CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	A prospective study of placental growth factor in twin pregnancy and development of a dichorionic twin pregnancy specific reference range
Authors	Hayes-Ryan, Deirdre;Meaney, Sarah;Fitzgerald, Anthony P.;O'Mahony, E.;Normile, C.;Kenny, Louise C.;O'Donogue, Keelin
Publication date	2020-09-18
Original Citation	Hayes-Ryan, D., Meaney, S., Fitzgerald, A., O'Mahony, E., Normile, C., Kenny, L. and O'Donoghue, K. (2020) 'A prospective study of placental growth factor in twin pregnancy and development of a dichorionic twin pregnancy specific reference range', BJOG: An International Journal of Obstetrics & Gynaecology, (9 pp). doi: 10.1111/1471-0528.16518
Type of publication	Article (peer-reviewed)
Link to publisher's version	https://obgyn.onlinelibrary.wiley.com/doi/epdf/10.1111/1471-0528.16518 - 10.1111/1471-0528.16518
Rights	© 2020 John Wiley & Sons Ltd. This is the peer reviewed version of the following article: 'A prospective study of placental growth factor in twin pregnancy and development of a dichorionic twin pregnancy specific reference range', BJOG 2020, which has been published in final form at https://doi.org/10.1111/1471-0528.16518. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.
Download date	2026-10-30 11:25:54
Item downloaded from	https://hdl.handle.net/10468/10688



University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

A prospective study of placental growth factor in twin pregnancy and development of a dichorionic twin pregnancy specific reference range

Deirdre Hayes-Ryan[1, 2]*, Sarah Meaney*[3]*, Anthony P Fitzgerald*[4,5]*, Eve O'Mahony*[1]*, Caoimhe Normile*[1]*, Louise C Kenny*[6] *& Keelin O'Donoghue* [1, 2]

[1]The Irish Centre for Fetal and Neonatal Translational Research (INFANT), Cork, Ireland

[2]Cork University Maternity Hospital (CUMH), Ireland

[3] National Perinatal Epidemiology Centre, University College Cork , Ireland

[4] School of Public Health, University College Cork, Ireland

[5] Department of Statistics, University College Cork, Ireland

[6]Faculty of Health & Life Sciences, University of Liverpool, United Kingdom

Corresponding author details:

Deirdre Hayes-Ryan,

Clinical Research Fellow, INFANT Research Centre, CUMH, Wilton, Cork, Ireland.

+353 21 420 5058

Deirdre.hayesryan@ucc.ie

Running Title: Placental growth factor in twin pregnancy

Abstract

Objective: The aim of this study was twofold; to develop a dichorionic twin pregnancy specific reference range for placental growth factor, and to compare gestational specific placental growth factor levels in twin pregnancies later complicated by preeclampsia, hypertensive disorder of pregnancy or fetal growth restriction to controls

Design: Prospective observational study

Setting: Single large tertiary maternity unit in Ireland

Population or Sample: Women with a twin pregnancy

Methods: Consenting pregnant women, across a variety of gestations, had a single blood sample taken at one time point only during their pregnancy. The plasma was initially biobanked and PlGF was measured later in batches using the point of care Triage® PlGF test

Main Outcome Measures: Development of preeclampsia, hypertensive disorder of pregnancy or fetal growth restriction

Results: PlGF levels in uncomplicated dichorionic twin pregnancies were significantly lower in the women who later developed preeclampsia than in the controls at all gestational intervals. In those that later developed any hypertensive disorder of pregnancy median PlGF was lower only in those recruited before 24 weeks' gestation

while in infants with a customised birthweight below the 3rd centile, PlGF was lower only in those sampled after 24 weeks' gestation.

Conclusions: PlGF levels in twin pregnancy differ significantly between those women with a pregnancy that will later be complicated by preeclampsia and those that will not. This difference is present many weeks before clinical signs or symptoms of disease are present. Using cross sectional values from uncomplicated twin pregnancies, we have developed a dichorionic twin pregnancy specific reference range for PlGF.

Funding: The Irish Centre for Fetal and Neonatal Translational Research (INFANT), Cork, Ireland provided financial support for this study. The funder had no role in the study design; collection, analysis or interpretation of data; or in the writing of the report; or in the decision to submit the article for publication

Keywords:

- Hypertensive Disorders of Pregnancy
- Placental Growth Factor
- Preeclampsia
- Reference Range
- Twin Pregnancy

Tweetable Abstract: PlGF levels in twin pregnancy differ significantly between women that will later develop preeclampsia and those that will not.

Introduction

Preeclampsia is a common complication of pregnancy characterised by new onset hypertension and either proteinuria or other maternal organ dysfunction after 20 weeks' gestation (1). Along with other hypertensive disorders of pregnancy (HDP), it is a major contributor to maternal and neonatal morbidity and mortality (2, 3). Potentially serious maternal morbidity may arise in the form of seizures, cerebral haemorrhage, renal failure, liver rupture and disseminated intravascular coagulation (4). The only definitive treatment for preeclampsia is removal of the placenta, often resulting in iatrogenic pre-term delivery and subsequent fetal morbidity (5). Women with a twin pregnancy are at a two to three fold increased risk of developing preeclampsia, possibly due to a combination of larger placental mass and use of assisted reproductive therapy (ART), especially use of non-autologous gametes (6-8). Rates of twin pregnancy have risen over the last number of decades globally (9-12).

Although the exact aetiology of preeclampsia is not fully understood, a growing body of evidence suggests that an imbalance of angiogenic factors of placental origin play a crucial role in its development (13-17). Placental growth factor (PlGF) is an angiogenic protein and a member of the vascular endothelial growth factor family (18). Studies in singleton pregnancies have shown lowered levels of PlGF and increased levels of its soluble receptor sFlt-1 in maternal plasma, weeks prior to the clinical onset of preeclampsia (19, 20). The UK National Institute for Clinical Excellence (NICE) advocates PlGF testing, combined with routine clinical care, to help rule out pre-term preeclampsia in singleton pregnancies (21). A number of international randomised control trials (RCTs) are currently on-going, investigating the clinical impact of the integration of PlGF into clinical care pathways (22). The international INSPIRE trial evaluated the use of sFlt-1/PlGF ratio in women presenting with suspected

preeclampsia and showed that use of the ratio, in conjunction with standard clinical practice, significantly improved clinical precision without changing admission rates (23). The UK PARROT study, demonstrated a reduction in time taken to diagnosis preeclampsia and reduced maternal morbidity when PlGF is integrated into clinical care algorithms (24).

Few studies to date have evaluated the levels of circulating angiogenic factors during twin pregnancy. In those that have been described, huge variations exist in; the primary outcome (i.e preeclampsia, fetal growth restriction or other adverse clinical outcome); the definition/classification of the primary outcome; the gestational age at time of sampling; and the immunoassay used for quantification. (25-33). The aim of this study was twofold; to develop a dichorionic twin pregnancy specific reference range for PlGF and secondly to compare gestational specific PlGF levels in twin pregnancies complicated by preeclampsia, any hypertensive disorder of pregnancy (HDP) or fetal growth restriction to controls.

Materials & Methods

Setting and Design

This study was conducted in a single maternity hospital in Ireland with over 8000 deliveries per annum. The study was a prospective cross-sectional cohort study of PlGF in twin pregnancy. From the start of July 2015 to the end of December 2017, women attending the hospital's dedicated twin pregnancy clinic were approached to participate in the study. Any woman with an uncomplicated twin pregnancy from 12+0-36+6 weeks' gestation inclusive, without signs/symptoms or a diagnosis of preeclampsia was eligible for inclusion. Those with complications such as a known

congenital anomaly in either baby, severe early onset growth restriction or twin-to-twin transfusion syndrome (TTTS) were excluded from recruitment. Following informed patient consent, a 3ml ethylenediaminetetraacetic acid (EDTA) blood sample was taken, centrifuged, divided into aliquots and the plasma biobanked at -80C within 3 hours of sampling. All sampling, processing and biobanking was carried out within the same building according to previously published Standard Operating Procedures (SOPs) (34). Women had venepuncture performed at one random gestational time point only. Clinically relevant outcome data such as the diagnosis of any HDP (chronic hypertension, gestational hypertension, preeclampsia or superimposed preeclampsia) and infant birthweights were taken from medical notes following delivery. Anonymised clinical and demographic data pertaining to the participant and their offspring were recorded in the study database. For our study, the NICE definitions of hypertensive disorders of pregnancy were utilised (35).Fetal growth restriction was calculated based on actual birthweight, gestation at birth, fetal gender and maternal ethnicity, parity and BMI using the Gestation Related Optimal Weight (GROW) centile calculator (36). Patients were not involved in the development of this research study and a core outcome set was not used.

Placental Growth Factor Immunoassay

Biobanked plasma samples were analysed in batches for circulating levels of PlGF using a point of care immunoassay; the Triage® PlGF test (Quidel Inc., San Diego). This test is not routinely available in the hospital for clinical use. It was purchased by our research centre for the purpose of this study. The test manufacturers had no part in the study design, conduct, analysis or manuscript development. The immunoassay was performed as per manufacturer's instructions, in a single freeze thaw cycle to minimise protein denaturation. The results are displayed on the meter screen in

approximately 15 minutes and have a measurable range from 12-3000 pg/ml. The Triage® has a reported measurable range from 12-3000 pg/ml. The manufacturers report total precision on plasma controls at concentrations of 85.2 and 1300 pg/mL as 12.8% and 13.2% respectively. For the purposes of this study, any result obtained <12 pg/ml was allocated the value of 10 pg/ml.

Statistics

SPSS Version 23 and Stata 15 were used to analyse the data.

Part 1: Descriptive statistics were employed to examine the baseline maternal demographics, clinical outcomes and the PlGF distribution in the cohort. When developing a reference range for PlGF, all cases where a stillbirth was diagnosed in either of the twins as well as cases where any form of HDP later developed were removed. Secondly in order to facilitate development of a reference range for PlGF in an uncomplicated dichorionic twin pregnancy all monochorionic twin pregnancies were removed. Lastly, cases that developed fetal growth restriction resulting in both twins having a customised birthweight of less than the 3rd centile were then removed. The remaining women were divided according to gestational age at recruitment and PlGF ranges calculated for each gestational week were calculated. A heterogeneous regression of log(PlGF) on gestational age of was used to develop a reference range for PlGF in uncomplicated dichorionic twin pregnancies.. The mean of log(PlGF) was modelled using fractional polynomials and the variance was modelled as proportional to some power of gestational age (37). The residuals were assumed to follow a Normal distribution at each gestational age. Centiles of log(PIGF) were exponentiated to yield centiles

Part 2: To examine the effect of hypertensive disorders and placental dysfunction on PlGF, the entire cohort including abnormal cases, was divided into 2 groups based on the woman's gestational age at time of her enrolment to the study and hence sampling of maternal plasma PlGF; <24 weeks' gestation and ≥ 24 weeks' gestation. This gestational cut-off was employed as pregnancy related hypertensive complications are unusual prior to this timepoint and also it equated well with the median of the cohort.

Results

In total, 275 women with a twin pregnancy were recruited. There were no withdrawals or losses to follow up. Three women (1% of the cohort) had a stillbirth occur in one of the twins while in 4.7% (n=12) of women, an anomaly of one or both twins was diagnosed. Given with twin pregnancy there is differing placental volumes present dependent on chorionicity, circulating levels of PlGF may also vary in line with chorionicity. We found that PlGF was lower in monochorionic twin pregnancy (data not shown) but given the high incidence of complications as well as the small numbers present (n=40) in this subgroup, further analysis was not possible. We limited our analysis to dichorionic cases only for development of the reference range.

Part 1:

Reference Range Demographics

Removal of those with an abnormal pregnancy outcome (preeclampsia or HDP in the mother, stillbirth of either twin or where both twins had a customised birthweight of <3rd centile at delivery), or a monochorionic pregnancy left 173 women with an uneventful

dichorionic twin pregnancy for inclusion in the reference range analysis (Supplemental Material Figure 1). Median maternal age was 34 years, booking BMI was <30Kg/m^2 for the majority (81.5%; n=141) and most were Caucasian (93.6%; n=162). Over half of the group were multiparous (56.1%; n=97), just over a third (35.1.1%; n=60) had conceived the twin pregnancy with use of ART. All women with pre-existing renal disease or essential hypertension developed superimposed preeclampsia in their pregnancies and hence were not included in the reference range cohort (Supplemental Material Table 1). Comparison of participant characteristics between each gestational group showed no significant difference in enrolment characteristics (Supplemental Material Table 2).

Reference Range Development

The 3rd-97th centiles of PIGF as a function of gestational age concentrations were calculated (Table 1). With progressing gestational age the median PlGF was seen to rise, simultaneously to the development and maturation of the placentae, peaking at 25 weeks gestation, and then steadily decreased towards term. The lowest acceptable PlGF value for each gestational week is presented (Figure 1). Removal of cases where women developed any form of HDP or where both twins had a customised birthweight <3rd centile did not alter the reference range significantly. These data provide a valid reference range for PlGF in a normal dichorionic twin pregnancy (Figure 2).

Part 2:

Comparison of Gestational PlGF

The second aim of this study was to compare gestational PlGF in twin pregnancies complicated by preeclampsia, HDP or customised birthweight of both twins <3rd centile, to controls. To this end, the entire cohort (n=275) was divided into 2 groups

based on the woman's gestational age at time of her enrolment to the study and hence gestational age at time of sampling of maternal plasma PlGF; <24 weeks' gestation and ≥ 24 weeks' gestation. Just under half the cohort (43.6%; n=120) were recruited at <24 weeks' gestation with the remainder (56.4%, n=155) recruited at ≥ 24 gestational weeks. The groups were then stratified by presence of preeclampsia, HDP or customised birthweight <3rd centile for both infants.

Demographics of Entire Cohort

The maternal age of the study group ranged from 20 to 50 years, with 134 women (48.7%) aged >35 years at booking. The majority of the cohort had a Body Mass Index (BMI) of <30Kg/m^2 at booking (78.9%; n=217) and were of Caucasian ethnicity (93.8%; n=258). Just under half the cohort were nulliparous (46.9%; n=129). The majority of the group were dichorionic twin pregnancies (81.5%; n=224) and approximately two thirds (65.5%; n=180) of the population studied had conceived the twin pregnancy spontaneously. Where assisted reproductive therapy (ART) was utilised, almost a fifth (17.1%; n=47) had conceived through the assistance of In Vitro Fertilisation (IVF) and a large proportion of these using a donor oocyte (12%; n=33). There was a small number of women with pre-existing renal disease or hypertension (1.8%; n=5). The two gestational groups were well matched, with no differences seen in BMI <30, ethnicity, parity or chorionicity. However, there were significantly more women with ART assisted pregnancies (40.2%; n=48 v 27.8%; n=42, p=0.04), oocyte donation (18.5%; n=22 v 7.3%; n=11, p=0.009) and those with a maternal age >35 years (57.5%; n=69 v 41.9%; n=65, p=0.01) sampled in the <24 weeks' gestational group compared to the ≥ 24 weeks group (Supplemental Material Table 3).

Clinical Outcomes

Overall, the incidence of a subsequent diagnosis of HDP was 15.3% (n=42) and of these 11.3% (n=31) developed preeclampsia (Supplemental Material Table 4). Of the 532 infants with maternal BMI information available, 11.8% (n=65) had a customised birthweight <3rd centile with both twins <3rd customised birthweight in twelve cases. Gestation at delivery ranged from 23 to 38 weeks' gestation, with two thirds of the cohort delivered via Caesarean section (66.5%, n=183). Pre-term delivery at <35 weeks occurred in almost a fifth of the cohort (17.8%, n=49) and in over half of cases was iatrogenic (59.2%, n=20). Preterm delivery at <32 weeks was less common (6.9%, n=19), and again half of cases were iatrogenic (47.4%, n=9). There were no significant differences between the two gestational groups in terms of incidence of HDP or preeclampsia, nor were there any differences in pre-term delivery or mode of delivery.

Comparison of PlGF

The median PlGF was 230.5 pg/mL when sampling occurred at <24 weeks and 276 pg/mL when sampling was ≥24 weeks. The cohort was then stratified by subsequent diagnosis of preeclampsia, HDP or customised birthweight <3rd centile in both twins. PlGF levels were 0.6 times higher in the controls than in the women who later developed preeclampsia at <24 weeks gestation *(247 pg/ml vs 153 pg/ml)* and 2.0 times higher at >24 weeks gestation (*304 pg/ml vs 99.8 pg/ml.)*(Table 2). In those that subsequently developed any form of HDP, PlGF was 0.7 times higher in the >24 weeks group (*250 pg/ml vs 150 pg/ml)* (Table 3). In those that subsequently had either twin born at a birthweight <3rd customised centile, PlGF was 0.8 times higher in the group recruited <24 weeks *(170 pg/ml vs 304pg/ml)*(Table 4).

Discussion:

Main Findings

This study demonstrates that maternal plasma PlGF in twin pregnancy follows the same gestational pattern as described in singletons (38, 39); a steady rise corresponding with development of the placenta, peaking slightly earlier at approximately 25 weeks' gestation, and then declining thereafter. It also shows that maternal plasma PlGF is significantly lower in twin pregnancies that will later develop preeclampsia but not other HDP, independent of gestational age at time of sampling of PlGF, compared to controls. PlGF was also noted to be lower in those babies with a customised birthweight <3rd centile when maternal sampling occurred after 24 weeks gestation.

Interpretation

To our knowledge, this is the largest prospective study of PlGF in twin pregnancy from a single site. This allows us to describe the twin pregnancy specific distribution of gestational PlGF, as well as develop a dichorionic specific reference range for PlGF in twin pregnancy, which has not been previously described. This is also the only study to date examining PlGF in twin pregnancies specifically using the Triage® PlGF test. The Triage® PlGF test is currently the only point of care test on the market for measuring PlGF, is CE marked and has been endorsed by NICE for use in further research (21).

Previous studies of angiogenic factors in twin pregnancy have had limited numbers of participants, varied gestations at quantification, varied outcome measures and often involve pooled results from a number of sites or countries across a variety of time periods (40-42). Often these studies require shipment of specimens to laboratories in

other countries, which may affect the quality of samples. In contrast, all of the laboratory analysis in our study was performed on site, by a single researcher, in a single freeze thaw cycle, to minimise the chance of protein denaturation.

A Spanish study in 2011 examined first trimester levels of circulating angiogenic factors in 61 women with a twin pregnancy (40). Using a R&D systems immunoassay, they reported higher serum concentrations of both PlGF and sFlt-1 in twins compared to matched singletons. They also reported maternal serum sFlt-1 levels were higher in twin pregnancies conceived through ART compared to spontaneous twin conceptions, supporting the well-accepted concept that ART pregnancies are at increased risk of preeclampsia development.

A study from Boston in 2012 (41) described 79 women with a twin pregnancy presenting with suspected preeclampsia in the third trimester. Serum PlGF and sFlt-1 from the women was quantified using the Roche Elecsys immunoassay Ratio test. The outcome measure utilised was the diagnosis of an adverse clinical event in the subsequent fortnight, of which 52 women met the criteria. The authors reported median PlGF was significantly reduced, while median sFlt-1 was elevated in those that did develop an adverse event indicating that these angiogenic factors have potential utility as prognostic indicators in twin pregnancies with suspected preeclampsia.

A German group in 2014 published on a small cohort of 49 women with a twin pregnancy, 18 of which developed preeclampsia. Maternal serum PlGF and sFlt-1 was quantified again using the Roche Elecsys immunoassay Ratio test. The researchers reported PlGF levels were decreased and sFlt-1 levels increased in the preeclampsia cases at time of presentation with preeclampsia symptoms compared to the twin

controls, indicating the potential for integration of angiogenic factors into clinical care pathways for investigation of suspected preeclampsia in twin pregnancy (42).

Clearly, potential exists for use of PlGF and sFlt-1 as biomarkers for prediction of preeclampsia in twin pregnancies. However, before these biomarkers are introduced into clinical use for twins, it is important that relevant cut-offs are developed and validated specifically for this group and specific to each PlGF platform. Differences in PlGF results may arise between commercially available platforms owing to measurement of different PlGF isoforms by each assay gene (43). The Triage immunoassay predominantly measures PlGF Isoform-1, the R&D System assay detects PIGF-2 and PIGF-3 isoforms in addition to PlGF-1 while the Roche is a ratio of PlGF to sFLT-1 (44, 45).

Several large prospective observational studies have published on clinically relevant cut-offs for use in singletons. The PROGNOSIS study, using the Roche Elecsys immunoassay Ratio test in 550 women with suspected pre-eclampsia, reported a sFlt-1:PlGF ratio of ≤38 as having a negative predictive value for preeclampsia in singletons in the subsequent 7 days of 99.3% (46). The PELICAN study, using the Triage® PlGF test in 625 women with suspected pre-eclampsia, reported a PlGF of >100 pg/ml as having a 98% negative predictive value for preeclampsia in the subsequent 14 days in singletons presenting at < 35 weeks' gestation (47).

A 2018 Dutch study compared PlGF and sFlt-1 levels in normotensive and pre-eclamptic singleton and twin pregnancies using the Roche Elecsys immunoassay Ratio test (48). Numbers were small, with only 22 twin pregnancies included. Again, differences in serum sFlt-1 and PlGF levels were noted in the normotensive twins compared to the matched singletons and in the pre-eclamptic twin cases compared to

the twin controls. Importantly, they demonstrated that the previously defined sFlt-1/PlGF ratio cut-off of ≤38 for predicting short-term absence of preeclampsia in singleton pregnancies is not applicable to twin pregnancies. Importantly this demonstrates that established reference ranges for PlGF/sFlt-1 in singletons are not transferrable to twin or higher order multiple pregnancies. This highlights the need for quality prospective observational studies of women with twin pregnancy presenting with suspected preeclampsia, in order to develop and validate clinically useful cut-offs for PlGF/sFlt-1 in twins.

It appears there may be a role for PlGF in identification of pathologically related growth restriction in twin pregnancy, given the lower PlGF levels we observed in the maternal plasma of those women that later went on to deliver babies <3rd CBW centile. Studies in singleton pregnancies have demonstrated the utility of PlGF in discriminating pathological growth restriction from constitutional smallness and in the prediction of adverse perinatal outcomes (49, 50).

Strengths and Limitations.

We recognise there are limitations to our study specifically the use of a customised birthweight centile not specific to twin pregnancy and the exclusion of cases where only both twins were <3rd customised centile. This choice was pragmatic given our numbers however we recognise that reduced placental volume in either twin may affect the circulating maternal PlGF levels. Normal twin growth patterns are the subject of much debate with differing opinion as to which is the most appropriate growth curve to use in clinical practice (51, 52). Concerns exist that twin specific growth charts, adjusted to reflect the smallness of twins compared to singletons, may not identify growth restricted twins with underlying placental pathology, thereby resulting in

increased perinatal morbidity (53). There is no consensus as to whether fetal growth charts should be customised by factors such as ethnicity, height, weight and parity or not and there is also no agreement regarding which is the most appropriate growth calculator to use (54-59).

A second limitation of the study was single sampling of participants. Serial sampling of maternal PlGF may have provided a much more robust, informative account of PlGF distribution. However repeated phlebotomy, solely for the purposes of a research study, may have deterred many women from taken part and given that participation was truly altruistic, a single timepoint only approach was adopted. Our population is largely homogenous; white Caucasian and non-obese which potentially limits extrapolation to minority ethnic groups however this is representative of the twin pregnancy population that attends our unit. Although a large number of women with a twin pregnancy were enrolled, we do not have sufficient power at present to develop a monochorionic twin pregnancy specific reference range, although it would be possible to expand on the study and add to our numbers in the future to achieve this. An additional limitation of our study is the use of only one automated commercial platform for quantification of PlGF alone and not s-FLT1, rather than on multiple commercially available platforms such as the DELFIA Xpress PlGF 1-2-3 test, Brahms Kryptor and the Roche Elecsys ratio test, as advocated by NICE (21). Comparative studies performed in singleton pregnancies have shown similar performance of all three platforms in ability to rule out preeclampsia (60). As sufficient plasma remains biobanked in our site, this is an area for potential future research subject to funding and ethical approval.

Conclusion

We have shown that PlGF levels in twin pregnancy differ between those pregnancies that later will be complicated by preeclampsia and those that will not. This difference is present many weeks before clinical signs or symptoms of disease are present. We provide a valid overall reference range for PlGF in a normal twin pregnancy and specifically in a normal dichorionic twin pregnancy. With further research, PlGF has potential as an adjunct to clinical care as a predictor of evolving preeclampsia and/or adverse clinical outcomes in twin pregnancy.

Financial Disclosure

All authors report no financial disclosures

Conflict of Interest

All authors report no conflicts of interest

Acknowledgements

The authors would like to thank the women who participated in this study, generously donated their time and making the study possible.

Contribution to Authorship

All authors contributed to the overall study design and specific methodologies. KOD and LK conceived and designed the study with DHR. DHR conducted the data collection with assistance from CN and EOM. DHR conducted the analysis with assistance from SM and AF. DHR drafted the manuscript with assistance from SM and

KOD. All authors have critically read, contributed with inputs and revisions and approved the final manuscript**.**

Details of Ethics Approval

Ethical approval for the study was granted from the Cork Research Ethics committee (ECM 3 (PPP) 19/05/15).

Funding

The Irish Centre for Maternal and Child Health Research (INFANT), Cork, Ireland provided financial support for this study. The funder had no role in the study design; collection, analysis or interpretation of data; or in the writing of the report; or in the decision to submit the article for publication.

References

1. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive Disorders of Pregnancy. Hypertension. 2018;72(1):24-43.
2. Duley L. The Global Impact of Pre-eclampsia and Eclampsia. Seminars in Perinatology. 2009;33(3):130-7.
3. Khan KS, Wojdyla D, Say L, Gülmezoglu AM, Van Look PFA. WHO analysis of causes of maternal death: a systematic review. The Lancet. 2006;367(9516):1066-74.
4. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet. 2005;365(9461):785-99.
5. Steegers EAP, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. The Lancet. 2010;376(9741):631-44.
6. Sibai BM, Hauth J, Caritis S, Lindheimer MD, MacPherson C, Klebanoff M, et al. Hypertensive disorders in twin versus singleton gestations. American Journal of Obstetrics and Gynecology. 2000;182(4):938-42.
7. Ananth CV, Chauhan SP. Epidemiology of twinning in developed countries. Semin Perinatol. 2012;36(3):156-61.
8. Prapas N, Kalogiannidis I, Prapas I, Xiromeritis P, Karagiannidis A, Makedos G. Twin gestation in older women: antepartum, intrapartum complications, and perinatal outcomes. Archives of gynecology and obstetrics. 2006;273(5):293-7.
9. Central Statistics Office 2016 [Available from: www.cso.ie.
10. Martin JA, Park MM. Trends in twin and triplet births: 1980-97. National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System. 1999;47(24):1-16.
11. Office for National Statistics. Births: maternities with multiple births: rates per 1 m, age of mother, 1938e2004, all maternities. London: ONS; 2006.; 2017.
12. Smith KE, Ravikumar N, Hession M, Morrison JJ. Trends in the obstetric features and management of twin pregnancies. Irish medical journal. 2010;103(3):70-2.
13. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction hypertension, and proteinuria in preeclampsia. Journal of Clinical Investigation. 2003;111(5):649-58.
14. Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? J Clin Invest. 1997;99(9):2152-64.
15. Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, et al. First Trimester Placental Growth Factor and Soluble Fms-Like Tyrosine Kinase 1 and Risk for Preeclampsia. Journal of Clinical Endocrinology and Metabolism. 2004;89(2):770-5.
16. Koga K, Osuga Y, Yoshino O, Hirota Y, Ruimeng X, Hirata T, et al. Elevated serum soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) levels in women with preeclampsia. The Journal of clinical endocrinology and metabolism. 2003;88(5):2348-51.
17. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA. Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. Am J Obstet Gynecol. 2003;188(1):177-82.
18. Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG. Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. Proceedings of the National Academy of Sciences. 1991;88(20):9267-71.
19. Chappell LC, Seed PT, Briley A, Kelly FJ, Hunt BJ, Charnock-Jones DS, et al. A longitudinal study of biochemical variables in women at risk of preeclampsia. Am J Obstet Gynecol. 2002;187(1):127-36.
20. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating Angiogenic Factors and the Risk of Preeclampsia. New England Journal of Medicine. 2004;350(7):672-83.
21. NICE. PlGF-based testing to help diagnose suspected pre-eclampsia (Triage PlGF test, Elecsys immunoassay sFlt-1/PlGF ratio, DELFIA Xpress PlGF 1-2-3 test, and BRAHMS sFlt-1 Kryptor/BRAHMS

PlGF plus Kryptor PE ra 2016 [Diagnostics guidance [DG23] Published date: May 2016:[Available from: https://www.nice.org.uk/guidance/dg23/chapter/1-Recommendations.
22. Ireland P. PARROT Ireland 1997 [Available from: https://parrotireland.medscinet.com/.
23. Cerdeira AS, O'Sullivan J, Ohuma EO, Harrington D, Szafranski P, Black R, et al. Randomized Interventional Study on Prediction of Preeclampsia/Eclampsia in Women With Suspected Preeclampsia: INSPIRE. Hypertension (Dallas, Tex : 1979). 2019;74(4):983-90.
24. Duhig KE, Myers J, Seed PT, Sparkes J, Lowe J, Hunter RM, et al. Placental growth factor testing to assess women with suspected pre-eclampsia: a multicentre, pragmatic, stepped-wedge cluster-randomised controlled trial. Lancet (London, England). 2019.
25. Ruiz-Sacedon N, Perales-Puchalt A, Borras D, Gomez R, Perales A. Angiogenic growth factors in maternal and fetal serum in concordant and discordant twin pregnancies. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2014;27(9):870-3.
26. Nevo O, Many A, Xu J, Kingdom J, Piccoli E, Zamudio S, et al. Placental expression of soluble fms-like tyrosine kinase 1 is increased in singletons and twin pregnancies with intrauterine growth restriction. The Journal of clinical endocrinology and metabolism. 2008;93(1):285-92.
27. Bdolah Y, Lam C, Rajakumar A, Shivalingappa V, Mutter W, Sachs BP, et al. Twin pregnancy and the risk of preeclampsia: bigger placenta or relative ischemia? American Journal of Obstetrics and Gynecology. 2008;198(4).
28. Maynard SE, Moore Simas TA, Solitro MJ, Rajan A, Crawford S, Soderland P, et al. Circulating angiogenic factors in singleton vs multiple-gestation pregnancies. Am J Obstet Gynecol. 2008;198(2):200.e1-7.
29. Boucoiran I, Thissier-Levy S, Wu YQ, Wei SQ, Luo ZC, Delvin E, et al. Risks for Preeclampsia and Small for Gestational Age: Predictive Values of Placental Growth Factor, Soluble fms-like Tyrosine Kinase-1, and Inhibin A in Singleton and Multiple-Gestation Pregnancies. American journal of perinatology. 2013;30(7):607-12.
30. Kusanovic JP, Romero R, Espinoza J, Nien JK, Kim CJ, Mittal P, et al. Twin-to-twin transfusion syndrome: an antiangiogenic state? American Journal of Obstetrics and Gynecology. 2008;198(4).
31. Yinon Y, Ben Meir E, Berezowsky A, Weisz B, Schiff E, Mazaki-Tovi S, et al. Circulating angiogenic factors in monochorionic twin pregnancies complicated by twin-to-twin transfusion syndrome and selective intrauterine growth restriction. American Journal of Obstetrics and Gynecology. 2014;210(2).
32. Fox CE, Sekizawa A, Pretlove SJ, Chan BC, Okai T, Kilby MD. Maternal cell-free messenger RNA in twin pregnancies: the effects of chorionicity and severe twin to twin transfusion syndrome (TTTS). Acta Obstet Gynecol Scand. 2012;91(10):1206-11.
33. Faupel-Badger JM, McElrath TF, Lauria M, Houghton LC, Lim KH, Parry S, et al. Maternal circulating angiogenic factors in twin and singleton pregnancies. American Journal of Obstetrics and Gynecology. 2015;212(5).
34. Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, et al. Early Pregnancy Prediction of Preeclampsia in Nulliparous Women, Combining Clinical Risk and Biomarkers. Hypertension. 2014;64(3):644-52.
35. NICE. Hypertension in Pregnancy 2010 [Available from: https://www.nice.org.uk/guidance/cg107.
36. Weight GRO. [Available from: https://www.gestation.net/cc/about.htm.
37. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. International Journal of Epidemiology. 1999;28(5):964-74.
38. Knudsen UB, Kronborg CS, von Dadelszen P, Kupfer K, Lee SW, Vittinghus E, et al. A single rapid point-of-care placental growth factor determination as an aid in the diagnosis of preeclampsia. Pregnancy Hypertens. 2012;2(1):8-15.

39. Saffer C, Olson G, Boggess KA, Beyerlein R, Eubank C, Sibai BM, et al. Determination of placental growth factor (PlGF) levels in healthy pregnant women without signs or symptoms of preeclampsia. Pregnancy Hypertension-an International Journal of Womens Cardiovascular Health. 2013;3(2):124-32.
40. Sanchez O, Llurba E, Marsal G, Dominguez C, Aulesa C, Sanchez-Duran MA, et al. First trimester serum angiogenic/anti-angiogenic status in twin pregnancies: relationship with assisted reproduction technology. Human Reproduction. 2012;27(2):358-65.
41. Rana S, Hacker MR, Modest AM, Salahuddin S, Lim KH, Verlohren S, et al. Circulating Angiogenic Factors and Risk of Adverse Maternal and Perinatal Outcomes in Twin Pregnancies With Suspected Preeclampsia. Hypertension. 2012;60(2):451-8.
42. Droge L, Herraiz I, Zeisler H, Schlembach D, Stepan H, Kussel L, et al. Maternal serum sFlt-1/PlGF ratio in twin pregnancies with and without pre-eclampsia in comparison with singleton pregnancies. Ultrasound in Obstetrics & Gynecology. 2015;45(3):286-93.
43. Cao YH, Ji WDR, Qi P, Rosin A, Cao YM. Placenta growth factor: Identification and characterization of a novel isoform generated by RNA alternative splicing. Biochemical and Biophysical Research Communications. 1997;235(3):493-8.
44. Alere. 2013 [Available from: https://www.quidel.com/immunoassays/triage-test-kits/triage-plgf-test.
45. R&D. 2016 [Available from: https://www.rndsystems.com/products/human-plgf-quantikine-elisa-kit_dpg00.
46. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennstrom M, et al. Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia. New England Journal of Medicine. 2016;374(1):13-22.
47. Chappell LC, Duckworth S, Seed PT, Griffin M, Myers J, Mackillop L, et al. Diagnostic Accuracy of Placental Growth Factor in Women With Suspected Preeclampsia A Prospective Multicenter Study. Circulation. 2013;128(19):2121-31.
48. Saleh L, Tahitu SIM, Danser AHJ, van den Meiracker AH, Visser W. The predictive value of the sFlt-1/PlGF ratio on short-term absence of preeclampsia and maternal and fetal or neonatal complications in twin pregnancies. Pregnancy Hypertens. 2018;14:222-7.
49. Anderson N, De Laat M, Benton S, von Dadelszen P, McCowan L. Placental growth factor as an indicator of fetal growth restriction in late-onset small-for-gestational age pregnancies. The Australian & New Zealand journal of obstetrics & gynaecology. 2019;59(1):89-95.
50. Benton SJ, McCowan LM, Heazell AE, Grynspan D, Hutcheon JA, Senger C, et al. Placental growth factor as a marker of fetal growth restriction caused by placental dysfunction. Placenta. 2016;42:1-8.
51. Ghi T, Prefumo F, Fichera A, Lanna M, Periti E, Persico N, et al. Development of customized fetal growth charts in twins. American Journal of Obstetrics & Gynecology. 2017;216(5):514.e1-.e17.
52. Kalafat E, Sebghati M, Thilaganathan B, Khalil A, Collaborative tSTOR. Predictive accuracy of Southwest Thames Obstetric Research Collaborative (STORK) chorionicity-specific twin growth charts for stillbirth: a validation study. Ultrasound in Obstetrics & Gynecology. 2019;53(2):193-9.
53. Lockie E, McCarthy E, Hui L, Churilov L, Walker S. Feasibility of using self-reported ethnicity in pregnancy according to the gestation-related optimal weight classification: a cross-sectional study. BJOG: An International Journal of Obstetrics & Gynaecology. 2018;125(6):704-9.
54. Collaboration NCDRF. A century of trends in adult human height. Elife. 2016;5:e13410.
55. Smith G, Shah I, White I, Pell J, Crossley J, Dobbie R. Maternal and biochemical predictors of antepartum stillbirth among nulliparous women in relation to gestational age of fetal death. BJOG: An International Journal of Obstetrics & Gynaecology. 2007;114(6):705-14.
56. Broere-Brown ZA, Baan E, Schalekamp-Timmermans S, Verburg BO, Jaddoe VWV, Steegers EAP. Sex-specific differences in fetal and infant growth patterns: a prospective population-based cohort study. Biol Sex Differ. 2016;7:65-.

57. Altman DG, Chitty LS. Charts of fetal size: 1. Methodology. British journal of obstetrics and gynaecology. 1994;101(1):29-34.
58. Papageorghiou AT, Ohuma EO, Gravett MG, Hirst J, da Silveira MF, Lambert A, et al. International standards for symphysis-fundal height based on serial measurements from the Fetal Growth Longitudinal Study of the INTERGROWTH-21st Project: prospective cohort study in eight countries. Bmj. 2016;355:i5662.
59. Kiserud T, Piaggio G, Carroli G, Widmer M, Carvalho J, Neerup Jensen L, et al. Correction: The World Health Organization Fetal Growth Charts: A Multinational Longitudinal Study of Ultrasound Biometric Measurements and Estimated Fetal Weight. PLoS medicine. 2017;14(3):e1002284.
60. McCarthy FP, Gill C, Seed PT, Bramham K, Chappell LC. Comparison of three commercially available placental growth factor-based tests in women with suspected preterm pre-eclampsia: the COMPARE study. 2019;53(1):62-7.

Figure Legend

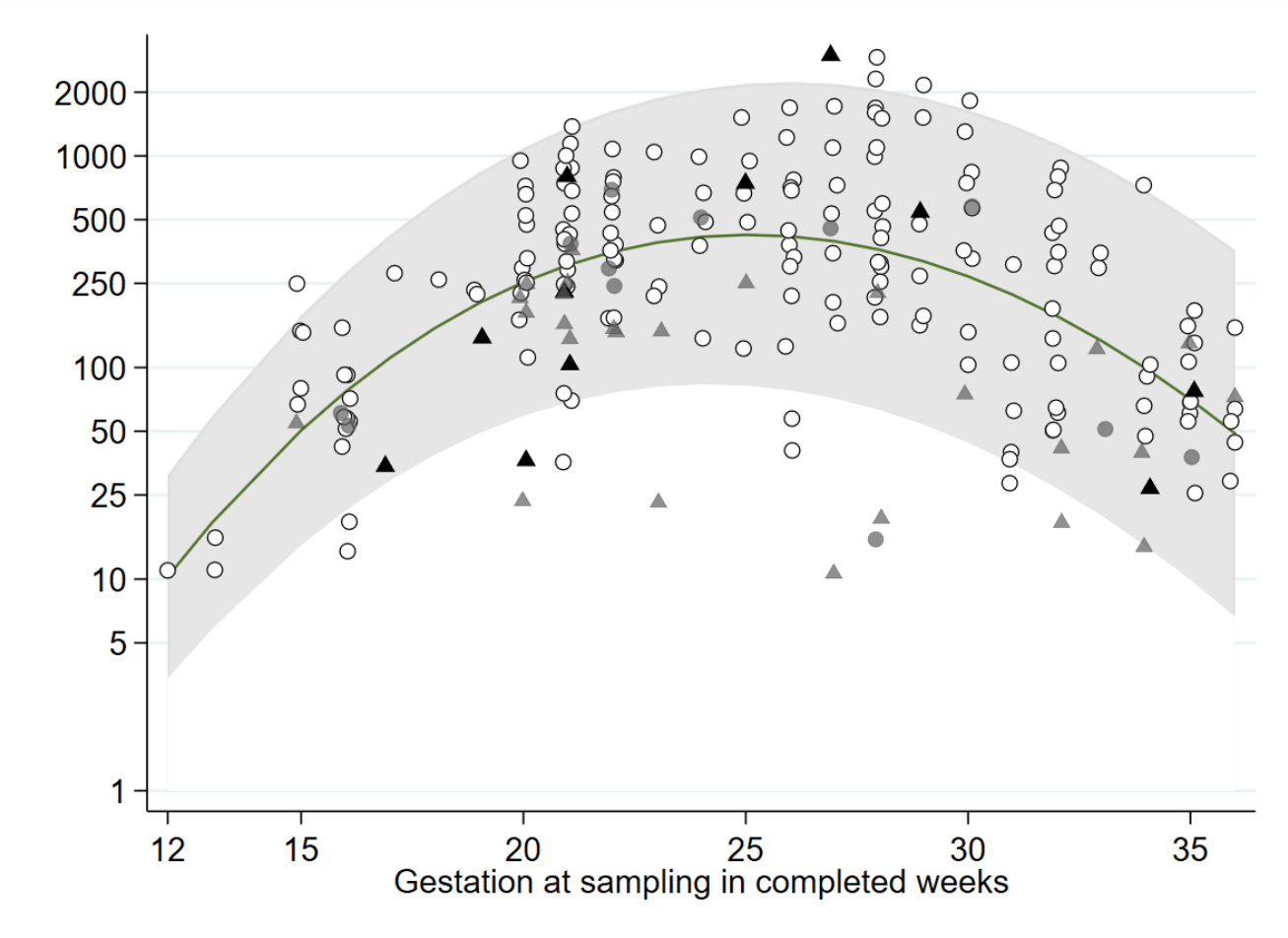


Figure 1: Scatter plot of gestational PlGF. Shaded area represents the reference range from the 5th to 95th percentiles (n=222).

○ uncomplicated dichorionic twin pregnancy cohort, ▲HDP and PET present, ▲ HDP present, ● both neonates <3rd CBW

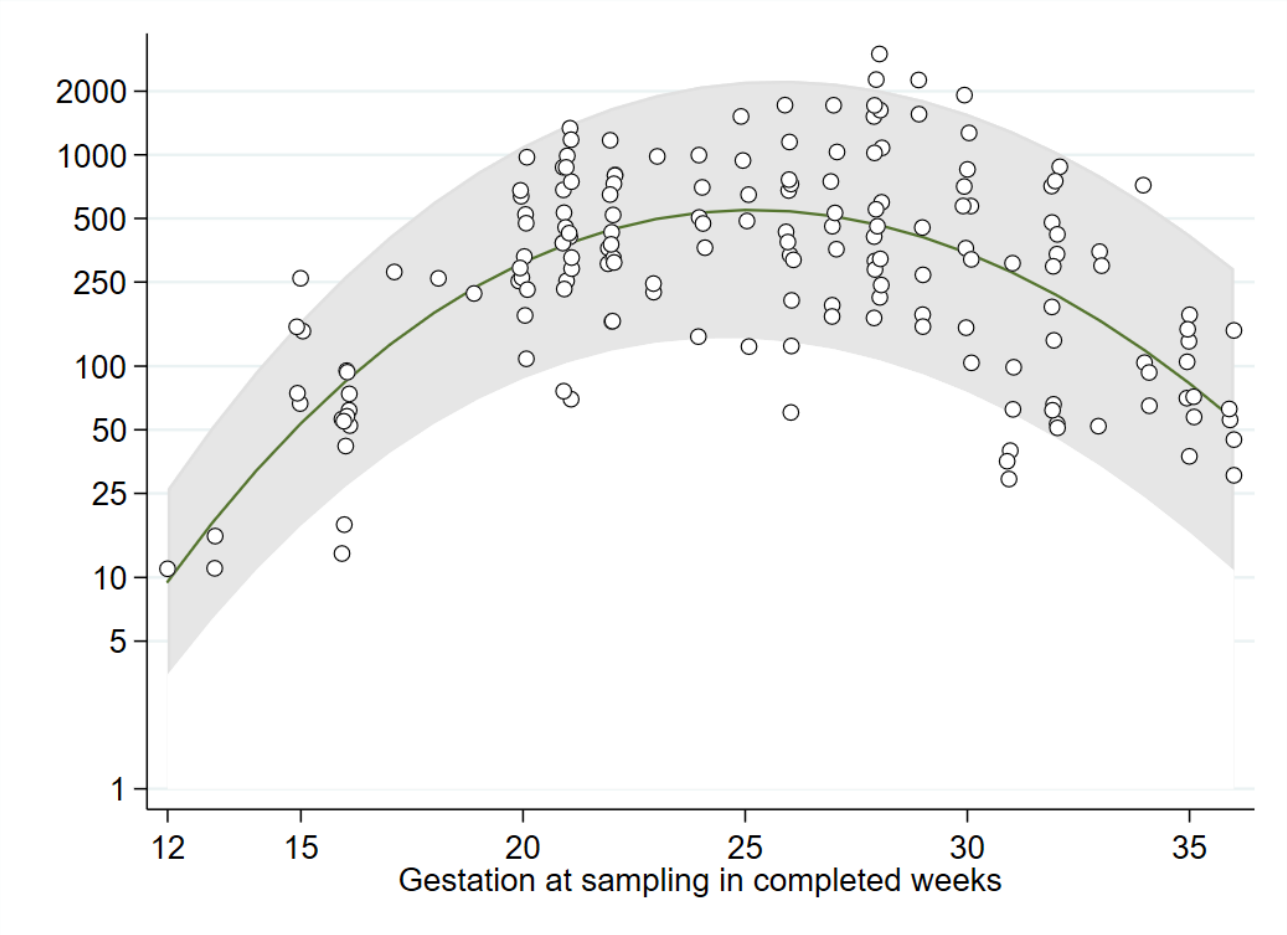


Figure 2: Scatter plot of gestational PlGF for the uncomplicated dichorionic twin pregnancy cohort. Shaded area represents the reference range from the 5th to 95th percentiles (n=173).

Table 1: Normal Reference Range percentiles of PlGF by gestational age interval quantified using the Triage® PlGF test (n=173)

Gestational age at sampling (weeks)	**Percentile of PlGF (pg/ml)**								
	3rd	**5th**	**10th**	**25th**	**50th**	**75th**	**90th**	**95th**	**97th**
12	3.0	3.5	4.3	6.3	9.5	14.5	21.0	26.2	30.3
13	5.5	6.3	8.0	11.8	18.1	27.8	40.9	51.5	59.9
14	9.3	10.9	13.8	20.6	32.1	50.0	74.6	94.7	110.7
15	15.0	17.6	22.5	33.9	53.6	84.7	127.9	163.7	192.1
16	22.7	26.8	34.5	52.8	84.5	135.4	206.9	266.6	314.4
17	32.8	38.8	50.3	77.8	126.2	204.8	316.5	410.7	486.5
18	44.8	53.3	69.7	108.9	178.9	293.8	459.3	600.0	713.7
19	58.5	69.8	91.8	145.1	241.1	400.8	633.3	832.7	994.7
20	72.7	87.1	115.3	184.1	309.6	520.7	831.3	1100.0	1319.4
21	86.2	103.8	138.2	223.0	379.3	645.2	1040.7	1385.5	1668.4
22	97.9	118.3	158.5	258.2	444.0	763.7	1244.1	1666.1	2014.0
23	106.4	129.1	173.9	286.0	497.2	864.4	1422.0	1915.3	2324.1
24	110.8	134.9	182.8	303.5	533.3	936.8	1555.7	2107.4	2566.6
25	110.7	135.3	184.3	308.9	548.2	973.0	1630.8	2221.4	2715.2
26	106.2	130.2	178.3	301.6	540.7	969.5	1639.6	2245.4	2754.2
27	97.8	120.4	165.8	282.9	512.1	927.3	1582.1	2178.3	2681.0
28	86.7	107.0	148.1	255.0	466.1	852.1	1466.5	2029.5	2506.3
29	73.9	91.5	127.3	221.0	407.9	752.7	1306.5	1817.3	2251.7
30	60.6	75.4	105.3	184.4	343.4	639.6	1119.5	1565.0	1945.3
31	47.9	59.7	83.9	148.1	278.3	523.1	923.1	1296.8	1617.1
32	36.5	45.6	64.4	114.6	217.3	412.0	732.9	1034.6	1294.1
33	26.8	33.6	47.6	85.4	163.4	312.6	560.6	795.0	997.5
34	19.0	23.9	34.0	61.4	118.5	228.7	413.2	588.7	740.9
35	12.9	16.3	23.4	42.6	82.9	161.3	293.6	420.3	530.5
36	8.5	10.8	15.5	28.5	55.9	109.7	201.3	289.4	366.3

Centiles were calculated based on a Normal distribution for log(PIGFR) where

$\mu = exp\left(-19.8165 + 6.9434 \times \sqrt{G} - 0.01375 \times G^2\right)$

$\sigma = exp\left(-1.5851 + 0.4422 \times log(G)\right)$ *and G = gestational age (weeks)*

Table 2: PlGF by gestational group at enrolment in twin pregnancies complicated by [A] Preeclampsia (de novo or superimposed) compared to those that were not, quantified using the Triage® PlGF test (n=275)

Gestation at recruitment (weeks)	**Median** (IQR) **PlGF pg/mL (n=275)**	**Median** (IQR) **PlGF PE[A] present pg/mL (n=31)**	**Median** (IQR) **PlGF PE not present pg/mL (n=244)**
<24	230.5 (79.4-437.8)	153 (54-224)	247 (81-489)
≥24	276 (71.6-577)	99.8 (24-273)	304 (73-652)

Table 3: PlGF by gestational group at enrolment in twin pregnancies complicated by [B] Hypertensive Disorder of Pregnancy compared to those that were not, quantified using the Triage® PlGF test (n=275)

Gestation at recruitment (weeks)	**Median** (IQR) **PlGF pg/mL (n=275)**	**Median** (IQR) **PlGF HDP[B] present pg/mL (n=42)**	**Median** (IQR) **PlGF HDP not present pg/mL (n=233)**
<24	230.5 (79.4-437.8)	150 (45-229)	250 (84-490)
≥24	276 (71.6-577)	123 (32-425)	304 (73-598)

Table 4: PlGF by gestational group at enrolment in offspring of twin pregnancies complicated by [C] birthweight <3rd customised centile compared to those that were not, quantified using the Triage® PlGF test (n=532)

Gestation at recruitment (weeks)	**Median** (IQR) **PlGF pg/mL (n=532)***	**Median** (IQR) **PlGF pg/mL CBW <3rd[C] (n=109)**	**Median** (IQR) **PlGF pg/mL CBW not <3rd[C] (n=423)**
<24	230.5 (79.4-437.8)	234 (54.2-460.5)	231 (105-413)
≥24	276 (71.6-577)	170 (42.7-462)	304 (73-652)

**BMI not available for 9 women so CWB not available for 18 offspring*