Progesterone Receptor Isoform Expression in the Guinea Pig Myometrium from Normal and Growth Restricted Pregnancies

Hannah K Palliser[1,3], PhD, Tamas Zakar[2,3], MD, PhD, Ian M Symonds[2,3], MD and Jonathan J Hirst[1,3], PhD.

From the School of Biomedical Sciences[1], School of Medicine and Public Health[2] and Mothers and Babies Research Centre[3], University of Newcastle, Newcastle, New South Wales, Australia

Corresponding Author:
Hannah Palliser, PhD
MBRC/Endo Unit
JHH
Locked bag 1
Hunter Region Mail Centre
NSW 2310
Australia

Ph +61 2 4985 5642
Fax +61 2 4921 4394

Abstract

Despite the increased incidence of preterm labour with intrauterine growth restriction, the mechanisms of the relationship are unclear. In women, functional progesterone withdrawal mediated by changing myometrial progesterone receptor (PR) expression is linked to labour. The objectives of this study were to assess myometrial PR isoform abundance in guinea pig pregnancies associated with growth restriction, induced by disruption of placental blood supply, and in non gravid uterine horns during late gestation and with labour. Myometrial progesterone receptor isoform A (PRA) and B (PRB) abundance were down regulated as labour approached and the expression of both isoforms were markedly higher in the non-gravid compared to the gravid uterine horns. The fall in myometrial PRA and B protein levels was delayed in growth restricted pregnancies despite these pregnancies delivering significantly earlier. The results suggest a PR-mediated functional progesterone withdrawal mechanism in guinea pigs that may initiate uterine activation but does not directly stimulate labour, and an unexpected role of PR regulation in IUGR associated pregnancies.

3-5 keywords:

Growth restriction

Progesterone receptor

Myometrium

Preterm labour

Guinea pig

INTRODUCTION

Preterm birth remains the major cause of neonatal morbidity and mortality in the developed world. Intrauterine growth restricted (IUGR) infants are at a 2-3 fold increased risk of being delivered spontaneously preterm than infants appropriately sized for age and the rate of spontaneous delivery for small for gestational age infants is greater preterm than at term [1-5]. IUGR in itself is a major contributor to neonatal mortality and morbidity and commonly arises due to a compromised supply of nutrients and oxygen through the placenta to the fetus.

Progesterone acts through its receptor to maintain quiescence by limiting the activation of stimulatory pathways involving prostaglandins and their receptors, oxytocin, and gap junctions [6]. In most species, this progesterone block is lost prior to labour with the fall of circulating progesterone releasing the myometrium from its quiescent state. In primates including the human and in guinea pigs however, progesterone levels remain elevated throughout late pregnancy and labour [7-9] despite the increasing expression of prolabour genes leading to delivery. A decrease in progesterone responsiveness, termed "functional" progesterone withdrawal has been proposed and whilst the literature supports a modification in the sensitivity of the myometrium to progesterone in human labour, the mechanisms remain elusive. The proposed mechanisms of progesterone withdrawal, which are often conflicting and none of which are equivocally supported, include decreased nuclear PR expression [10], increased nuclear PR expression [11,12], changes in nuclear PR isoform ratios [13,14], reduction in steroid receptor coactivators [15], increase in PR repressors [16], decreased binding of PR to its nuclear response element [17] and more recently the putative role of membrane-bound PR receptors [18].

Defining the mechanisms controlling gestational length and labour onset in humans has proved difficult. The guinea pig, like the human, has a haemochorial placenta and maintains high circulating progesterone concentrations until delivery. The similarities between the two species make the guinea pig a suitable experimental model of the endocrine regulation of human pregnancy including pathologies such as IUGR [19]. For example, PR isoforms similar to those in women, have been identified in the guinea pig myometrium and an analogous role of prostaglandins in the birth process has been demonstrated [20-22]. In addition, the guinea pig uterus is bicornuate, often producing single horn pregnancies. This provides the opportunity to examine receptor expression in gravid and non-gravid myometrium simultaneously and assess the contribution of systemic and local factors and stretch to the changing myometrial phenotype during pregnancy and labour.

In this study we have established a guinea pig model where disruption of maternal placental blood flow was used to induce placental insufficiency and cause mild fetal growth restriction. The objectives of this study were to assess myometrial PR isoform abundance in pregnancies associated with growth restriction and in non gravid uterine horns during late gestation and with labour onset.

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. In order to establish placental insufficiency and subsequent IUGR in guinea pig fetuses, a modification of the method of Turner and Trudinger was used [23]. Surgery was performed at day 32-35 of gestation (term approximately 70 days) under 1-3% isoflurane in medical grade E.P. oxygen. 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 half the arteries supplying each placenta. Sham surgeries in which the fat pad and uterine artery branches were exposed but not ablated were performed in order to obtain control tissues. Dams were monitored daily until euthanasia at day 62 of gestation (control n=5; GR n=6), day 65 (control n=5; GR n=6), day 68 (control n=7; GR n=5) and during labour (control n=6; GR n=4) by CO_2 inhalation. Labour was identified by the birth of the first pup at which time the dam was immediately euthanased. The uterus was exposed and fetuses were removed, sexed, and placement in horn, body, brain and liver weights were recorded. Myometrial samples were collected from the site of each fetal head and stored at -80°C. Non gravid myometrium was also collected at 62 days (n=5), 65 days (n=3), 68 days (n=4) and at labour (n=6) from control (sham) pregnancies. Guinea pigs require a marked relaxation and opening of the pubic symphysis to allow passage of the fetus through the birth canal at labour. Pubic symphysis separation was monitored throughout pregnancy and classified as opening (day 1) when manual palpation by a constant observer detected the initial separation of this joint.

Determination of IUGR

Fetuses were categorized into IUGR (growth restriction or small for gestational age) or control (size appropriate for age) groups by the assessment of their body weight and calculation of their brain to liver ratio (BLR) at the time of tissue collection. Fetuses were classified as IUGR if their BLR was greater than 0.9 [24] or if their body weight was greater than one standard deviation below the mean for that age group, as calculated from our extended cohort of animals. No significant difference was found in litter size nor in the sex of the fetuses (data not presented). No more than one myometrial sample was used from each pregnancy (obtained from the site of either the first or second fetus at the cervical end of the horn).

Western blotting

Frozen myometrial samples were pulverized on dry ice and protein extracted. Briefly, samples (0.1mg) were homogenized in 1ml ice cold buffer (50mM Tris-HCl (pH7.5), 150mM NaCl, 1% NP-40, 0.5% Na Deoxycholate, 0.1% SDS) containing Complete Protease Inhibitor Cocktail and PhosphoSTOP Phosphatase Inhibitor Cocktail (Roche Diagnostics, Castle Hill, Australia). After centrifugation, the supernatant was removed and protein content determined using colorimetric detection and quantitation (Pierce Protein Assay kit, ThermoFisher Scientific, Rockford, USA). Protein (100μg) was separated using 10% Bis-Tris polyacrylamide pre cast gels (Invitrogen, Mt Waverley, Australia) and transferred to PVDF (Hybond-P, GE Healthcare, Sydney, Australia) by electroblotting. Membranes were then blocked in 5% skim milk in TBST (25mM Tris-HCl, 15mM NaCl, 0.1% v/v Tween-20) at room temperature for 1 hour. Membranes were incubated overnight at 4°C in a 1:300 dilution of PR antibody (MAI-410, Affinity Bioreagents, Thermo Fisher Scientific) in TBST containing 5% skim milk. After washing in TBST, the membranes were incubated in a 1:1000 dilution in 5% skim milk in TBST of anti-mouse IgG (HRP-conjugated, Zymed, Invitrogen) for 1 hour at room temperature. The immune

complexes were visualized using SuperSignal West Pico chemiluminescent substrate (Pierce, Thermo Fisher Scientific) detection system and captured using the LAS-3000 Imaging System (Fuji Photo Film, Tokyo, Japan). Pre-adsorbed antibody-peptide (MA1-410 neutralizing peptide, Affinity Bioreagents) controls were run to determine specificity of PR isoform detection in the guinea pig myometrium (Figure 1A). Relative amounts of PRA (80kDa) and PRB (100kDa) 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 control (pooled guinea pig myometrial protein sample used on all blots) to allow for comparison between blots. The PR ratio (PRA:B) is an arbitrary unit calculated on an individual myometrial sample basis using the paired PRA and PRB protein expression values.

Statistical analyses

Data are shown as mean ± SEM. All data were analyzed using PASW statistical software (Version 18, SPSS Inc., Chicago, IL, USA). One way ANOVA and subsequent LSD post hoc tests were used to assess differences between groups. $P<0.05$ was considered to be statistically significant.

RESULTS

Gestational Age and Physical Characteristics

As expected, body weight was significantly lower at all gestational ages in the fetuses subjected to placental artery ablation compared to the sham-operated controls (Table 1). Placental weight was also reduced at 62 days of gestation and at labour and showed a trend to lower weights in the other ages examined. Whilst BLR appeared lower at all time points, the difference was only significant at 62 and 65 days of gestation. As shown in Table 1, gestational length was significantly shorter in the growth restricted group compared to control. In contrast, duration for which the pubic symphysis was detectably separated prior to labour or at any other time point did not differ.

PRA, PRB and total PR abundance in the gravid and non-gravid uterine horns

Specific PRA and PRB bands were detected in the guinea pig myometrium at approximately 80kDa and 100kDa respectively (Figure 1A). Control myometrium (gravid horns from sham-operated guinea pigs) showed a decrease in PRA, PRB and total PR from 62 days of gestation to 65 days with no further change at 68 days or with labour onset (Figure 1B-D). Uterine tissue from the non-gravid horn showed a significant decrease in PRA and total PR at 68 days with a further significant decrease in total PR abundance at labour. PRB abundance fell significantly from 62 days and at the time of labour. PRB and total PR abundance was markedly higher in the non-gravid uterine horn than in the gravid horn at 62, 65 and 68 days, but not in labour. PRA abundance was significantly higher in the non-gravid horn than in the gravid horn at 62 and 68 days.

Effect of growth restriction on myometrial PRA, PRB and total PR abundance

Myometrium from growth restricted pregnancies did not show a decrease in PRA, PRB or total PR abundance between 62 and 65 days; however, there was a significant fall between 65 and 68 days with no further change with labour (Figure 1B-D). The levels of PRA and total PR abundance did not differ between control and IUGR myometrium (Figures 1B and 1D; the tendency for PRA to increase with GR did not reach significance at 65 days), but PRB was significantly more abundant in the growth restriction-associated myometrium at 65 days and at 68 days (Figure 1C).

PRA/PRB isoform ratio

No change was seen in the PRA/PRB ratio with advancing gestation or at labour within each of the myometrial types. Between the myometrial types, the PR ratio was found to be lower in the GR group and in the non-gravid horns than in the gravid uterine horns of the sham-operated animals (Control, Figure 1E).

DISCUSSION

The key findings of this study are that both PRA and PRB levels were down regulated in guinea pig myometrium as labour approached and that the expression of both isoforms were markedly higher in the non-gravid compared to the gravid uterine horns. We have also observed that the fall in myometrial PRA and PRB protein levels in intrauterine growth restricted pregnancies was markedly delayed despite these pregnancies delivering significantly earlier than sham-operated controls.

The fall of total PR expression with advancing gestation and approaching labour is consistent with a reduction in myometrial progesterone responsiveness, the subsequent release from myometrial quiescence and is a potential mechanism of controlling functional progesterone withdrawal and responsiveness. It is also consistent with previous studies in the guinea pig in which myometrial progesterone nuclear receptor binding was found to decrease at approximately 62 days of gestation [25] and immunohistochemical analysis that revealed total uterine PR expression decreased at labour [26]. It also provides support to previous studies in women, who also labour in high circulating progesterone environments, that demonstrated marked decreases in PR expression at term and at labour [10]. Our observations also indicate that PR action in guinea pig myometrium may be controlled by PRA and B acting synergistically. This is in contrast to the possible antagonistic interaction between PRA and PRB in human myometrium in which PRA expression has been proposed to increase relative to PRB with advancing gestation, resulting in an increased PRA/B ratio and an inhibition of PR action leading to labour [13, 14, 27]. Alternatively either PRA or PRB may be of primary importance in the control of labour onset in this species. Indeed, studies in PR knockout mice have revealed that only PRA is necessary to mediate actions of progesterone during pregnancy and labour [28].

Our findings support total PR downregulation as a possible functional progesterone withdrawal mechanism that prepares the uterus for subsequent labour onset. PR and the nuclear transcription factor NFκB, which regulates the transcription of a number of labour associated genes, have a mutually negative interaction [29-31]. For example, ablation of PR A and B in T47D cells results in enhanced NFκB activation and COX-2 expression [31]. Hence the release of myometrial PR dominance in late gestation may allow for the subsequent activation and preparation of the uterus for labour. We suggest this may occur via NFκB mediated increase in PG production and subsequent myometrial activity, the latter of which is known to occur in the lead up to guinea pig labour [21]. Further studies are required to substantiate these possibilities.

Clinical studies have shown that spontaneous preterm labour is associated with an excess of IUGR infants and that a large proportion of fetuses destined to be delivered preterm do not reach their growth potential [1, 5]. The causal mechanisms underlying the association of growth restriction and spontaneous preterm birth have not been determined. The observation that the growth restricted guinea pigs delivered earlier than control animals may indicate similar mechanisms operate in both species. Interestingly, myometrium associated with growth restricted pregnancies exhibited a delay in the down regulation of PRA and PRB beyond 65 days of gestation compared to 62 days in control myometrium (term 71 days). We speculate that this relative maintenance of PR expression may be a protective mechanism in which IUGR pregnancies attempt to maintain myometrial quiescence possibly in the face of IUGR induced upregulation of other labour associated pathways. However, the finding that delivery occurred at a significantly earlier gestational age in the growth restricted pregnancies suggests these PR-preserving mechanisms were not sufficient to prolong IUGR associated pregnancies to term. This indicates that the

intervention causing IUGR in our model may affect gestational length prior to the functional progesterone withdrawal stage. Following the marked decrease in myometrial PR abundance, there was a significantly shorter period of time to delivery in pregnancies associated with IUGR (4 days) than in controls (9 days). This suggests either increased sensitivity or increased exposure of the myometrium to contractile agents (or both) in IUGR pregnancies and further supports the hypothesis of IUGR inducing other prolabour pathways and prolonged PR expression as a mechanism to protect and prolong gestational length following this compromise. Alternatively, IUGR may have affected other unexamined means of functional progesterone withdrawal. Due to the known association of IUGR and preterm birth, further examination of the effect of IUGR on uterine activational pathways and progesterone withdrawal is warranted.

The observations that there were 1) no further reductions in PR abundance from 68 days to labour onset in either control-gravid or IUGR associated myometrium, 2) the delay between decreased PR expression and labour and 3) the prolonged maintenance of high PR expression in IUGR pregnancies suggests that changes in PR expression do not directly mediate labour onset. Decreased PR may release the myometrium from its state of relative quiescence and upregulate uterine activation pathways however other factors directly signal the initiation of contractile events. Like humans, guinea pigs respond to progesterone antagonists with an increase in myometrial responsiveness (particularly to prostaglandins) and cervical ripening, but not always by preterm delivery suggesting that the myometrium undergoes a conditioning step which may be mediated through a fall in progesterone action [9, 20].

The finding that PR levels in the non-gravid uterine horn were markedly higher than in the gravid horn (Figure 1 B-D) of the uterus in the late gestation time period studied is

interesting and suggests that local placental and fetal factors may be exerting influence over PR expression in order to prepare the myometrium for approaching contractile activity and thereby reducing PR expression in late gestation. PR abundance fell in both horns however, prior to term indicating that the late gestational PR down regulation may be caused by systemic factors acting on the whole uterus, albeit not uniformly. These data suggest that stretch is unlikely to play a role in PR downregulation as the non-gravid horn is not under the influence of stretch yet markedly decreases its PR expression at 68 days and again at labour.

In conclusion, we have demonstrated that PRA and PRB abundance decreases in guinea pig uterine tissue with approaching labour. Falling PR levels may result in functional progesterone withdrawal, preparing the uterus and increasing myometrial sensitivity to stimulation [32]. The prolonged maintenance of PR abundance in the GR myometrium, despite the earlier delivery in these pregnancies, may be a protective mechanism to prolong gestation and diminish the consequences of IUGR on gestational length. The current study suggests decreases in total PR expression mediates functional progesterone withdrawal to prepare the myometrium for approaching labour but does not itself directly initiate myometrial contractions and labour onset in the guinea pig. Whilst extrapolation of results between species should be made with proper caution, the unexpected role of PR in IUGR associated pregnancies should be further investigated.

Table 1. Gestational age and physical characteristics of the fetus at the site of myometrial sampling in control and GR associated pregnancies

Gestational group	Surgical intervention	Gestational age (days)[1]	Pubic symphysis (days open)	Body weight (g)	BLR	Placenta (g)
62 days	Control (n=5)	62 ± 0.0	1.4 ± 0.6	74.6 ± 3.5	0.59 ± 0.04	4.8 ± 0.3
	IUGR (n=6)	62.8 ± 0.2*	1.6 ± 0.4	55.3 ± 2.2*	0.99 ± 0.07*	3.7 ± 0.4*
65 days	Control (n=5)	64.6 ± 0.3	3.4 ± 0.9	83.6 ± 2.7	0.65 ± 0.05	4.9 ± 0.5
	IUGR (n=6)	64.8 ± 0.3	4.2 ± 0.6	54.1 ± 3.8*	1.03 ± 0.12*	3.8 ± 0.5
68 days	Control (n=7)	68.1 ± 0.5	7.4 ± 0.8	102.5 ± 5.1	0.59 ± 0.05	5.4 ± 0.5
	IUGR (n=5)	67.8 ± 0.5	7.7 ± 0.9	78.3 ± 2.1*	0.65 ± 0.07	4.5 ± 0.6
Labour	Control (n=6)	71 ± 0.5	9 ± 0.7	114.7 ± 6.8	0.47 ± 0.03	7.7 ± 0.2
	IUGR (n=4)	69.2 ±.8*	8.5 ± 0.9	74.9 ± 3.1*	0.71 ± 0.11	3.5 ± 0.3*

[1]Gestational age at which tissues were collected or when labor occurred and in labor tissues were collected. Control = sham operated, IUGR = surgically ablated, growth restricted, BLR = brain to liver ratio. Data presented as mean ± SEM. * Indicates significant difference within a gestational age group (either 62 days, 65 days, 68 days or at labor) between control and GR associated groups at the level of $P<0.05$.

Figure 1. PRA, PRB, total PR and PR ratio in control (gravid), IUGR associated and non-gravid myometrium at late gestation and with labour.

A) Representative western blot demonstrating PRA, PRB and β actin expression in the guinea pig myometrium (duplicate samples as labelled) and control blot showing specificity of PRA and PRB by preadsorbed blocking peptide. Abundance of B) PR isoform A, C) PR isoform B, D) total PR and PRA:PRB ratio expression as assessed by western blot at 62 days of gestation (black bar), 65 days of gestation (white bar), 68 days (grey bar) and at labour (lined bar) in the non gravid myometrium (collected from the non gravid horn of sham operated dams), control (sham operated, gravid horn) and growth restriction (IUGR) associated pregnancies. Data presented as mean ± SEM with significant differences reported at $P<0.05$. Values with different letters represent significant differences within each myometrial group over gestation. * represents significant difference between IUGR or non-gravid with control myometrium within a gestational time point.

FUNDING

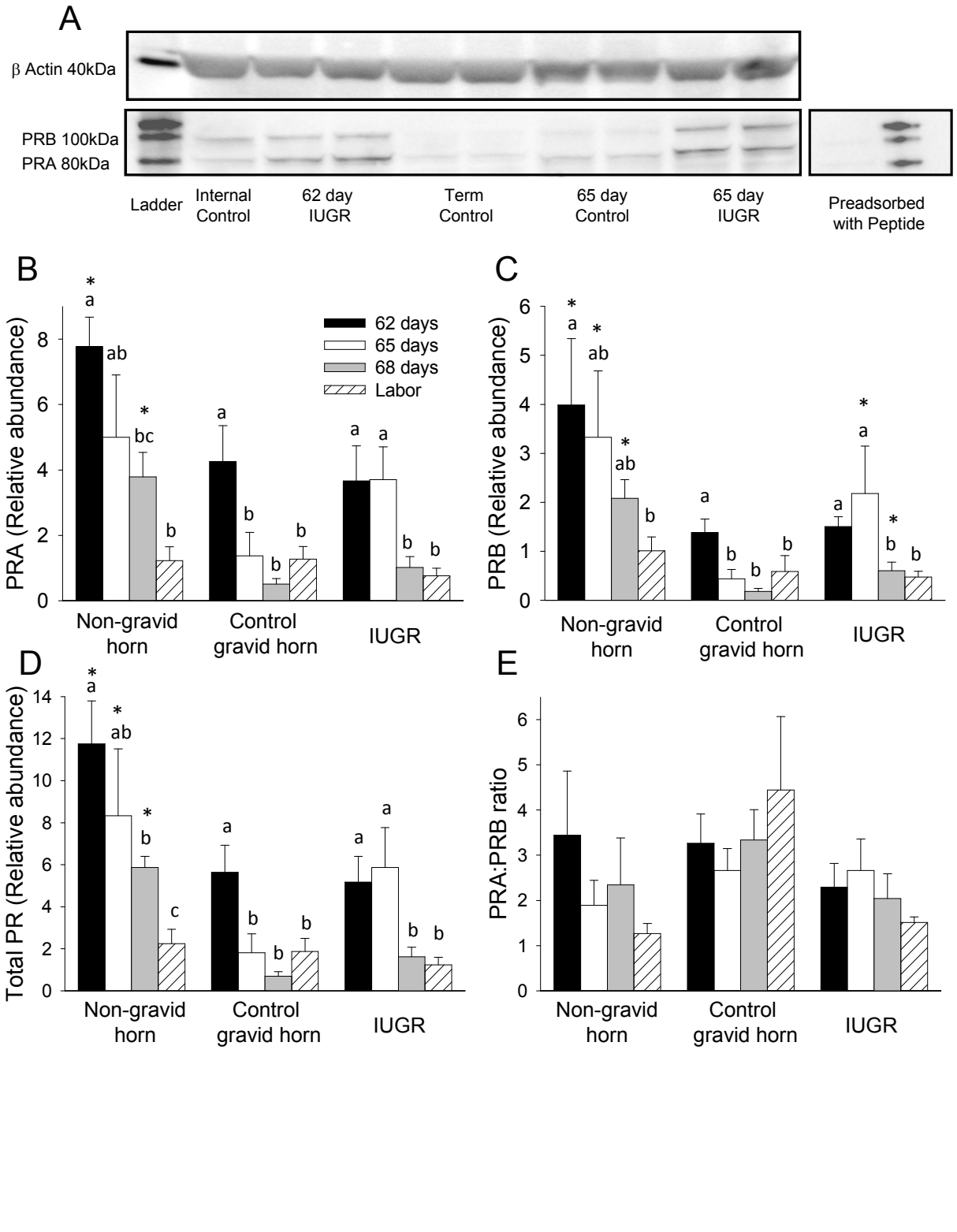
This work was supported by the John Hunter Hospital Charitable Trust and a University of Newcastle Research Fellowship awarded to HKP.

REFERENCES

1. Bukowski R, Gahn D, Denning J, Saade G. Impairment of growth in fetuses destined to deliver preterm. *Am J of Obstet Gynec 2001;185(2):463-67.*
2. Zeitlin JA, Ancel P-Y, Saurel-Cubizolles M-J, Papiernik E. Are risk factors the same for small for gestational age versus other preterm births? *Am J Obstet Gynec* 2001;185(1):208-15.
3. Lackman F, Capewell V, Richardson B, daSilva O, Gagnon R. The risks of spontaneous preterm delivery and perinatal mortality in relation to size at birth according to fetal versus neonatal growth standards. *Am J Obstet Gynec* 2001;184(5):946-53.
4. Hershkovitz R, Erez O, Sheiner E, Bashiri A, Furman B, Shoham-Vardi I, et al. Comparison study between induced and spontaneous term and preterm births of small-for-gestational-age neonates. *Eur J Obstet & Gynec and Reproductive Biology* 2001;97(2):141-46.
5. Morken N-H, Kallen K, Jacobsson B. Fetal growth and onset of delivery: A nationwide population-based study of preterm infants. *Am J Obstet Gynec* 2006;195(1):154-61.
6. Zakar T, Hertelendy F. Progesterone withdrawal: key to parturition. *Am J Obstet Gynec* 2007;196(4):289-96.
7. Csapo AI, Knobil E, Van Der Molen HJ, Wiest WG. Peripheral plasma progesterone levels during human pregnancy and labor. *Am J Obstet Gynec* 1971;110(5):630-32.
8. Illingworth DV, Challis JRG, Ackland N, Burton AM, Heap RB, 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(3):557-70.
9. Rodriguez HA, Kass L, Varayoud J, Ramos JG, Ortega HH, Durando M, et al. Collagen remodelling in the guinea-pig uterine cervix at term is associated with a decrease in progesterone receptor expression. *Mol Human Reprod* 2003;9(12):807-13.
10. How H, Huang ZH, Zuo J, Lei ZM, Spinnato JA, 2nd, Rao CV. Myometrial estradiol and progesterone receptor changes in preterm and term pregnancies. *Obstet Gynec* 1995;86(6):936-40.
11. Rezapour M, Backstrom T, Lindblom B, Ulmsten U. Sex steroid receptors and human parturition. *Obstet Gynec* 1997;89(6):918-24.
12. Bernard A, Duffek L, Torok I, Kosa Z. Progesterone and oestradiol levels and cytoplasmic receptor concentrations in the human myometrium at term, before labour and during labour. *Acta Physiologica Hungarica* 1988;71(4):507-10.
13. Pieber D, Allport VC, Hills F, Johnson M, Bennett PR. Interactions between progesterone receptor isoforms in myometrial cells in human labour. *Mol Human Reprod* 2001;7(9):875-79.
14. Mesiano S, Chan EC, Fitter JT, Kwek K, Yeo G, Smith R. Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. *J Clin Endocrinol Metab* 2002;87(6):2924-30.
15. Condon JC, Jeyasuria P, Faust JM, Wilson JW, Mendelson CR. A decline in the levels of progesterone receptor coactivators in the pregnant uterus at term may antagonize progesterone receptor function and contribute to the initiation of parturition. *Proc Nat Acad Sci USA* 2003;100(16):9518-23.
16. Dong X, Shylnova O, Challis JRG, Lye SJ. Identification and characterization of the protein-associated splicing factor as a negative co-regulator of the progesterone receptor. J *Biol Chem* 2005;280(14):13329-40.

17. Henderson D, Wilson T. Reduced binding of progesterone receptor to its nuclear response element after human labor onset. *Am J Obstet Gynec* 2001;185(3):579-85.
18. Chapman NR, Kennelly MM, Harper KA, Europe-Finner GN, Robson SC. Examining the spatio-temporal expression of mRNA encoding the membrane-bound progesterone receptor-alpha isoform in human cervix and myometrium during pregnancy and labour. *Mol Human Reprod* 2006:gah248.
19. Mitchell BF, Taggart MJ. Are animal models relevant to key aspects of human parturition? *Am J Physiol Regul Integr Comp Physiol* 2009;297(3):R525-45.
20. Elger W, Fahnrich M, Beier S, Qing SS, Chwalisz K. Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs. *Am J Obstet Gynec*1987;157(4 Pt 2):1065-74.
21. Schellenberg JC, Kirkby W. Production of prostaglandin F_{2a} and E_2 in explants of intrauterine tissues of guinea pigs during late pregnancy and labor. *Prostaglandins* 1997;54(3):625-38.
22. Welsh T, Mitchell CM, Walters WA, Mesiano S, Zakar T. Prostaglandin H2 synthase-1 and -2 expression in guinea pig gestational tissues during late pregnancy and parturition. *J Physiol* 2005;569(3):903-12.
23. Turner AJ, Trudinger BJ. A Modification of the Uterine Artery Restriction Technique in the Guinea Pig Fetus Produces Asymmetrical Ultrasound Growth. *Placenta* 2009;30(3):236-40.
24. Carter AM. Restriction of placental and fetal growth in the guinea-pig. *Placenta* 1993;14:125-35.
25. Glasier MA, Hobkirk R. Nuclear receptors for progesterone and estradiol in the guinea pig uterine compartment during gestation. *Steroids* 1993;58(10):478-83.
26. Rodriguez HA, Ramos JG, Ortega HH, Munoz-de-Toro M, Luque EH. Regional changes in the spatio-temporal pattern of progesterone receptor (PR) expression in the guinea-pig genital tract as parturition approaches. *J Steroid Biochem Molec Biol* 2008;111(3-5):247-54.
27. Merlino AA, Welsh TN, Tan H, Yi LJ, Cannon V, Mercer BM, et al. Nuclear progesterone receptors in the human pregnancy myometrium: evidence that parturition involves functional progesterone withdrawal mediated by increased expression of progesterone receptor-A. *J Clin Endocrinol Metab* 2007;92(5):1927-33.
28. Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM. Defective Mammary Gland Morphogenesis in Mice Lacking the Progesterone Receptor B Isoform. Proceedings of the National Academy of Sciences of the United States of America 2003;100(17):9744-49.
29. Allport VC, Pieber D, Slater DM, Newton R, White JO, Bennett PR. Human labour is associated with nuclear factor kB activity which mediates cyclo-oxygenase-2 expression and is involved with the 'functional progesterone withdrawal'. *Mol Human Reprod* 2001;7(6):581-86.
30. Madsen G, Zakar T, Ku CY, Sanborn BM, Smith R, Mesiano S. Prostaglandins differentially modulate progesterone receptor-A and -B expression in human myometrial cells: evidence for prostaglandin-induced functional progesterone withdrawal. *J Clin Endocrinol Metab* 2004;89(2):1010-13.
31. Hardy DB, Janowski BA, Corey DR, Mendelson CR. Progesterone receptor plays a major antiinflammatory role in human myometrial cells by antagonism of NFk-B activation of cyclooxygenase 2 expression. *Molec Endocrinol* 2006;20(11):2724-33.
32. Chwalisz K, Fahrenholz F, Hackenberg M, Garfield R, Elger W. The progesterone antagonist onapristone increases the effectiveness of oxytocin to produce delivery

without changing the myometrial oxytocin receptor concentrations. *Am J Obstet Gynec* 1760;165(6 Pt 1):1760-70.


A
β Actin 40kDa
PRB 100kDa
PRA 80kDa
Ladder
Internal Control
62 day IUGR
Term Control
65 day Control
65 day IUGR
Preadsorbed with Peptide
B
PRA (Relative abundance)
62 days
65 days
68 days
Labor
Non-gravid horn
Control gravid horn
IUGR
C
PRB (Relative abundance)
D
Total PR (Relative abundance)
E
PRA:PRB ratio