniselle MC, Gonzalez SL, Ibanez C, Labombarda F, Coirini H, Baulieu EE, and De Nicola AF*:* Local synthesis and dual actions of progesterone in the nervous system: neuroprotection and myelination. Growth Hormone & Igf Research 2004; 14: S18-33.

19. Ghoumari AM, Baulieu EE, and Schumacher M*:* Progesterone increases oligodendroglial cell proliferation in rat cerebellar slice cultures. Neuroscience 2005; 135: 47-58.

20. Swamydas M, Bessert D, and Skoff R*:* Sexual dimorphism of oligodendrocytes is mediated by differential regulation of signaling pathways. J Neurosci Res 2008; 15 December 2008, Epub ahead of print.

21. Ghoumari AM, Ibanez C, El-Etr M, Leclerc P, Eychenne B, O'Malley BW, Baulieu EE, and Schumacher M*:* Progesterone and its metabolites increase myelin basic protein expression in organotypic slice cultures of rat cerebellum. J Neurochem 2003; 86: 848-859.

22. Nitsos I and Rees S*:* The effects of intrauterine growth retardation on the development of neuroglia in fetal guinea pigs. An immunohistochemical and ultrastructural study. Int J Dev Neurosci 1990; 8: 233-244.

23. Olivier P, Baud O, Evrard P, Gressens P, and Verney C*:* Prenatal ischemia and white matter damage in rats. J Neuropathol Exp Neurol 2005; 64: 998-1006.

24. Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, and Chambon P*:* Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. The EMBO Journal 1990; 9: 1603-1614.

25. Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW, and McDonnell DP*:* Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. Mol Endocrinol 1993; 7: 1244-1255.

26. Camacho-Arroyo I, Guerra-Araiza C, and Cerbon MA*:* Progesterone receptor isoforms are differentially regulated by sex steroids in the rat forebrain. Neuroreport 1998; 9: 3993-3996.

27. Guerra-Araiza C, Coyoy-Salgado A, and Camacho-Arroyo I*:* Sex differences in the regulation of progesterone receptor isoforms expression in the rat brain. Brain Research Bulletin 2002; 59: 105-109.

28. Camacho-Arroyo I, González-Arenas A, González-Agüero G, Guerra-Araiza C, and González-Morán G*:* Changes in the content of progesterone receptor isoforms and estrogen receptor alpha in the chick brain during embryonic development. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 2003; 136: 447-452.

29. Guerra-Araiza C, Villamar-Cruz O, González-Arenas A, Chavira R, and Camacho-Arroyo I*:* Changes in Progesterone Receptor Isoforms Content in the Rat Brain During the Oestrous Cycle and After Oestradiol and Progesterone Treatments. J Neuroendocrinol 2003; 15: 984-990.

30. Quadros PS, Pfau JL, and Wagner CK*:* Distribution of progesterone receptor immunoreactivity in the fetal and neonatal rat forebrain. The Journal of Comparative Neurology 2007; 504: 42-56.

31. Dobbing J and Sands J*:* Growth and development of the brain and spinal cord of the guinea pig. Brain Research 1970; 17: 115-123.

32. Illingworth DV, Challis JRG, Ackland N, Burton AM, Heap RB, and Perry JS*:* Parturition in the guinea pig; plasma levles of steroid hormones, steroid-binding proteins, and oxytocin, and the effect of corticosteroids, prostaglandins and adrenocorticotrophin. J Endocrinol 1974; 63: 557-570.

33. Lafeber HN, Rolph TP, and Jones CT*:* 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 1984; 6: 441-459.

34. Turner AJ and Trudinger BJ*:* A Modification of the Uterine Artery Restriction Technique in the Guinea Pig Fetus Produces Asymmetrical Ultrasound Growth. Placenta 2009; 30: 236-240.

35. Jones CT and Parer JT*:* The effect of alterations in placental blood flow on the growth of and nutrient supply to the fetal guinea-pig. J Physiol 1983; 343: 525-537.

36. Jensen A, Klonne HJ, Detmer A, and Carter AM*:* Catecholamine and serotonin concentrations in fetal guinea-pig brain: relation to regional cerebral blood flow and oxygen deliver in the growth-restricted fetus. Reprod Fert & Dev 1996; 8: 355-364.

37. Inder TE and Volpe JJ*:* Mechanisms of perinatal brain injury. Semin Neonat 2000; 5: 3-16.

38. Skoff RP, Bessert DA, Barks JDE, Song D, Cerghet M, and Silverstein FS*:* Hypoxic-ischemic injury results in acute disruption of myelin gene expression and death of oligodendroglial precursors in neonatal mice. Int J Dev Neurosci 2001; 19: 197-208.

39. Volpe JJ*:* Neurobiology of periventricular leukomalacia in the premature infant. Pediatric Research 2001; 50: 553-562.

40. Back SA, Han BH, Luo NL, Chricton CA, Xanthoudakis S, Tam J, Arvin KL, and Holtzman DM*:* Selective vulnerability of late oligodendrocyte progenitors to hypoxia-ischemia. J Neurosci 2002; 22: 455-463.

41. Tung L, Mohamed MK, Hoeffler JP, Takimoto GS, and Horwitz KB*:* Antagonist-occupied human progesterone B-receptors activate transcription without binding to progesterone response elements and are dominantly inhibited by A-receptors. Mol Endocrinol 1993; 7: 1256-1265.

42. Mani SK, Reyna AM, Chen JZ, Mulac-Jericevic B, and Conneely OM*:* Differential Response of Progesterone Receptor Isoforms in Hormone-Dependent and -Independent Facilitation of Female Sexual Receptivity. Mol Endocrinol 2006; 20: 1322-1332.

43. Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM, and Horwitz KB*:* Differential Gene Regulation by the Two Progesterone Receptor Isoforms in Human Breast Cancer Cells. J Biol Chem 2002; 277: 5209-5218.

44. Mallard EC, Rehn A, Rees S, Tolcos M, and Copolov D*:* Ventriculomegaly and reduced hippocampal volume following intrauterine growth-restriction: implication for the aetiology of schizophrenia. Schizophrenia Research 1999; 40: 11-21.

45. Mallard C, Loeliger M, Copolov D, and Rees S*:* Reduced number of neurons in the hippocampus and the cerebellum in the postnatal guinea-pig following intrauterine growth-restriction. Neuroscience 2000; 100: 327-333.

46. Dieni S and Rees S*:* Dendritic morphology is altered in hippocampal neurons following prenatal compromise. J Neurobiol 2003; 55: 41-52.

47. Wright DW, Kellermann AL, Hertzberg VS, Clark PL, Frankel M, Goldstein FC, Salomone JP, Dent LL, Harris OA, Ander DS, Lowery DW, Patel MM, Denson DD, Gordon AB, Wald MM, Gupta S, Hoffman SW, and Stein DG*:* ProTECT: A Randomized Clinical Trial of Progesterone for Acute Traumatic Brain Injury. Annals of Emergency Medicine 2007; 49: 391-402.e392.

48. Xiao G, Wei J, Yan W, Wang W, and Lu Z*:* Improved outcomes from the administration of progesterone for patients with acute severe traumatic brain injury: a randomized controlled trial. Critical Care 2008; 12: R61.

49. Meis PJ, Klebanoff M, Thom E, Dombrowski MP, Sibai B, Moawad AH, Spong CY, Hauth JC, Miodovnik M, Varner MW, Leveno KJ, Caritis SN, Iams JD, Wapner RJ, Conway D, O'Sullivan MJ, Carpenter M, Mercer B, Ramin SM, Thorp JM, Peaceman AM, Gabbe S, and National Institute of Child Health and Human Development Maternal-Fetal Medicine Units N*:* Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. N Engl J Med 2003; 348: 2379-2385.

50. Ness A, Dias T, Damus K, Burd I, and Berghella V*:* Impact of the recent randomized trials on the use of progesterone to prevent preterm birth: a 2005 follow-up survey. Am J Obstet Gynecol 2006; 195: 1174-1179.

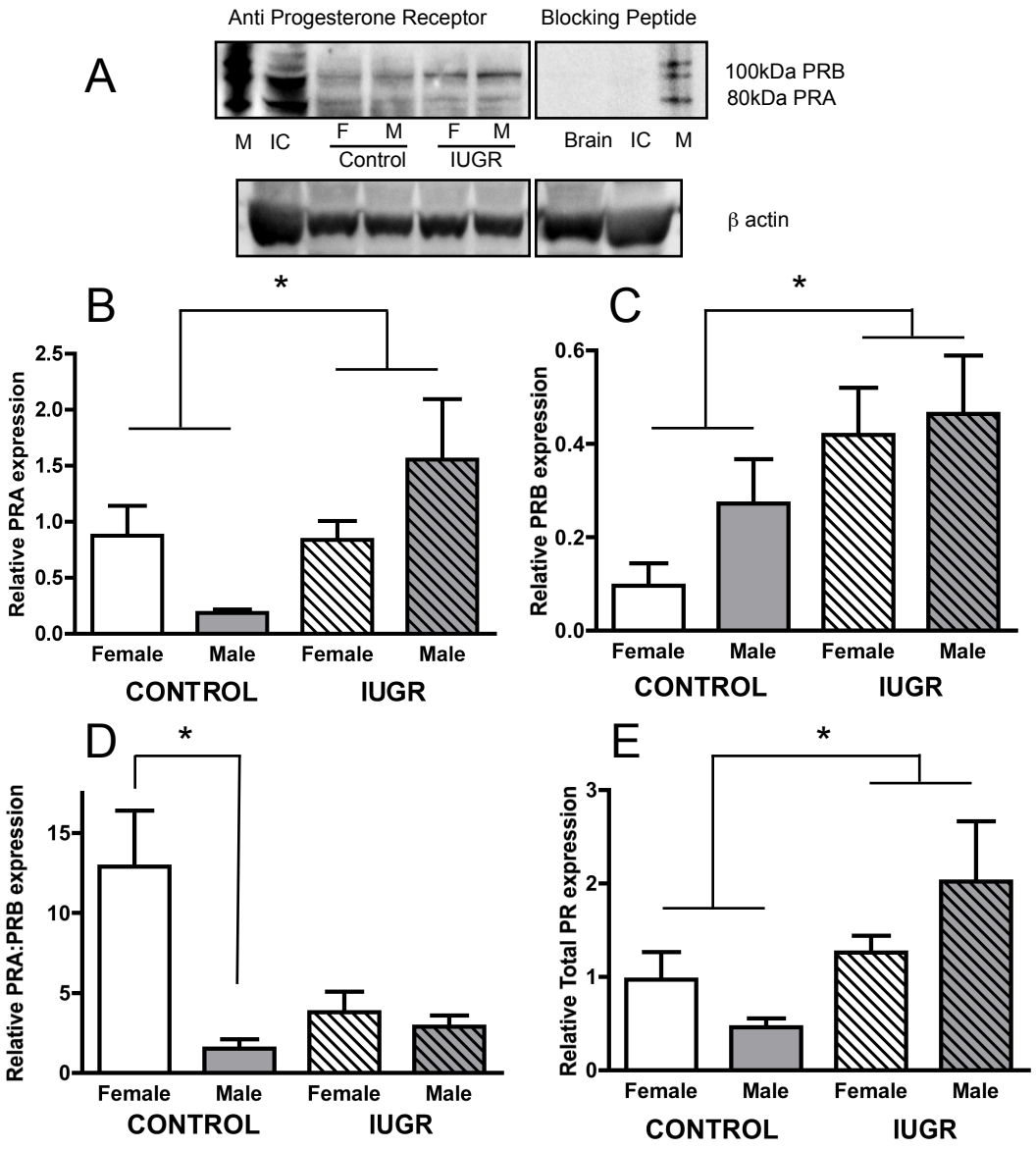
51. Dodd JM, Flenady VJ, Cincotta R, and Crowther CA*:* Progesterone for the prevention of preterm birth: a systematic review. Obstet Gynecol 2008; 112: 127-134.

52. Trotter A, Bokelmann B, Sorgo W, Bechinger-Kornhuber D, Heinemann H, Schmucker G, Oesterle M, Kohntop B, Brisch K-H, and Pohlandt F*:* Follow-Up Examination at the Age of 15 Months of Extremely Preterm Infants after Postnatal Estradiol and Progesterone Replacement. J Clin Endocrinol Metab 2001; 86: 601-603.

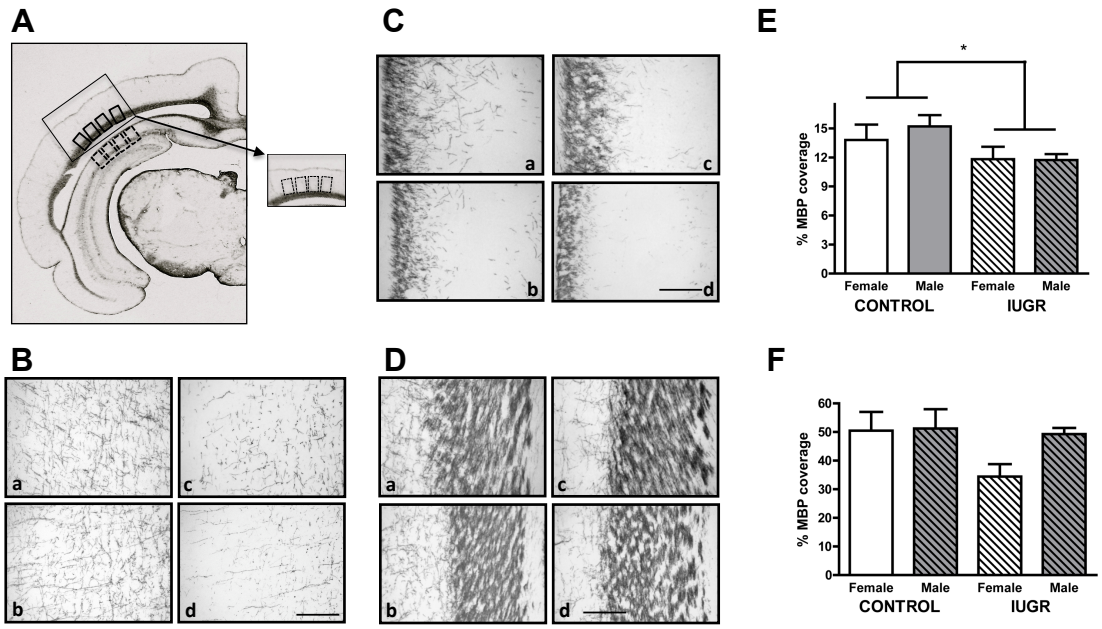
FIGURE LEGENDS

Figure 1. Representative western blot (A) demonstrating expression of progesterone receptor isoforms PRA (80kDa) and PRB (100kDa) in the late gestation female (F) and male (M), control and IUGR guinea pig brain in the upper left hand panel. A pooled brain sample and the internal control (IC, adult myometrium) are shown in the presence of the blocking peptide in the upper right hand panel and corresponding B-acting loading control in the bottom panel. The expression of progesterone receptor isoform A (B), isoform B (C) the progesterone receptor ratio (PRA:B) (D) and total progesterone receptor expression (E) in female (white), male (grey), control (open bar) and IUGR (lined bar) late gestation guinea pig brain (region containing hippocampus and cortex). PRA:B is calculated on an individual animal basis. M = molecular weight marker, myo = myometrium. Data presented as mean ± SEM with * $P<0.05$.

Figure 2. Representative photomicrograph demonstrating site of cortical and hippocampal MBP analyses (A) and representative images of the organisation of axons staining for MPB in the subcortical white matter (A, represented in the outset dashed boxes, and B) MBP expression in the hippocampal CA1 (A, region represented in dashed boxes and C) and subcortical white matter (A, region represented in solid boxes, and D) in control female (a), IUGR female (b), control male (c) and IUGR male (d) late gestation fetal guinea pig brains. MBP expression (represented as percentage area coverage) in the CA1 (E) and subcortical white matter (F) of female (white bar), male (grey bar), control (open bar) and IUGR (lined bar) late gestation fetal guinea pigs. Scale bar = 0.1mm. A = 2.5x magnification, B, C and D = 40x magnification. Data presented as mean ± SEM with * $P<0.05$.



585
586 Figure 1



587
588 Figure 2
589