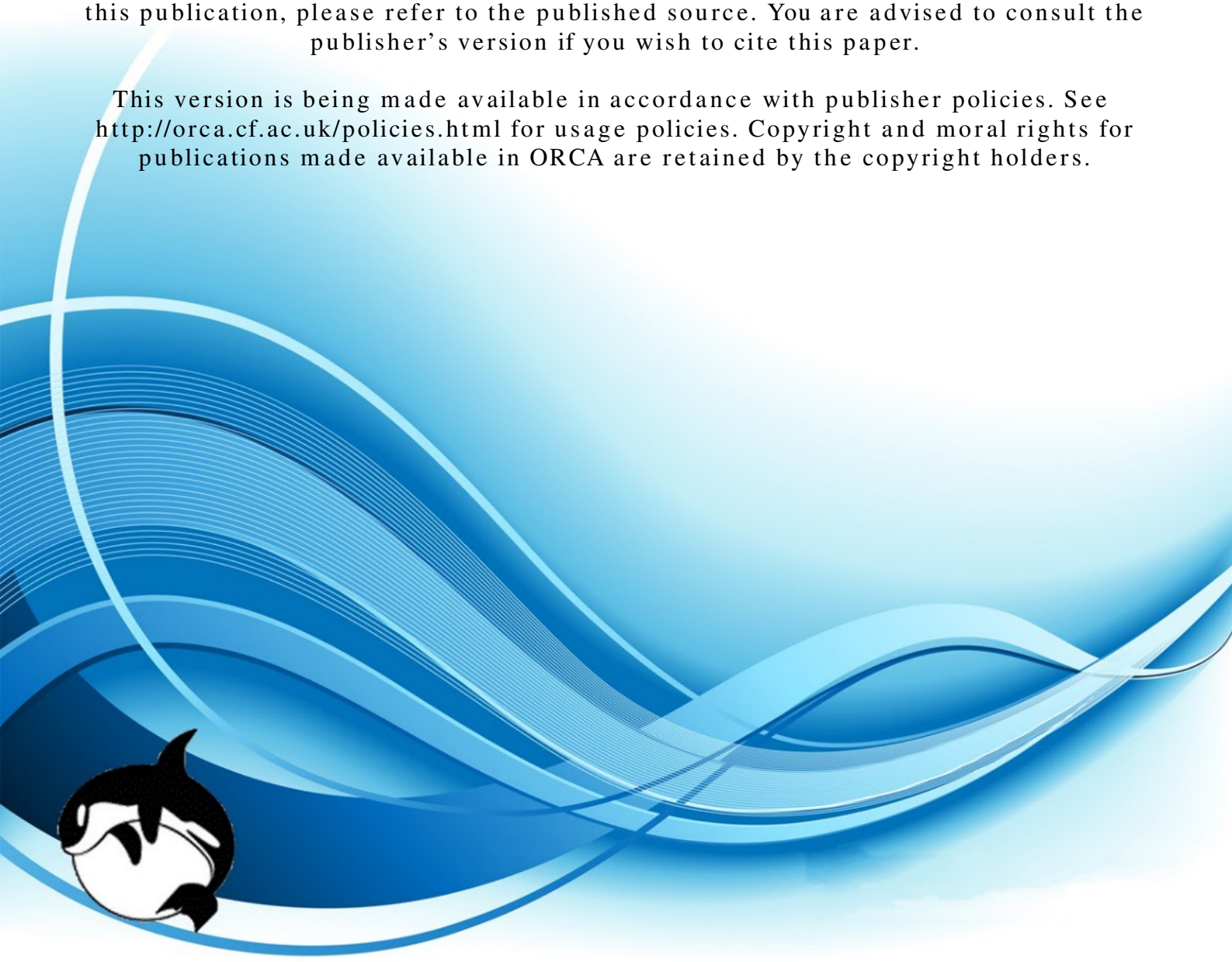

Low serum placental lactogen at term predicts postnatal symptoms of depression and anxiety in women delivering female infants

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Running title: Serum placental lactogen predict postnatal depression

Abstract

Background. Placental endocrine insufficiency may increase the risk of depression and anxiety during pregnancy and/or after birth. This study investigated the association between serum human placental lactogen (hPL) and measures of perinatal mental health, accounting for selective serotonin-reuptake inhibitor (SSRI) usage.

Method. Caucasian women with singleton, term pregnancies recruited at their pre-surgical appointment prior to an elective caesarean section (ELCS) were studied. Serum hPL levels in maternal blood collected at recruitment were measured by ELISA. Depression and anxiety scores were derived from Edinburgh Postnatal Depression Scale (EPDS) and the trait subscale of the State-Trait Anxiety Inventory (STAI) questionnaires completed at recruitment and three postnatal time points. Data was analysed by unadjusted and adjusted multiple linear regression.

Results. In adjusted linear regressions, term maternal serum hPL levels were negatively associated with postnatal EPDS and STAI score ten weeks postnatal for mothers who had girls (B= -.367 , *p*= .022, 95% CI -.679, -.056; and B= -.776, *p*= .030, 95% CI -1.475, -.077 respectively). Excluding women prescribed SSRIs strengthened the relationship at 10 weeks and uncovered an earlier association between hPL and mood scores within one week of delivery (EPDS B= -.357 , *p*= .041, 95% CI -.698, -.015; and STAI B= -.737, *p*= .027, 95% CI -1.387, -.086). In mothers who had boys, there were no associations between hPL and mood scores at any time point.

Conclusion. Low hPL predicted postnatal depression and anxiety symptoms exclusively in mothers of girls. Insufficiency in hPL may contribute to maternal mood symptoms.

Key words: human placental lactogen, sex differences, postnatal depression, postnatal anxiety

1. Introduction

During pregnancy, women are highly vulnerable to perinatal mental health problems, with depression and anxiety among the most common (Bauer et al., 2014) and associated with substantial morbidity to both mother and child (Stewart, 2011). Poor mental health in pregnancy is strongly linked to postnatal depression, both having negative effects on mother–child interactions and child development in the short and longer term (Brand and Brennan, 2009; Kinsella and Monk, 2009). A recent study in the UK estimated that maternal mental health disorders have a long-term cost to UK society of £8.1 billion for each one-year cohort of births with nearly three-quarters of this cost relating to adverse impacts on the child (Bauer et al., 2014). Despite the considerable clinical, financial and emotional burden to society, and the reporting of many risk factors, the biological mechanisms are unknown. Consequently, our ability to predict which women will experience significant mental health symptoms related to pregnancy is severely limited.

Hormonal changes represent prime candidates linking pregnancy to the higher vulnerability for depression because their levels change dramatically, driven in part by the placenta acting as an endocrine organ (Burton and Fowden, 2015). The human placenta is the source of 102 polypeptide hormones contributing to the co-ordinated induction and maintenance of maternal physiology and behaviour (Liu et al., 2018). Studies in rodent models directly demonstrate the importance of prolonged exposure to oestrogen, progesterone, and the lactogenic hormones prolactin and placental lactogens in the induction of maternal care behaviours (Creeth et al., 2019). To date there is little direct evidence from human studies to support altered levels of a specific pregnancy hormone in perinatal depression or anxiety. However, there is indirect evidence to suggest that lactogenic signalling may be important. Human placental lactogen (hPL) is coded for by three separate genes with the majority of term hPL derived from the human chorionic somatomammotropin 1 (CSH1) gene (Männik et al., 2010). hPL is present at considerably higher levels than the pituitary hormone prolactin in the maternal serum at term (5–7 vs. 0.15–0.18 μg/ml) (Newbern and Freemark, 2011) and both hormones bind and activate the prolactin receptor (Gurtunca and Sperling, 2017). Maternal serum hPL levels and placental *hPL* expression have previously been demonstrated to be significantly reduced in pregnancies complicated by fetal growth restriction (Männik et al., 2010; Roh et al., 2005). We reported an association between lower maternal serum hPL and being born small for gestational age (Janssen et al., 2016b). Fetal growth restriction is more common when mothers experience prenatal depression (Diego et al., 2009). Maternal obesity has been associated with decreased placental *hPL* expression at term (Vakili et al., 2013) potentially as a consequence of changes in the interactions between key regulatory elements at the gene locus (Jin et al., 2018). Maternal obesity is a risk factor for maternal depression and anxiety

(Molyneaux et al., 2014). Decreased maternal serum levels of prolactin (Gurtunca and Sperling, 2017), have been reported in human mothers with postnatal depression symptoms (Groer and Morgan, 2007; Ingram et al., 2003). Maternal serum levels of prolactin have been inversely correlated with anxiety scores during pregnancy (Asher et al., 1995). We reported lower placental *hPL* gene expression in term placenta from women with clinically diagnosed depression and women self-reporting clinically significant symptoms of depression (Janssen et al., 2016a). In this 2016 study, we were not able to compare depression symptoms to serum levels of hPL as either the sample numbers were too low or the data was not collected. An additional factor which may have not been considered in previous studies in the confounder of antidepressant usage in pregnancy. Selective serotonin reuptake inhibitors (SSRIs), which are commonly prescribed in pregnancy to manage mood symptoms (Dubovicky et al., 2017), have a lactogenic activity (Goodnick et al., 2005), potentially masking any relationship between placental lactogens levels and mood symptoms

The aims of this study were to investigate the relationship between maternal serum levels of hPL in term pregnancies and both maternally reported symptoms of depression and anxiety. A sensitivity analyses was performed to further understand the relationship between mood symptoms and hPL levels. These analyses were performed using data from the Grown in Wales cohort (Janssen et al., 2018).

2. Method

2.1 Cohort

Full ethical approval for the Grown in Wales (GiW) Study cohort (Janssen et al., 2018) was obtained from the Wales Research Ethics Committee REC reference 15/WA/0004. Research was carried out in line with the principles of the Declaration of the Helsinki as revised in 2008. Briefly, the GiW study is a longitudinal birth cohort based in South East Wales, United Kingdom that began in September 2015 and ended recruitment in November 2016. Women aged between 18 and 45 with a singleton term pregnancy without fetal abnormalities or infectious diseases were recruited at their morning pre-surgical appointment prior to an elective caesarean section (ELCS) at the University Hospital of Wales by two trained research midwives.

2.2 Participant numbers

355 women were recruited into the study and seven withdrew. hPL concentrations were available for 272 of these women of whom 251 had fully complete antenatal self-reported mental health data at term. However not all of the participants completed mental health questionnaires at all time points (Supplementary Table 2). 91% of participants in the Grown in

Wales cohort reported Caucasian ethnicity (Janssen et al., 2018), and the current study focused on this group of 233 women.

2.3 Materials

2.3.1 Maternal depression and anxiety symptoms

Maternal mental health was measured through two self-reporting questionnaires, both validated for pre- and post-natal use. Depression was measured using the Edinburgh Postnatal Depression Scale (EPDS) which comprises of ten questions each scored between zero and three, with total scores 13 and above indicating probable depression (Cox et al., 1987; Matthey et al., 2006). Anxiety was measured using the Trait subscale of the State Trait Anxiety Index to measure general anxiety levels (Meades and Ayers, 2011; Spielberger et al., 1983). This subscale contains 20 questions scored between one and four, with final scores of 40 and above indicating high anxiety levels. Both questionnaires were filled in at the pre-surgical appointment (A1), within seven days of birth (P1), ten weeks (P2) and 12 months postnatally (Y1). Missing data was addressed using participant level mean substitution for those missing <20% of data.

2.3.2 Selective Serotonin Re-uptake Inhibitor (SSRI) use

SSRI prescription records were recorded by research midwives from the medical notes after birth.

2.3.3 Human placental lactogen

Maternal venous serum samples were obtained at recruitment from blood taken as part of an anaesthetic review. Serum was obtained by centrifugation of maternal venous blood which was then frozen at −80°C. hPL levels were assayed in duplicate using the Leinco Technologies Human Placental Lactogen (HPL) Micro-ELISA test kit (Universal Biologicals product code T115-96 tests). Assays were performed by the NIHR Cambridge Biomedical Research Centre, Core Biochemical Assay Laboratory.

2.3.4 Maternal demographics and birth outcomes

Maternal lifestyle and demographics were reported by the mother in the questionnaire at recruitment. Data included ethnicity, education, income, age, and whether they smoked or drank alcohol during their pregnancy. Welsh Index of Multiple Deprivation (WIMD) 2014 scores were calculated from anonymised postcodes (http://wimd.wales.gov.uk). Delivery information, fetal and placental biometry, body mass index at initial booking and parity were taken from the research midwifes notes after birth.

2.4 Statistical analysis

All statistical tests were carried out in IBM SPSS Statistics Version 23. Normality was assessed using Shapiro-Wilk test, Kolmogorov-Smirnov test, histograms and normal Q-Q plots. EPDS, STAI scores and hPL concentration were all found to be not normally distributed, and therefore non-parametric tests were used going forward. Chi squared tests and Spearman's correlations were used when analysing demographic data for categorical and continuous data respectively. Both unadjusted and adjusted linear regression was used to assess the relationship between hPL and maternal perinatal mental health. Potential confounders that had a significant relationship with A1 EPDS or A1 STAI through univariate linear regressions were taken forward to a multiple linear regression with either A1 EPDS or STAI. Due to the large number of potential confounding variables, backward selection was then used to choose the final variables to include. To simplify analyses, those that were significant in either A1 EPDS or A1 STAI were taken forward to all the subsequent adjusted linear regressions to ensure the same confounders were used throughout all analysis. A sensitivity analysis was performed to determine the effect of SSRI usage during pregnancy on hPL levels. Women that were prescribed SSRIs at any point during their pregnancy were removed from the dataset and the unadjusted and adjusted multiple linear regressions were performed again. All unadjusted and adjusted linear regressions were run separately for participants who had girls and boys. Figure was created in R Studio Version 1.1.463.

3. Results

Demographics of the 233 women in this study are provided in Table 1. The relationship between these variables and maternal hPL levels are described, by either chi-squared or Spearman's correlation tests for categorical and continuous data respectively (Table 1). Serum hPL levels were significantly associated with birth weight as previously reported (Männik et al., 2010; Roh et al., 2005), head circumference and placental weight. Two maternal characteristics associated with serum hPL were maternal BMI, as reported in other studies (Vakili et al., 2013), and WIMD score. WIMD is designed to identify the small areas of Wales that are the most deprived related to multiple indicators including income, employment status, health status and educational achievement. Higher scores are associated with an increased percentage of people being treated for mental health conditions (http://wimd.wales.gov.uk). However, individually mental health history, education or income were not associated with serum hPL.

A1 EPDS and STAI scores recorded just prior to the term delivery were strongly associated with depression/anxiety scores recorded at all three postnatal time points ($p > 0.001$ for all

comparisons; Supplementary Table 1). Prenatal mood scores were chosen to decide the confounding variables for testing. Maternal depression and anxiety scores were recorded at four time points. Not all of women completed all four questionnaires, with completion rate lowest at Y1 (Supplementary Table 2). The relationships between both mood scores and potential confounders were assessed using univariate linear regressions (Supplementary Table 3). Those that were significant at the $p<.05$ level were taken forward to a multiple linear regression analysis with EPDS and STAI independently. A backward selection process was performed to choose the final confounders to enter into the adjusted model. The confounders significant with A1 EPDS at $p<0.05$ were 'history of mental health' and 'highest education level'. The potential confounders significant with A1 STAI at $p<0.05$ were 'history of mental health' and 'WIMD'. All three factors were taken forward into the adjusted linear regressions.

To investigate the relationship between hPL and perinatal anxiety and depression, multiple linear regression was used. Unadjusted, there was no significant associations when all participants were included (Table 2). However, when splitting analyses by fetal sex, term anxiety scores (p=.041) and both ten week depression (p=.037) and anxiety scores (p=.047) were significantly associated with hPL concentration when the infant was a girl but not when the infant was a boy (Table 2). After adjustment for the three confounders, the relationship between hPL and depression and anxiety scores at ten weeks postnatal for mothers of girls remained significantly negatively associated (EPDS p=.022 and STAI p=.030) (Table 2). After controlling for a history of mental health, WIMD and highest education level, hPL concentrations predicted a decrease in depression scores (B= -.367) and more strongly in anxiety (B= -.776) in women with girls.

To determine if there were an effect of SSRI usage on the relationship between hPL and maternal mental health, a sensitivity analysis was performed. Those who were prescribed SSRIs at any point during their pregnancy were removed from the analyses and the linear regressions repeated. There were 21 women prescribed SSRIs, 13 of which had boys and eight who had girls. The unadjusted linear regressions uncovered a significant association between hPL concentrations at three time points: just prior to delivery (A1), within one week of delivery (P1) and 10 weeks after delivery (P2) for both depression and anxiety when the infant was female (Table 3; Figure 1). After adjustment for the three confounders, the relationship between hPL and depression and anxiety scores at one week and ten weeks postnatal remained significantly negatively associated (Table 3). Again, this was only significant for mothers of girls and not mothers of boys. hPL predicted a larger decrease in depression scores at P2 compared to P1 (B= -.357 and B= -.737 respectively), and the reverse was true of anxiety scores (B= -.718 and B= -.337, respectively). Sex-specific association was not related to inherent, naturally occurring differences in the levels of hPL between mothers

pregnant with male versus female infants (EPDS <13; male infant/without SSRIs 8.33 ± .3 versus female infant/without SSRIs 8.58 ± .3 female; p=.533).

4. Discussion

Here we report that lower levels of maternal serum human placental lactogen (hPL) measured at term are predictive of higher maternal depression and anxiety scores postnatally, but only in women who gave birth to girls. This negative association was initially only significant for mood scores reported ten weeks after delivery. However, removing data from participants prescribed SSRIs during their pregnancy strengthened the results and additionally predicted mood scores within one week of the birth. For mothers of boys, there were no association between maternally reported symptoms of depression or anxiety with hPL.

This is the first study to report a sex-specific relationship between serum human placental lactogen levels and postnatal depression and anxiety symptoms. Although the unit of change was small, hPL concentrations were significantly negatively associated with postnatal depression and anxiety. Furthermore, this association was only present for women who had girls. We previously reported an association between lower expression of the *hPL* gene in the placenta of women with prenatal symptoms of depression (Janssen et al., 2016a) highlighting the same inverse relationship in two independent studies. Considerable experimental data exists to demonstrate that lactogenic hormones, both prolactin and placental lactogen, are important for the appropriate induction of maternal behaviour in rodents. In particular, infusion of placental lactogen into the brain of virgin female rodents causes them to adopt maternal behaviours whereas blocking lactogenic signalling by ablating the prolactin receptor, to which the hormone binds, results in a lack of pup-induced maternal behaviour and altered indicating a key contribution of lactogenic signalling to maternal behaviour and changes in the brain (Creeth et al., 2019). *In vivo*, placental lactogens are proposed to enter the maternal brain through the choroid plexus, which accumulates the short and long isoforms of the prolactin receptor to facilitate their entry into the brain (Augustine et al., 2003; Bridges and Hays, 2005; Grattan, 2002; Pi et al., 2003). Placental lactogens are thought to bind the prolactin receptor in the medial preoptic area (MPOA) and paraventricular nucleus (PVN) of the hypothalamus and the hippocampus (Bridges et al., 1990; Brown et al., 2017; Kokay et al., 2006; Torner et al., 2009; Vergara-Castañeda et al., 2016), regions important for maternal behaviour (Kimble et al., 1967; Stack et al., 2002). We have experimentally demonstrated, again in rodents, that altering the endocrine function of the mouse placenta alters maternal caregiving behaviour (Creeth et al., 2018) and that a smaller endocrine compartment is associated with higher levels of maternal anxiety (McNamara et al., 2018). These data suggest a causal relationship

between lower levels of hPL and the development of symptoms of depression and anxiety in human pregnancy because women do not undergo the appropriate brain adaptations required to initiate maternal behaviour.

A second important finding of this study was that removing data from women prescribed SSRIs in pregnancy strengthened the relationship between mood symptoms and hPL levels. SSRIs function by selectively binding the 5-hydroxytryptamine transporter blocking the reuptake of serotonin by pre-synaptic neurons resulting in enhanced serotonergic function in the brain (Sangkuhl et al., 2009). Serotonin induces the release of prolactin-releasing factors from the hypothalamus stimulating the pituitary to release prolactin causing prolactinaemia, at least in rodents (Gil-ad et al., 1976; Kamberi et al., 1971). Although human literature is conflicting, a large pharmacoepidemiological study reported a clear association between SSRIs and a rise in serum prolactin (Petit et al., 2003). If SSRI usage is associated with prolactinaemia in pregnancy, this could explain why removing data from women prescribed SSRIs strengthened the association between mood symptoms and hPL levels. Together, these data suggest a mechanism through which placental endocrine dysfunction could increase the risk of depression in mothers both during pregnancy and immediately after birth and also suggest a mechanism through which SSRIs might function to alleviate maternal symptoms.

In this study we observed a sex-specific association between serum placental lactogens and postnatal mood symptoms. Although some placental hormones show inherent sex differences, such as human chorionic gonadotropin which is higher when the fetus is female (Adibi et al., 2015), we did not find significant sex-differences in the level of hPL. Lower maternal serum levels of hPL have been reported in small for gestational age pregnancies, but only when the infant was female (Lagerström et al., 1990). Similarly, maternal smoking has been associated with lower maternal hPL in mothers carrying girls but not boys (Bremme et al., 1990). These relationships may reflect sex-specific endocrine responses by the male and female placenta.

4.1 Limitations

Both a strength and a limitation of our study is the defined nature of the cohort. Grown in Wales is set in South Wales where the majority of the population are Caucasian. We examined data exclusively from Caucasian women to avoid confounders that can be introduced by ethnic differences (Bornstein et al., 2013; Capron et al., 2018). We had too few women in our study representing distinct other ethnicities. All women in the cohort were recruited at a single site by the same two highly trained research midwives where they completed the EPDS and STAI questionnaires, and where blood was collected in advance of their planned surgery. Therefore, all women were in the same environment when responding to questionnaires and providing blood samples, and anticipating a surgical delivery. The homogenous nature of the cohort

provided a clear advantage uncovering the reported associations with relatively small numbers of participants. However, our findings may not apply to other ethnicities or to other modes of delivery. It will therefore be important to replicate these findings in more diverse cohorts, and across other modes of delivery. A second limitation of this study are the smaller sample sizes at the later time points (P2=124, Y1=95). The negative associations between hPL and mood were only significant in women with girls and there were higher numbers for this group at each time point. With more samples from boys, a similar association between low hPL and maternal mood symptoms may be present where the fetus is male. A third limitation is that serum hPL was measured at a single time point. We do not know whether low hPL is present at earlier time points. This knowledge has important implications for the use of hPL levels as a predictive biomarker.

5. Conclusions

This study set out to assess the relationship between maternal hPL concentrations at term and perinatal mental health measures in the Grown in Wales Study cohort. hPL serum levels at term were found to predict postnatal depression and anxiety scores. The inverse relationships between low hPL and high scores for depression and anxiety were significant for mothers who had girls, and not those who had boys. Excluding those prescribed SSRIs during pregnancy strengthened the relationship. Our data is consistent with the hypothesis that low lactogenic signalling contributes to perinatal mood disorders rescued, in part, by the prolactinaemic function of SSRIs.

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Declarations of interest:

None.

References

Adibi, J.J., Lee, M.K., Saha, S., Boscardin, W.J., Apfel, A., Currier, R.J., 2015. Fetal sex differences in human chorionic gonadotropin fluctuate by maternal race, age, weight and by gestational age. Journal of Developmental Origins of Health and Disease 6, 493-500.

Asher, I., Kaplan, B., Modai, I., Neri, A., Valevski, A., Weizman, A., 1995. Mood and hormonal changes during late pregnancy and puerperium. Clinical and Experimental Obstetrics and Gynecology 22, 321-325.

Augustine, R.A., Kokay, I.C., Andrews, Z.B., Ladyman, S.R., Grattan, D.R., 2003. Quantitation of prolactin receptor mRNA in the maternal rat brain during pregnancy and lactation. Journal of Molecular Endocrinology 31, 221-232.

Bauer, A., Parsonage, M., Knapp, M., Iemmi, V., Adelaja, B., 2014. The costs of perinatal mental health problems. London School of Economics and Political Science, London.

Bornstein, M.H., Jager, J., Putnick, D.L., 2013. Sampling in developmental science: situations, shortcomings, solutions, and standards. Developmental Review 33, 257-270.

Brand, S.R., Brennan, P.A., 2009. Impact of antenatal and postpartum maternal mental illness: how are the children? Clinical Obstetric and Gynecology 52, 441-455.

Bremme, K., Lagerstrom, M., Andersson, O., Johansson, S., Eneroth, P., 1990. Influences of maternal smoking and fetal sex on maternal serum oestriol, prolactin, hCG, and hPL levels. Archives of Gynecology ad Obstetrics 247, 95-103.

Bridges, R.S., Hays, L.E., 2005. Steroid-induced alterations in mRNA expression of the long form of the prolactin receptor in the medial preoptic area of female rats: Effects of exposure to a pregnancy-like regimen of progesterone and estradiol. Brain Research. Molecular Brain Research 140, 10-16.

Bridges, R.S., Numan, M., Ronsheim, P.M., Mann, P.E., Lupini, C.E., 1990. Central prolactin infusions stimulate maternal behavior in steroid-treated, nulliparous female rats. PNAS 87, 8003-8007.

Brown, R.S.E., Aoki, M., Ladyman, S.R., Phillipps, H.R., Wyatt, A., Boehm, U., Grattan, D.R., 2017. Prolactin action in the medial preoptic area is necessary for postpartum maternal nursing behavior. PNAS 114, 10779-10784.

Burton, G.J., Fowden, A.L., 2015. The placenta: a multifaceted, transient organ. Philosophical Transactions of the Royal Society of London, Series B Biological Sciences 370, 20140066.

Capron, L.E., Ramchandani, P.G., Glover, V., 2018. Maternal prenatal stress and placental gene expression of NR3C1 and HSD11B2: The effects of maternal ethnicity Psychoneuroendocrinology 87, 166-172.

Cox, J.L., Holden, J.M., Sagovsky, R., 1987. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. The British Journal of Psychiatry 150, 782-786.

Creeth, H.D.J., McNamara, G.I., Isles, A.R., John, R.M., 2019. Imprinted genes influencing the quality of maternal care. Frontiers in Neuroendocrinology 53, 100732.

Creeth, H.D.J., McNamara, G.I., Tunster, S.J., Boque-Sastre, R., Allen, B., Sumption, L., Eddy, J.B., Isles, A.R., John, R.M., 2018. Maternal care boosted by paternal imprinting in mammals. PLoS Biology 16, e2006599.

Diego, M.A., Field, T., Hernandez-Reif, M., Schanberg, S., Kuhn, C., Gonzalez-Quintero, V.H., 2009. Prenatal depression restricts fetal growth. Early Human Development 85, 65-70.

Dubovicky, M., Belovicova, K., Csatlosova, K., Bogi, E., 2017. Risks of using SSRI / SNRI antidepressants during pregnancy and lactation. Interdisciplinary Toxicology 10, 30-34.

Gil-ad, I., Zambotti, F., Carruba, M.O., Vicentini, L., Muller, E.E., 1976. Stimulatory role for brain serotoninergic system on prolactin secretion in the male rat. Proceedings of the Society for Experimental Biology and Medicine 151, 512-518.

Goodnick, P.J., Chaudry, T., Artadi, J., Arcey, S., 2005. Women's issues in mood disorders. Expert Opinions in Pharmacotherapy 1, 903-916.

Grattan, D.R., 2002. Behavioural significance of prolactin signalling in the central nervous system during pregnancy and lactation. Reproduction 123, 497-506.

Groer, M.W., Morgan, K., 2007. Immune, health and endocrine characteristics of depressed postpartum mothers. Psychoneuroendocrinology 32, 133-139.

Gurtunca, N., Sperling, M.A., 2017. Growth Hormone, Prolactin, and Placental Lactogen in the Fetus and Newborn, Fetal and Neonatal Physiology (Fifth Edition), pp. 1470-1476.

Ingram, J.C., Greenwood, R.J., Woolridge, M.W., 2003. Hormonal predictors of postnatal depression at 6 months in breastfeeding women. Journal of Reproductive and Infant Psychology 21, 61-68.

Janssen, A.B., Capron, L.E., O'Donnell, K., Tunster, S.J., Ramchandani, P.G., Heazell, A.E., Glover, V., John, R.M., 2016a. Maternal prenatal depression is associated with decreased placental expression of the imprinted gene PEG3. Psychological Medicine 46, 2999-3011.

Janssen, A.B., Savory, K.A., Garay, S.M., Sumption, L., Watkins, W., Garcia-Martin, I., Savory, N., Rideway, A., Isles, A., Penketh, R., Jones, I., John, R.M., 2018. Persistence of anxiety symptoms after elective caesarean delivery. BJPsych Open.

Janssen, A.B., Tunster, S.J., Heazell, A.E.P., John, R.M., 2016b. Placental PHLDA2 expression is increased in cases of fetal growth restriction following reduced fetal movements. BMC Medical Genetics 17, s12881-12016-10279-12881.

Jin, Y., Vakili, H., Liu, S.Y., Menticoglou, S., Bock, M.E., Cattini, P.A., 2018. Chromosomal architecture and placental expression of the human growth hormone gene family are targeted

385 by pre-pregnancy maternal obesity. American Journal of Physiology. Endocrinology and
386 Metabolism 315, E435-445.
387 Kamberi, I.A., Mical, R.S., Porter, J.C., 1971. Effects of melatonin and serotonin on the release
388 of FSH and prolactin. Endocrinology 88, 1288-1293.
389 Kimble, D.P., Rogers, L., Hendrickson, C.W., 1967. Hippocampal lesions disrupt maternal, not
390 sexual, behavior in the albino rat. Journal of Comparative of Physiological Psychology 63,
391 401-407.
392 Kinsella, M.T., Monk, C., 2009. Impact of maternal stress, depression and anxiety on fetal
393 neurobehavioural development. Clinical Obstetrics and Gynecology 52, 425-440.
394 Kokay, I.C., Bull, P.M., Davis, R.L., Ludwig, M., Grattan, D.R., 2006. Expression of the long
395 form of the prolactin receptor in magnocellular oxytocin neurons is associated with specific
396 prolactin regulation of oxytocin neurons. Americal Journal of Physiology, Regulatory
397 Integrative and Comparitive Physiology 290, R1216-1225.
398 Lagerström, M., Bremme, K., Eneroth, P., 1990. Maternal serum levels of estriol, prolactin,
399 human placental lactogen and chorionic gonadotrophin related to fetal sex in normal and
400 abnormal pregnancies. Gynecologic and Obsetetric Investigations 30, 198-203.
401 Liu, Y., Fan, X., Wang, R., Lu, X., Dang, Y.L., Wang, H., Lin, H.Y., Zhu, C., Ge, H., Cross,
402 J.C., Wang, H., 2018. Single-cell RNA-seq reveals the diversity of trophoblast subtypes and
403 patterns of differentiation in the human placenta. Cell Research 28, 819-832.
404 Männik, J., Vaas, P., Rull, K., Teesalu, P., Rebane, T., Laan, M., 2010. Differential expression
405 profile of growth hormone/chorionic somatomammotropin genes in placenta of small- and
406 large-for-gestational-age newborns. Journal of Clinical Endocrinology and Metabolism 95,
407 2433-2442.
408 Matthey, S., Henshaw, C., Elliott, S., Barnett, B., 2006. Variability in use of cut-off scores and
409 formats on the Edinburgh Postnatal Depression Scale: implications for clinical and research
410 practice. Archives of Women's Mental Health 9, 309-315.
411 McNamara, G.I., Creeth, H.D.J., Harrison, D.J., Tansey, K.E., Andrews, R.M., Isles, A.R.,
412 John, R.M., 2018. Loss of offspring Peg3 reduces neonatal ultrasonic vocalisations and
413 increases maternal anxiety in wild type mothers. Human Molecular Genetics 27, 440-450.
414 Meades, R., Ayers, S., 2011. Anxiety measures validated in perinatal populations: a
415 systematic review. Journal of Affective Disorders 133, 1-15.
416 Molyneaux, E., Poston, L., Ashurst-Williams, S., Howard, L.M., 2014. Obesity and mental
417 disorders during pregnancy and postpartum: a systematic review and meta-analysis.
418 Obstetrics and Gynecology 123, 857-867.
419 Newbern, D., Freemark, M., 2011. Placental hormones and the control of maternal metabolism
420 and fetal growth. Current Opinions in Endocrinology, Diabetes and Obesity 18, 490-416.

Petit, A., Piednoir, D., Germain, M.L., Trenque, T., 2003. Drug-induced hyperprolactinemia: a case-non-case study from the national pharmacovigilance database. Therapie 58, 159-163.

Pi, X., Zhang, B., Li, J., Voogt, J.L., 2003. Promoter usage and estrogen regulation of prolactin receptor gene in the brain of the female rat. Neuroendocrinology 77, 187-197.

Roh, C.R., Budhraja, V., Kim, H.S., Nelson, D.M., Sadovsky, Y., 2005. Microarray-based identification of differentially expressed genes in hypoxic term human trophoblasts and in placental villi of pregnancies with growth restricted fetuses. Placenta 26, 319-328.

Sangkuhl, K., Klein, T., Altman, R., 2009. Selective Serotonin Reuptake Inhibitors (SSRI) Pathway. Pharmacogenetics and Genomics 19, 907-909.

Spielberger, C., Gorsuch, R., Lushene, R., Vagg, P., Jacobs, G., 1983. Manual for the State-trait Anxiety Inventory. Consulting Psychologists Press. Inc., Palo Alto, C. A.

Stack, E.C., Balakrishnan, R., Numan, M.J., Numan, M., 2002. A functional neuroanatomical investigation of the role of the medial preoptic area in neural circuits regulating maternal behavior. Behavioural Brain Research 131, 17-36.

Stewart, D.E., 2011. Clinical practice. Depression during pregnancy. New England Journal of Medicine 365, 1605-1611.

Torner, L., Karg, S., Blume, A., Kandasamy, M., Kuhn, H.G., Winkler, J., Aigner, L., Neumann, I.D., 2009. Prolactin prevents chronic stress-induced decrease of adult hippocampal neurogenesis and promotes neuronal fate. Journal of Neuroscience 29, 1826-1833.

Vakili, H., Jin, Y., Menticoglou, S., Cattini, P.A., 2013. CCAAT-enhancer-binding protein β (C/EBPβ) and downstream human placental growth hormone genes are targets for dysregulation in pregnancies complicated by maternal obesity. Journal of Biological Chemistry 288, 22849-22861.

Vergara-Castañeda, E., Grattan, D.R., Pasantes-Morales, H., Pérez-Domínguez, M., Cabrera-Reyes, E.A., Morales, T., Cerbón, M., 2016. Prolactin mediates neuroprotection against excitotoxicity in primary cell cultures of hippocampal neurons via its receptor. Brain Research 1636, 193-199.

Tables

Table 1. Demographic and birth data for 233 participants and relationship with maternal hPL

Demographics	***% (n) or median (IQR)***	***p***
Maternal age	33 (7)	.348
Parity	1 (1)	.109
Maternal BMI at booking	26.4 (7.3)	**.009****
WIMD score [a]	1270 (1200)	**.002****
History of mental health, *% (n)*		.408
No	69.5 (161)	
Yes	30.5 (71)	
SSRI use in pregnancy, *% (n)*		.879
No	91 (212)	
Yes	9 (21)	
SSRI use at term, *% (n)*		.789
No	94.8 (221)	
Yes	5.2 (12)	
Highest education level, *% (n)*		.443
Left before GCSE	6 (14)	
GCSE or vocational	22.7 (53)	
A-level	12.4 (29)	
University	30 (70)	
Postgraduate	26.2 (61)	
Not recorded	2.6 (6)	
Family income, *% (n)*		.909
<£18,000	7.3 (17)	
£18-25,000	10.3 (24)	
£25-43,000	19.3 (45)	
>£43,000	51.1 (119)	
Not recorded	12 (28)	
Smoking in pregnancy [b], *% (n)*		.785
No	89.7 (209)	
Yes	10.3 (24)	
Alcohol in pregnancy [c], *% (n)*		.182
No	91.4 (213)	
Yes	6.9 (16)	
Not recorded	1.7 (4)	
Season of birth, *% (n)*		.621
Spring	21 (49)	
Summer	23.2 (54)	
Autumn	33.9 (79)	
Winter	21.8 (51)	
Fetal sex, *% (n)*		.436
Female	45.5 (106)	
Male	54.5 (127)	
Gestational age *(days)*	274 (3)	.238
Placental weight *(g)*	654 (171)	**<.001*****
Birth weight *(g)*	3500 (665)	**<.001*****
Head circumference *(cm)*	35.5 (2.2)	**.002****

Categorical data compared with hPL using chi squared tests, and continuous data with Pearson's correlations. BMI (Body Mass Index), WIMD (Welsh Index of Multiple Deprivation),

SSRI (Selective Serotonin Reuptake Inhibitors). Data is for 233 participants with the exception of head circumference (n=231) and placental weight (n=228), WIMD (n=227) and BMI (n=222).

[a] WIMD scores range from 1-1909 with higher scores indicating a small area of a lower overall deprivation, and a low score indicative of higher overall deprivation.

[b] At any point in pregnancy

[c] Weekly at any point in pregnancy

Table 2. Unadjusted and adjusted multiple linear regression models analysing association between hPL concentration µg/ml and maternal mental health scores.

Mood scores	***All***			***Male***			***Female***		
	B	***95% CI***	***p***	***B***	***95% CI***	***p***	***B***	***95% CI***	***p***
Unadjusted linear regressions									
A1 EPDS	-.179	-.396, .038	.106	-.040	-.383, .303	.817	-.278	-.560, .004	.053
A1 STAI	-.172	-.587, .242	.413	.357	-.282, .996	.270	-.561	-1.100, -.023	**.041***
P1 EPDS	-.158	-.418, .103	.233	.005	-.409, .419	.980	.308	-.642, .026	.070
P1 STAI	-.393	-.946, .160	.162	-.349	-1.195, .497	.413	-.453	-1.190, .283	.224
P2 EPDS	-.188	-.473, .096	.193	.034	-.457, .526	.889	-.361	-.700, -.022	**.037***
P2 STAI	-.485	-1.107, .137	.125	-.106	-1.166, .955	.842	-.775	-1.538, -.012	**.047***
Y1 EPDS	-.104	-.465, .257	.567	.048	-.626, .721	.887	-.195	-.632, .241	.374
Y1 STAI	-.358	-1.166, .451	.382	.073	-1.458, 1.603	.923	-.583	-1.549, .383	.232
Adjusted linear regressions									
A1 EPDS	-.069	-.280, .143	.523	.094	-.233, .422	.569	-.216	-.500, .069	.136
A1 STAI	.012	-.385, .409	.953	.513	-.094, 1.119	.097	-.438	-.966, .090	.103
P1 EPDS	-.118	-.382, .146	.377	.064	-.373, .501	.771	-.311	-.660, .037	.079
P1 STAI	-.234	-.797, .329	.413	-.069	-.949, .811	.876	-.510	-1.285, .265	.193
P2 EPDS	-.101	-.375, .173	.466	.250	-.217, .716	.287	-.367	-.679, -.056	**.022***
P2 STAI	-.325	-.925, .274	.285	.171	-.861, 1.203	.740	-.776	-1.475, -.077	**.030***
Y1 EPDS	.077	-.269, .423	.659	.271	-.294, .836	.335	-.117	-.546, .311	.585
Y1 STAI	-.032	-.852, .788	.939	.420	-1.004, 1.844	.551	-.544	-1.518, .429	.429

A1 (at term pre-surgical appointment), P1 (up to seven days postnatally), P2 (ten weeks postnatally), Y1 (12 months postnatally), EPDS (Edinburgh Postnatal Depression Scale), STAI

(Speilberger State Trait Anxiety-Trait subscale). Adjusting for history of mental health, WIMD, and Highest education level.

Table 3. Unadjusted linear regression assessing relationship between hPL and perinatal mental health scores, in women not prescribed SSRIs.

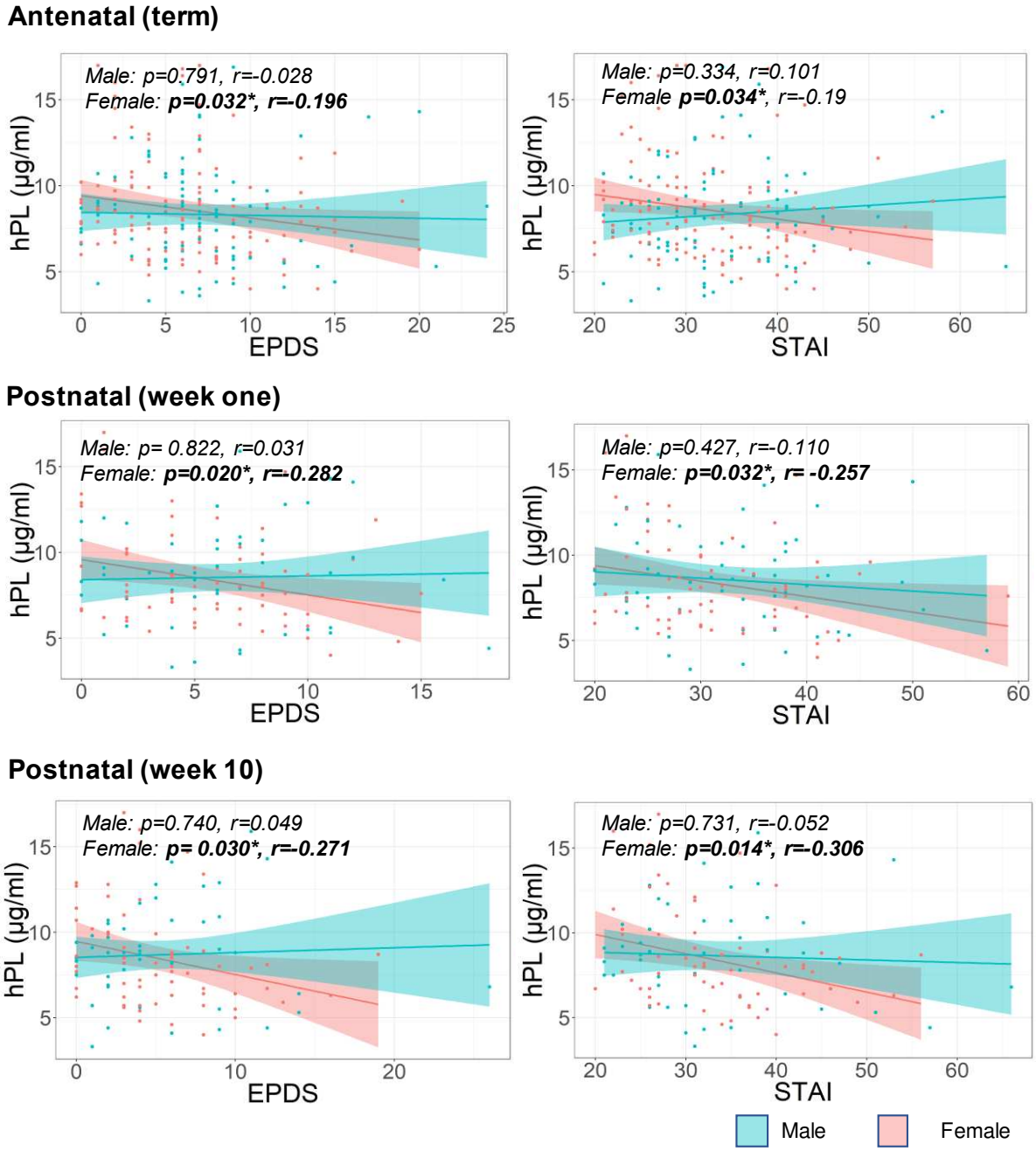
Mood scores	***All***			***Male***			***Female***		
	B	***95% CI***	***p***	***B***	***95% CI***	***p***	***B***	***95% CI***	***p***
Unadjusted linear regressions									
A1 EPDS	-.194	-.405, .016	.070	-.045	-.381, .291	.791	-.297	-.569, -.026	**.032***
A1 STAI	-.182	-.569, .205	.356	.307	-.321, .935	.334	-.527	-1.012, -.042	**.034***
P1 EPDS	-.183	-.435, .068	.151	.045	-.356, .447	.822	-.385	-.707, -.062	**.020***
P1 STAI	-.530	-1.039, -.020	**.042**	-.323	-1.131, .486	.427	-.728	-1.389, -.066	**.032***
P2 EPDS	-.181	-.470, .107	.216	.084	-.422, .590	.740	-.378	-.720, -.037	**.030***
P2 STAI	-.554	-1.118, .010	.054	-.177	-1.204, .850	.731	-.825	-1.477, -.173	**.014***
Y1 EPDS	-.098	-.447, .252	.580	.036	-.681, .752	.919	-.182	-.577, .213	.359
Y1 STAI	-.489	-1.237, .260	.198	-.191	-1.720, 1.338	.801	-.641	-1.496, .213	.138
Adjusted linear regressions									
A1 EPDS	-.090	-.301, .121	.400	.098	-.236, .432	.561	-.239	-.521, .043	.095
A1 STAI	-.025	-.413, .363	.901	.499	-.134, 1.131	.121	-.434	-.928, .060	.085
P1 EPDS	-.165	-.425, .097	.215	.040	-.402, .483	.855	-.357	-.698, -.015	**.041***
P1 STAI	-.363	-.902, .175	.184	-.059	-.941, .822	.893	-.718	-1.433, -.004	**.049***
P2 EPDS	-.069	-.350, .212	.626	.180	-.842, 1.202	.724	-.737	-1.387, -.086	**.027***
P2 STAI	-.336	-.898, .226	.238	.334	-.144, .812	.166	-.337	-.658, -.016	**.040***
Y1 EPDS	.065	-.280, .411	.708	.264	-.359, .888	.391	-.077	-.489, .334	.707
Y1 STAI	-.288	-1.076, .499	.468	.037	-1.417, 1.491	.959	-.588	-1.510, .335	.207

Adjusted for history of mental health, WIMD, and Highest education level.

Figure legends

Figure 1: Maternal serum hPL values correlate with EPDS and STAI scores for mothers of girls

Spearman's correlation of maternal serum hPL levels measured just prior to a term ELCS delivery and perinatal mental health scores in women not prescribed SSRIs analysing male and female data separately. Line of best fit is linear model. A1: at pre-surgical appointed prior to delivery; P1: within one week of delivery; P2: ten weeks after delivery.



Supplementary Tables

Supplementary Table 1: Spearman's correlations between perinatal mental health scores.

	A1 EPDS	***A1 STAI***	***P1 EPDS***	***P1 STAI***	***P2 EPDS***	***P2 STAI***	***Y1 EPDS***	***Y1 STAI***
A1 EPDS	-	-	-	-	-	-	-	-
A1 STAI	.722; **<.001*****	-	-	-	-	-	-	-
P1 EPDS	.644; **<.001*****	.595; **<.001*****	-	-	-	-	-	-
P1 STAI	.693; **<.001*****	.793; **<.001*****	.763; **<.001*****	-	-	-	-	-
P2 EPDS	.577; **<.001*****	.523; **<.001*****	.538; **<.001*****	.584; **<.001*****	-	-	-	-
P2 STAI	.633; **<.001*****	.752; **<.001*****	.587; **<.001*****	.697; **<.001*****	.739; **<.001*****	-	-	-
Y1 EPDS	.615; **<.001*****	.548; **<.001*****	.580; **<.001*****	.593; **<.001*****	.607; **<.001*****	.541; **<.001*****	-	-
Y1 STAI	.598; **<.001*****	.729; **<.001*****	.561; **<.001*****	.661; **<.001*****	.536; **<.001*****	.727; **<.001*****	.711; **<.001*****	-

(*Correlation coefficient*; *p* value)

A1 (at pre-surgical appointment), P1 (up to seven days postnatally), P2 (ten weeks postnatally), Y1 (12 months postnatally), EPDS (Edinburgh Postnatal Depression Scale), STAI (Speilberger State Trait Anxiety-Trait subscale).

Supplementary Table 2: Numbers of participants at each time point

	All	***Male***	***Female***
A1 EPDS	233	106	127
A1 STAI	233	106	127
P1 EPDS	133	62	71
P1 STAI	135	62	73
P2 EPDS	124	56	68
P2 STAI	122	54	68
Y1 EPDS	95	36	59
Y1 STAI	96	36	60

Supplementary Table 3: Univariate linear regression *p* values of potential confounders with antenatal mental health.

Demographics	***A1 EPDS p value***	***A1 STAI p value***
Maternal age	.347	.476
Parity	.996	.557
Maternal BMI at booking	.139	.265
WIMD score [a]	**.029***	**.004****
Highest education level Left before GCSE	**.033***	.177

GCSE or vocational	.686	.191
A-level	.661	.131
University	**.025***	.401
Postgraduate	*Ref.*	*Ref.*
Family income		
<£18,000	**.003****	**.003****
£18-25,000	.355	.182
£25-43,000	.374	.219
>£43,000	*Ref.*	*Ref.*
Smoking in pregnancy [b]	**.025***	**.040***
Alcohol in pregnancy [c]	.073	.069
Gestational age *(days)*	.692	.844
History of mental health	**>.001*****	**>.001*****

Ref. (reference category)

[a] WIMD score ranges from 1-1909, high score indicating a small area of a lower overall deprivation, and a low score of higher overall deprivation.

[b] At any point in pregnancy

[c] Weekly at any point in pregnancy