Lay summary

Early identification during pregnancy of small for gestational age (SGA) babies would enable interventions to lower risk of complications around birth (perinatal), but current detection rates of these at risk babies is low. Pregnancy hormones, prolactin and placental lactogen, are involved in metabolic changes required during pregnancy for the mother to support optimal growth and development of her offspring. The levels of these hormones may provide a measurable indicator (biomarker) to help identify these at risk pregnancies. Levels of these hormones were measured in samples from week 20 of gestation from women who went on to have small for gestational age babies and control pregnancies where babies were born at a size appropriate for gestation age. Despite the implications of prolactin and placental lactogen in maternal metabolism, no significant differences were detected suggesting that single measures of either prolactin or placental lactogen at 20 weeks gestation are unlikely to be useful biomarker to help detect SGA pregnancies.
Case-Control study of prolactin and placental lactogen in SGA pregnancies

Dr Sharon R Ladyman Ph.D.¹, ², Dr Caroline Larsen Ph.D.¹, Mrs Rennae S Taylor MHSc³,
Professor David R Grattan Ph.D.¹, ², Professor Lesley ME McCowan MBChB, FRANZCOG,
MD³

¹Centre for Neuroendocrinology and Department of Anatomy, School of Biomedical
Sciences, University of Otago, Dunedin, New Zealand.
²Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, New Zealand
³Department of Obstetrics and Gynaecology, Faculty of Medical and Health Science,
University of Auckland, Auckland, New Zealand.

Abbreviated title: Normotensive SGA and Prolactin Levels

Keywords: small for gestational age, prolactin, human placental lactogen, pregnancy

Word count: 2592

Corresponding author and reprint requests: Professor Dave Grattan, Dept of Anatomy,
School of Medical Sciences, University of Otago, PO Box 913, Dunedin 9054, New Zealand.
Tel: +64 3 479 7442; Fax: +64 3 479 7254; Email: dave.grattan@otago.ac.nz

Abstract
Prolactin and placental lactogens increase during pregnancy and are involved with many aspects of maternal metabolic adaptation to pregnancy, likely to impact on fetal growth. The aim of this study was to determine whether maternal plasma prolactin or placental lactogen concentrations at twenty weeks of gestation were associated with later birth of small-for-gestational-age babies (SGA). In a nested case-control study, prolactin and placental lactogen in plasma samples obtained at 20 weeks of gestation were compared between 40 women who gave birth to SGA babies, and 40 women with uncomplicated pregnancies and size appropriate-for-gestation-age (AGA) babies. Samples were collected as part of the “Screening of Pregnancy Endpoints” (SCOPE) prospective cohort study. SGA was defined as birthweight < 10th customised birthweight centile (adjusted for maternal weight, height, ethnicity, parity, infant sex and gestation age) in mothers who remained normotensive. No significant differences were observed in concentrations of prolactin or placental lactogen from women who gave birth to SGA babies compared with women with uncomplicated pregnancies. However, a sex specific association was observed in SGA pregnancies, whereby lower maternal prolactin concentration at twenty weeks of gestation was observed in SGA pregnancies that were carrying a male fetus ($132.0 \pm 46.7$ ng/ml vs $103.5 \pm 38.3$ ng/ml, mean ± SD, $p=0.036$ Student’s t-test) compared to control pregnancies carrying a female fetus. Despite the implications of these lactogenic hormones in maternal metabolism, single measurements of either prolactin or placental lactogen at 20 weeks of gestation are unlikely to be useful biomarkers for SGA pregnancies.

Clinical Trial Registration Number: Australian New Zealand Clinical Trials Registry, www.anzctr.org.au, ACTRN12607000551493, 26 October 2007 retrospectively registered
Introduction

Small for gestational age (SGA) infants account for about 30-50% of non-anomalous still-born infants and those that survive have an increase in the risk for neurodevelopmental delays, and cerebral palsy (Baschat 2011, PMMRC 2018). Furthermore, the effects of being born SGA impacts on health as an adult, with increased risk of cardiovascular complications and diabetes in later life (Barker, et al. 2007). Identification during pregnancy, leading to intervention and timely delivery has been reported to lead to a four-fold reduction in perinatal death and severe asphyxia (Lindqvist and Molin 2005). Identifying SGA before birth is difficult, however, and using population-based growth charts less than a quarter of all SGA babies are identified before birth (Wright, et al. 2006). Using customized antenatal growth charts that take into account a range of factors including maternal weight, height and ethnicity can improve ante-natal identification of SGA infants, but even with this improvement detection rates are reported to be only around 50% (Roex, et al. 2012, Wright, et al. 2006). Recent analysis of a multi-centre cohort study (SCOPE) has identified key clinical variables at 15 weeks that are associated with later development of SGA (McCowan, et al. 2013). In that study, only one quarter (24.5%) of all SGA infants were identified before birth, highlighting the need to have improved methods for detecting SGA during pregnancy (McCowan, et al. 2013). As indicated in McCowan et al (2013), the key next step for the development of a personalized algorithm for prediction of SGA is the identification of reliable biomarkers that can be combined with clinical risk factors (McCowan, et al. 2013).

One of the key hormones associated with pregnancy is prolactin. While prolactin is thought of primarily as a lactation hormone (Trott, et al. 2012), it has also been implicated in a wide range of other functions (Bole-Feyssot, et al. 1998, Grattan 2015), particularly during pregnancy (Grattan and Le Tissier 2015). Prolactin concentrations increase progressively throughout pregnancy in women (Aghaeepour, et al. 2018, Grattan 2001, Romero, et al. 2017,
Tyson, et al. 1972) and the placenta also contributes the closely related human placental lactogen (hPL, or chorionic somatomammotropin) as an additional source of circulating hormone that can activate the prolactin receptor. Human placental lactogen is secreted from the syncytiotrophoblast starting at 6 weeks of gestation and then increasing to extremely high levels as gestation advances (Braunstein, et al. 1980). Indeed, recent proteomic studies of human pregnancy blood samples have identified prolactin in the top 1% of proteins showing increased expression during pregnancy (Aghaeepour, et al. 2018, Romero, et al. 2017), with hPL also one of the highest induced proteins (Aghaeepour, et al. 2018). Prolactin and hPL play an important role in mediating the maternal metabolic adaptations that help establish a positive energy balance to meet the demands of fetal growth and also to prepare for subsequent demands of lactation (Augustine, et al. 2008, Grattan and Kokay 2008, Grattan and Le Tissier 2015, Newbern and Freemark 2011). These adaptations ensure appropriate glucose and amino acid availability to the fetus (Handwerger 1991). Prolactin may also have a more direct role in fetal growth by influencing trophoblast invasion in early pregnancy (Stefanoska, et al. 2013), which when impaired, has been associated with SGA pregnancies (Knofler 2010). In animal models, experimental suppression of placental lactogenic hormones is associated with fetal growth restriction (Lee, et al. 2015). Reduced expression of genes for hPL and the placental growth hormone variant (GH-V) has been observed in term placentas associated with SGA newborns (Mannik, et al. 2010), suggesting that secretion of these placental hormones may be compromised when fetal growth is impaired. Changes in expression of imprinted genes that control hPL expression (John 2013) have also been associated with changes in fetal growth (Janssen, et al. 2016, Jensen, et al. 2014). Similarly, animal models involving manipulation of these placental regulatory genes also result in changes in fetal growth (Tunster, et al. 2016). These data highlight the potential for placental hormones to serve as biomarkers of impaired fetal growth. Collectively, these data are
consistent with the hypothesis that changing levels of circulating hormones that act through the prolactin receptor, including prolactin and growth hormone from the maternal pituitary (and also decidua), and hPL (and to a lesser extent GH-V) from the placenta, impact on fetal growth and could potentially serve as biomarkers of impaired fetal growth. In previous work, we showed that while circulating GH-V correlated with fetal growth in large for gestational age babies, there was no detectable deficit in SGA pregnancies (Liao, et al. 2016). The aim of the current study was to investigate if hPL or pituitary-derived prolactin levels were reduced at 20 weeks of gestation in pregnancies that resulted in birth of a small for gestational age baby.

**Methods**

**Study design**

The participants were healthy, nulliparous women with singleton pregnancies who were recruited in Auckland, New Zealand, to be included in the multiple centre “Screening of Pregnancy Endpoints” (SCOPE) study. The SCOPE study was a prospective, multicentre international screening study which aimed to develop screening tests to predict preeclampsia, SGA infants and spontaneous preterm birth. Ethics approval and consent to participate was obtained from local ethics committees, including New Zealand (AKX/02/00/364) and all women provided written informed consent. Detailed methods of the SCOPE study are described elsewhere (McCowan, et al. 2010). For the current study, 40 case and 40 control samples were randomly selected from the participants who were recruited in Auckland, New Zealand, (total of 1296 uncomplicated AGA pregnancies and 159 normotensive SGA pregnancies) and who had a 20 week plasma specimen.

For the current investigation, plasma samples obtained at 20 weeks of gestation were used. Following birth (usually within 72h of giving birth), pregnancy outcome data and infant measurements were recorded by research midwives. As SGA outcome was conventionally
defined as a birthweight of less than the tenth customised centile, adjusted for maternal height, booking weight, ethnicity, sex of infant and gestation at birth. The control group in this nested case-control study comprised of 40 women with singleton, uncomplicated pregnancies who delivered babies with birthweight $\geq 10^{th}$ customised centile, while the SGA group was comprised of 40 women with singleton, normotensive pregnancies who delivered SGA babies. The number of 40 per group was determined based on power analysis (for an effect size of 0.8) based on unforeseen, preliminary data obtained from another study using samples from the SCOPE study.

**Assays**

Prolactin and placental lactogen concentrations were measured in duplicate in 20 weeks of gestation maternal plasma samples using commercially available ELISA assays (human prolactin ELISA (25-PROHU-E01) and human placental lactogen ELISA (20-HPLHU-E01) both from Alpco Diagnostics). Assays were performed as described in the manufacturer’s instructions. For the hPL assay, the intra-assay CV was 2.8% and inter-assay CV was 13.2%. For the prolactin assay, the intra-assay CV was 3.2% and the inter-assay CV was 5.1%. For each assay plate, both control and case studies were included.

**Statistical analysis**

All hormone data was analysed for normality using the D’Agostino and Pearson normality test. When data was normally distributed, a Student’s t-test was used to assess significance between groups, whereas when data was not normally distributed a Mann Whiney nonparametric test was used. The Mann Whitney test was used for human placental lactogen analysis of all samples combined and samples from mothers carrying female foetuses, and a two-tail Student’s t-test was used for the prolactin analysis along with analysis.
for human placental lactogen in samples from mothers carrying male foetuses. Since the hypothesis was that low lactogenic hormone activity would be associated with SGA pregnancies we also analyzed the data using a one-tail test. Previous work has suggested fetal sex-specific association of low hPL and reduced fetal growth (Lagerstrom, et al. 1990), therefore the data was also analyzed based on fetal sex. Each group was assessed for outliers using the ROUT outlier test. Only one sample from the control group of the hPL data was removed due to testing positive as an outlier (extremely high at 13.43ng/ml), however the outcome of the statistical analysis did not differ with or without the inclusion of this value (with: P=0.7736, without: P=0.7736 using two-tailed Mann-Whitney tests). Statistical differences in study population characteristics between the control pregnancies and SGA pregnancies were analysed with Student’s t-test for continuous variables and with Chi-square tests for categorical variables. For hormone data, all values are expressed as mean ± SD, and for the study population characteristics, continuous variables are expressed as mean (SD) while categorical variables are expressed as number (percentage).

Results

Maternal characteristics and pregnancy outcomes for the Normotensive SGA and uncomplicated pregnancy) groups are detailed in Table 1. There were no differences between groups for any maternal characteristics. SGA cases were delivered earlier than controls (P = 0.03).

No significant differences were detected in maternal plasma placental lactogen or prolactin concentrations between the uncomplicated pregnancy and SGA groups (Placental lactogen: P=0.7736 two-tailed Mann-Whitney tests, Prolactin: P=0.1164 two-tailed t-test) (figure 1). When specifically investigating the hypothesis that low lactogenic hormone
activity would be associated with SGA pregnancies, there was no statistically significant
difference in prolactin concentrations in pregnancies that went on to have a SGA infant
compared to those with uncomplicated pregnancies (P=0.058). To determine whether any
trend might become more pronounced with more severe SGA, the data was further examined
using only the samples from more extreme SGA cases (<5th centile). No significant
differences were found between control and SGA groups in this further analysis (figure 2).

No differences were seen at week 20 of gestation between the uncomplicated
pregnancy and SGA groups in maternal hPL and prolactin concentrations when the foetus was
female (Placental lactogen: P=0.1258 two-tailed Mann-Whitney tests, Prolactin: P=0.9586
two-tailed t-test)(figure 3). When the fetus was male, maternal prolactin concentrations were
significantly lower at 20 weeks of gestation in SGA pregnancies compared to uncomplicated
pregnancies (Prolactin: P=0.0361 two-tailed t-test) while maternal hPL was similar in both
groups (Placental lactogen: P=0.1749 two-tailed Mann-Whitney tests)(figure 3).

Discussion

In current clinical practice less than 50% of pregnancies with SGA infants are usually
detected before birth. Early detection of SGA pregnancies and timely delivery improves
outcomes and is therefore an important research priority. The aim of the present study was to
determine whether either prolactin or placental lactogen might serve as a useful bio-marker to
help predict SGA infants.

Lactogenic hormones such as prolactin and placental lactogen are markedly increased
during pregnancy, and play important roles in maternal adaptation to support the growing
development, prolactin receptor activation during pregnancy, by either prolactin or placental
lactogens, have been implicated in important roles in maternal metabolic adaptations, including induction of pregnancy-specific glucose regulation to direct glucose toward the fetus (Baeyens, et al. 2016, Sorenson and Brelje 1997), and increasing food intake to meet the energy demands of the growing fetus and prepare for lactation (Augustine and Grattan 2008, Augustine, et al. 2008). Prolactin action in the fetus is also involved in a range of developmental and growth functions (Hauth, et al. 1978, Pullano, et al. 1989, Winters, et al. 1975). Therefore, we hypothesized that inadequate prolactin and/or placental lactogen during pregnancy may be associated with pregnancies with SGA infants.

We found a trend (P=0.058) for lower prolactin concentrations at 20 weeks of gestation in pregnant women who went on to have SGA babies (<10 centile) however, this potential relationship was not apparent in more extreme SGA (<5 centile) and lends strength to the conclusion that a single measurement of prolactin at 20 weeks of gestation is not a useful biomarker of SGA. When the data was analysed based on sex of fetus, we found a significant reduction in maternal prolactin concentrations in SGA pregnancies compared to uncomplicated pregnancies. However, there was still quite an overlap between the prolactin concentrations in these two group, suggesting that maternal prolactin concentrations by itself is unlikely to be a useful biomarker per se even in pregnancies carrying male foetuses. Whether it might contribute to a wider risk score, associated with assessment of key clinical variables (McCowan, et al. 2013) requires further investigation. Overall, there was no correlation between plasma prolactin concentrations at 20 weeks and birth weight (data not shown). Whether plasma prolactin may be a useful biomarker of SGA at other time points during pregnancy cannot be ruled out by these data. At term, hPL has been significantly associated with infant birth weight (Janssen, et al. 2016) and potentially this relationship would be detectable at an earlier time, thus future work investigating time points between 20 weeks and term would be warranted.
Previous work has indicated that in pregnancies carrying female foetuses maternal hPL is lower in SGA pregnancies compared to uncomplicated controls (Lagerstrom, et al. 1990). We did not observe this difference in hPL and the reasons for this inconsistency between previous work and the current study are unknown. Previous work was a prospective cohort study and hence their SGA group size was smaller than the current study. It should be noted however that the difference in group size between our female SGA group (n=18) and the previous study (n=11) was not large. It is also possible that the definition of SGA in this current study and that of SFD (small for date) for the previous study are not comparable given the 30 year lapse in time between studies.

Our sample size was only 40 cases and 40 controls. Based on the apparent effect size and the high variability we observed for both hormones, a post hoc power analysis indicated that a larger study with at least 95 patients per group would be required to determine whether the observed trend was significant when data was not analysed by fetal sex. Within such a larger study, analysis of clinical and other biomarker variables that could be assessed alongside prolactin concentrations may strengthen the predictive value, especially for male foetuses. Our study only assessed one timepoint, and future work should also assess the relevance of other time points in pregnancy that may be of use for aiding diagnosis of SGA.

Overall, while maternal prolactin concentrations did tend to be lower in SGA pregnancies, there was great variation and overlap of prolactin concentrations in both groups. A significant sex-specific association was observed, with SGA pregnancies carrying male foetuses having lower maternal prolactin concentrations compared to uncomplicated pregnancies carrying male foetuses. Our work suggests that neither prolactin nor placental lactogen at 20 weeks gestation are likely to be useful biomarkers for SGA, however this does not rule out their potential to be useful at a different time point of pregnancy.
Declarations

Ethics approval and consent to participate: Ethics approval was obtained from local ethics committees, including New Zealand (AKX/02/00/364) and all women provided written informed consent.

Consent for publication: Not applicable.

Availability of data and material: The data sets from this manuscript is available by reasonable request from the corresponding author.

Competing interests: The authors declare that they have no competing interests.

Funding

This work was funded by the New Zealand Health Research Council. This funding body had no role in the design of the study, collection, analysis and interpretation of the data, nor in the writing of this manuscript.

Author contributions

CL, RST, DRG and LMEM were involved in the conceptualization of the study and the design of the work. SRL analysed the data and wrote the manuscript. SRL, RST, DRG and LMEM contributed to the interpretation of the data and edited the manuscript. All authors have read and approved of the manuscript.
Acknowledgements

We would like to thank the pregnant women who participated in the SCOPE study; Ms. Pene Knowles for performing the hormone assays, and Dr Jaijus Pallippadan Johny for statistical advice. We also thank the reviewer for the useful suggestion to analyse the data by fetal sex.
References


Janssen, AB, SJ Tunster, AE Heazell, and RM John 2016 Placental PHLDA2 expression is increased in cases of fetal growth restriction following reduced fetal movements. BMC Med Genet 17 17.


Liao, S, MH Vickers, RS Taylor, B Jones, M Fraser, LM McCowan, PN Baker, and JK Perry 2016 Human placental growth hormone is increased in maternal serum at 20 weeks of gestation in pregnancies with large-for-gestational-age babies. Growth Factors 34 203-209.


Tunster, SJ, HDJ Creeth, and RM John 2016 The imprinted Phlda2 gene modulates a major endocrine compartment of the placenta to regulate placental demands for maternal resources. Dev Biol 409 251-260.


Figure legends

Figure 1: Placental lactogen (A) and prolactin concentrations (B) in maternal plasma samples collected from pregnant women at 20 weeks of gestation who went on to have babies of normal birth weight (control, N=40) or small for gestational age (SGA, <10th centile, N=40). One sample was removed from the control placental lactogen group as it was tested to be an outlier. Placental lactogen, P=0.3868 (one-tail Mann Whitney nonparametric test as data were not normally distributed (SGA group P<0.05 D’Agostino and Pearson normality test)). Human prolactin, P=0.0582, (Student’s one-tail t-test). Data shown as mean ± SD.

Figure 2: Placental lactogen and prolactin concentrations in maternal plasma samples collected from pregnant women at 20 weeks of gestation who went on to have babies of normal birth weight (control, N=40) or small for gestational age (SGA, (<5th centile, N=10). One sample was removed from the control placental lactogen group as it was tested to be an outlier. Human placental lactogen P=0.75, Student’s t-test. Human prolactin P=0.30, Student’s t-test. Data shown as mean ± SD.

Figure 3: Placental lactogen (hPL) and prolactin concentrations in plasma samples from pregnant women carrying boys (top) or girls (bottom). Plasma samples were collected at 20 weeks of gestation from women who went on to have babies of normal birth weight (control, boys: N=20, girls: N=20) or small for gestational age (SGA, boys: N=22, girls: N=18). Boys: hPL P=0.164, prolactin P=0.036, Student’s t-test, Girls: hPL P=0.126, Mann Whitney nonparametric test as data were not normally distributed (SGA group P<0.05 D’Agostino and Pearson normality test)), prolactin P=0.959, Student’s t-test. Data shown as mean ± SD.
Table 1: Study population characteristics at 15 weeks and pregnancy outcome.
Figure 1: Placental lactogen (A) and prolactin concentrations (B) in maternal plasma samples collected from pregnant women at 20 weeks of gestation who went on to have babies of normal birth weight (control, N=40) or small for gestational age (SGA, <10th centile, N=40). One sample was removed from the control placental lactogen group as it was tested to be an outlier. Placental lactogen, \( P=0.3868 \) (one-tail Mann Whitney nonparametric test as data were not normally distributed (SGA group \( P<0.05 \) D’Agostino and Pearson normality test)). Human prolactin, \( P=0.0582 \), (Student’s one-tail t-test). Data shown as mean ± SD.
Figure 2: Placental lactogen and prolactin concentrations in maternal plasma samples collected from pregnant women at 20 weeks of gestation who went on to have babies of normal birth weight (control, N=40) or small for gestational age (SGA, <5th centile, N=10). One sample was removed from the control placental lactogen group as it was tested to be an outlier. Human placental lactogen P=0.75, Student’s t-test. Human prolactin P=0.30, Student’s t-test. Data shown as mean ± SD.
Figure 3: Placental lactogen (hPL) and prolactin concentrations in plasma samples from pregnant women carrying boys (top) or girls (bottom). Plasma samples were collected at 20 weeks of gestation from women who went on to have babies of normal birth weight (control, boys: N=20, girls: N=20) or small for gestational age (SGA, boys: N=22, girls: N=18). Boys: hPL P=0.164, prolactin P=0.036, Student’s t-test, Girls: hPL P=0.126, Mann Whitney nonparametric test as data were not normally distributed (SGA group P<0.05 D’Agostino and Pearson normality test)), prolactin P=0.959, Student’s t-test. Data shown as mean ± SD.

161x202mm (300 x 300 DPI)
Table 1. Study population characteristics at 15 weeks and pregnancy outcome. Results expressed as \( n \) (%) or mean (SD). Continuous variables: t-test; categorical variables: Chi-square or Fisher’s exact test.

<table>
<thead>
<tr>
<th>Maternal Details at 15 weeks</th>
<th>Normotensive SGA (( n=40 ))</th>
<th>Uncomplicated Pregnancy (( n=40 ))</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>31.6 ± 4.4</td>
<td>31.3 ± 4.4</td>
<td>0.78</td>
</tr>
<tr>
<td>Caucasian ethnicity</td>
<td>37 (93%)</td>
<td>37 (93%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Primigravid</td>
<td>29 (73%)</td>
<td>29 (73%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Single</td>
<td>2 (5%)</td>
<td>0</td>
<td>0.49</td>
</tr>
<tr>
<td>No paid employment</td>
<td>0</td>
<td>3 (7.5%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Socioeconomic index</td>
<td>51 ± 12</td>
<td>48 ± 12</td>
<td>0.29</td>
</tr>
<tr>
<td>Smoker</td>
<td>1 (2.5%)</td>
<td>1 (2.5%)</td>
<td>1.0</td>
</tr>
<tr>
<td>BMI category (kg/m(^2))</td>
<td></td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>&lt;24.9</td>
<td>26 (65%)</td>
<td>25 (62.5%)</td>
<td></td>
</tr>
<tr>
<td>25-29.9</td>
<td>10 (25%)</td>
<td>13 (32.5%)</td>
<td></td>
</tr>
<tr>
<td>≥30.0</td>
<td>4 (10%)</td>
<td>2 (5%)</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>106 ± 10</td>
<td>107 ± 9</td>
<td>0.82</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>63 ± 9</td>
<td>66 ± 8</td>
<td>0.19</td>
</tr>
<tr>
<td>15 wk extreme exercise (Yes)</td>
<td>1 (2.5%)</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Gestation at sampling (wks)</td>
<td>19.9 ± 0.6</td>
<td>19.9 ± 0.7</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Pregnancy Outcome

| Birthweight (g)             | 2710 ± 398                      | 3506 ± 353                          | <0.001  |
| Sex of baby (girl)          | 18 (45%)                        | 20 (50%)                            | 0.82    |
| Gestational age at delivery (wks) | 39.2 ± 2.4                  | 40.2 ± 1.1                          | 0.03    |
| Customised birthweight centile | 5.7 ± 2.6                    | 45.9 ± 23.7                         | <0.001  |
| Preterm births (<37wks)     | 6 (15%)                         | 0                                   | 0.08    |
| Neonatal unit admission     | 8 (20%)                         | 2 (5%)                              | 0.09    |

Specimen Analyses at 20 weeks*

| Prolactin (ng/L)            | 112.6 ± 44.9                    | 128.2 ± 43.2                        | 0.058 (0.116) |
| Human placental lactogen (mg/L) | 2.46 ± 0.74                | 2.64 ± 1.84                         | 0.464 (0.774) |

*Specimen analyses expressed as mean (SEM) and P values for both one-tailed and two tailed statistical testing is given, with the latter being in brackets