Supplementary material

Supplemental Table S1: Comparison of CONCEPTT participants who gave a sample for the biorepository (n=127) included in this analysis and all trial participants with a livebirth and neonatal outcomes n=225). Data are shown as mean ± SD or n (%); *geometric mean and range. The CGM time in range (TIR), time above range (TAR) and time below range (TBR) were defined according to international recommendations as TIR 3.5-7.8mmol/l (63-140mg/dl) and TBR <3.5 mmol/l (<63mg/dl)(16).

	CONCEPTT Mother/baby dyads; n=225	CONCEPTT Biorepository participants; n=127
BASELINE CHARACTERISTICS		
Maternal age years	31.4 ± 4.5	31.8 ± 4.4
Pre-pregnancy BMI kg/m2	25.8 ± 4.6	25.7 ± 4.6
Duration T1D yrs	16.5 ± 7.7	16.9 ± 7.7
Age of onset of T1D yrs	14.9 ± 8.0	14.9 ± 7.9
Insulin Pump %	110/225 (48.9%)	60/127 (47.2%)
Total daily insulin dose at 36 weeks (units per kg)	85.3 ± 39.1	84.4 ± 36.2
GLYCEMIA AT 12 WKS		
HbA1c %	6.9 ± 0.6	6.9 ± 0.6
HbA1c mmol/mol	51.8 ± 6.6	51.5 ± 6.3
Mean CGM glucose mg/dl	134.4 ± 21.0	134.8 ± 21.3
CGM Time in range %	51.7 ± 13.1	51.3 ± 13.0
CGM Time below range %	8.5 ± 7.1	8.8 ± 6.5
GLYCEMIA AT 24 WKS		
HbA1c %	6.3 ± 0.6	6.3 ± 0.6
HbA1c mmol/mol	45.6 ± 6.8	45.2 ± 6.8
Mean CGM glucose mg/dl	138.5 ± 22.9	137.9 ± 21.8
CGM Time in range %	51.1 ± 15.2	50.9 ± 15.1
CGM Time below range %	5.2 ± 5.3	5.5 ± 5.5
GLYCEMIA AT 34 WKS		
HbA1c 34w %	6.4 ± 0.6	6.4 ± 0.6
HbA1c 34w mmol/mol	46.7 ± 6.8	46.4 ± 6.6
Mean CGM glucose mg/dl	123.5 ± 18.7	123.6 ± 16.8
CGM Time in range 34w %	64.4 ± 14.7	64.7 ± 14.1
CGM Time below range %	5.1 ± 5.0	4.9 ± 4.8
PREGNANCY OUTCOMES		
Caesarean section	155/225 (68.9%)	86/127 (67.7%)
Vaginal delivery	51/225 (22.7%)	31/127 (24.4%)
Large for gestational age	139/225 (61.8%)	83/127 (65.4%)
Respiratory Distress	19/225 (8.4%)	6/127 (4.7%)
Neonatal hypoglycemia	57/225 (25.3%)	27/127 (21.3%)
NICU admission	83/225 (36.9%)	37/127 (29.1%)
Hyperbilirubinaemia	62/225 (27.6%)	31/127 (24.4%)
Cord blood C-peptide pmol/l	n=140 (62.2%); 836 (55-4965)*	n=85 (66.9%); 802 (55-4965)*

Supplemental Table S2: Associations of maternal and neonatal outcomes with maternal serum C-peptide pattern. Only results meeting $p \leq 0.05$ have been included. Results which show a trend ($p > 0.05$ & $p < 0.10$) are included in parentheses. Mean ± standard deviation. Pattern 1: undetectable maternal C-peptide throughout pregnancy. Pattern 2: detectable maternal C-peptide at 12 weeks' gestation. Pattern 3: Undetectable maternal C-peptide at 12 and 24 weeks which became detectable at 34 weeks' gestation. Data are shown as mean ± SD or n (%); *geometric mean and range. The CGM time in range (TIR), time above range (TAR) and time below range (TBR) were defined according to international recommendations as TIR 3.5-7.8mmol/l (63-140mg/dl) and TBR <3.5 mmol/l (<63mg/dl)(16).

	PATTERN 1 Undetectable C-peptide n=74 (58.3%)	PATTERN 2 Detectable C-peptide n=22 (17.3%)	PATTERN 3 C-peptide detectable at 34w only n=31 (24.4%)	P1 vs 2	P1 vs 3	P2 vs 3
BASELINE CHARACTERISTICS						
Maternal age years	31.8 ±4.4	33.1 ±3.8	30.8 ±4.9	ns	ns	ns (0.065)
Pre-pregnancy BMI kg/m^2	26.6 ±4.9	23.6 ±2.5	24.9 ±4.6	0.006	ns	
Duration T1D yrs	18.6 ±7.8	10.6 ±5.7	17.3 ±6.7	<0.001	ns	<0.001
Age of onset of T1D yrs	13.2 ±7.0	22.5 ±7.4	13.5 ±7.0	<0.001	ns	<0.001
Insulin Pump %	36/74 (48.7%)	9/22 (40.9%)	15/31 (48.4%)	ns	ns	ns
Total daily insulin dose at 36 weeks (units per kg)	87.4 ± 41.8	77.3 ± 22.9	82.4 ± 28.5	ns	ns	ns
GLYCEMIA AT 12 WKS						
HbA1c %	6.9 ±0.6	6.8 ±0.5	6.8 ±0.7	ns	ns	ns
HbA1c mmol/mol	51.9 ±6.3	50.7 ±5.3	51.1 ±7.1	ns	ns	ns
Mean CGM glucose mg/dl	135 ± 21.6	133 ± 19.8	137 ± 21.6	ns	ns	ns
Mean CGM glucose mmol/l	7.5 ±1.2	7.4 ±1.1	7.6 ±1.2	ns	ns	ns
CGM Time in range %	51.1 ±13.1	54.3 ±13.8	49.6 ±12.3	ns	ns	ns
CGM Time above range %	39.7 ± 14.2	38.2 ± 14.7	41.6 ± 15.0	ns	ns	ns
CGM Time below range %	9.3 ±6.5	7.3 ±7.0	8.8 ±6.4	ns	ns	ns
GLYCEMIA AT 24 WKS						
HbA1c %	6.3 ±0.6	6.2 ±0.6	6.4 ±0.7	ns	ns	ns
HbA1c mmol/mol	44.8 ±6.5	44.1 ±6.7	46.8 ±7.2	ns	ns	ns
Mean CGM glucose mg/dl	137 ± 19.8	132 ± 18.0	146 ± 25.2	ns	ns	0.027
Mean CGM glucose mmol/l	7.6 ±1.1	7.3 ±1.0	8.1 ±1.4	ns	ns	0.027
CGM Time in range %	51.9 ±13.9	55.0 ±14.8	45.3 ±16.8	ns	0.050	0.037
CGM Time above range %	42.7 ± 15.3	38.0 ± 14.8	49.8 ± 18.9	ns	ns (0.060)	0.021
CGM Time below range %	5.3 ±4.3	6.8 ±9.0	5.0 ±5.3	ns	ns	ns
GLYCEMIA AT 34 WKS						
HbA1c 34w %	6.4 ±0.6	6.3 ±0.6	6.5 ±0.6	ns	ns	ns
HbA1c 34w mmol/mol	46.1 ±6.6	45.7 ±6.2	47.7 ±7.1	ns	ns	ns
Mean CGM glucose mg/dl	123 ± 14.4	121 ± 18.0	128 ± 19.8	ns	ns	ns
Mean CGM glucose mmol/l	6.8 ±0.8	6.7 ±1.0	7.1 ±1.1	ns	ns	ns
CGM Time in range 34w %	65.2 ±13.7	66.6 ±15.9	62.3 ±13.9	ns	ns	ns
CGM Time above range %	29.1 ± 13.2	29.2 ± 15.6	34.0 ± 14.1	ns	ns	ns
CGM Time below range %	5.7 ±5.2	4.2 ±5.5	3.8 ±3.1	ns	ns	ns

PREGNANCY OUTCOMES						
Caesarean section	48/74 (64.9%)	13/22 (59.1%)	25/31 (80.6%)	ns	ns	ns (0.089)
Vaginal delivery	19/74 (25.7%)	8/22 (36.4%)	4/31 (12.9%)	ns	ns	0.045
Large for gestational age	44/74 (59.5%)	11/22 (50.0%)	28/31 (90.3%)	ns	0.002	<0.001
Respiratory Distress	2/74 (2.7%)	no events	4/31 (12.9%)	ns	0.040	ns (0.083)
Neonatal hypoglycemia	10/74 (13.5%)	4/22 (18.2%)	13/31 (41.9%)	ns	0.001	ns (0.070)
NICU admission	17/74 (23.0%)	6/22 (27.3%)	14/31 (45.2%)	ns	0.023	ns
Hyperbilirubinaemia	16/74 (21.6%)	3/22 (13.6%)	12/31 (38.7%)	ns	ns (0.072)	0.047
CORD C-PEPTIDE	n=46 (62.2%)	n=17 (78.9%)	n=22 (71.0%)	ns	ns	ns
Cord blood C-peptide >75th centile	6/46 (13.0%)	2/17 (11.8%)	11/22 (50.0%)	ns	0.001	0.012
Cord blood C-peptide pmol/l*	718 (172-4551)	570 (160-4518)	1319 (55-4965)	ns	0.007	0.010

Table S3 – Unadjusted logistic regression results comparing outcomes for pregnancies categorised according to maternal serum C-peptide pattern (patterns 1, 2 and 3). Pattern 1: undetectable maternal serum C-peptide throughout pregnancy. Pattern 2: detectable maternal serum C-peptide at 12 weeks' gestation. Pattern 3: Undetectable maternal serum C-peptide at 12 and 24 weeks which became detectable at 34 weeks' gestation.

	Pattern 2 vs Pattern 1 (reference) Odds ratio (95% CI)	Pattern 3 vs Pattern 1 (reference) Odds ratio (95% CI)	Pattern 3 vs Pattern 2 (reference) Odds ratio (95% CI)
Caesarean section	0.78 (0.30 to 2.07), p=0.622	2.26 (0.82 to 6.20), p=0.114	2.88 (0.84 to 9.88), p=0.092
Vaginal delivery	1.65 (0.60 to 4.56), p=0.330	0.43 (0.13 to 1.39), p=0.157	0.26 (0.07 to 1.01), p=0.052
Large for gestational age	0.68 (0.26 to 1.77), p=0.432	6.36 (1.77 to 22.84), p=0.005	9.33 (2.18 to 39.98), p=0.003
Respiratory distress	insufficient events	5.33 (0.92 to 30.82), p=0.061	insufficient events
Neonatal hypoglycemia	1.42 (0.40 to 5.07), p=0.587	4.62 (1.74 to 12.27), p=0.002	3.25 (0.89 to 11.89), p=0.075
Neonatal intensive care admission	1.26 (0.43 to 3.72), p=0.679	2.76 (1.13 to 6.73), p=0.025	2.20 (0.68 to 7.11), p=0.189
Hyperbilirubinemia	0.57 (0.15 to 2.18), p=0.414	2.29 (0.92 to 5.69), p=0.075	4.00 (0.97 to 16.48), p=0.055
Cord C-peptide >75th centile	0.89 (0.16 to 4.90), p=0.892	6.67 (2.01 to 22.09), p=0.002	7.50 (1.38 to 40.88), p=0.020

Figure S1: Total daily insulin doses (units/kg) at 12, 24 and 34 weeks for pregnancies categorised according to maternal serum C-peptide pattern (patterns 1, 2 and 3). Pattern 1: undetectable maternal serum C-peptide throughout pregnancy. Pattern 2: detectable maternal serum C-peptide at 12 weeks' gestation. Pattern 3: Undetectable maternal serum C-peptide at 12 and 24 weeks which became detectable at 34 weeks' gestation.

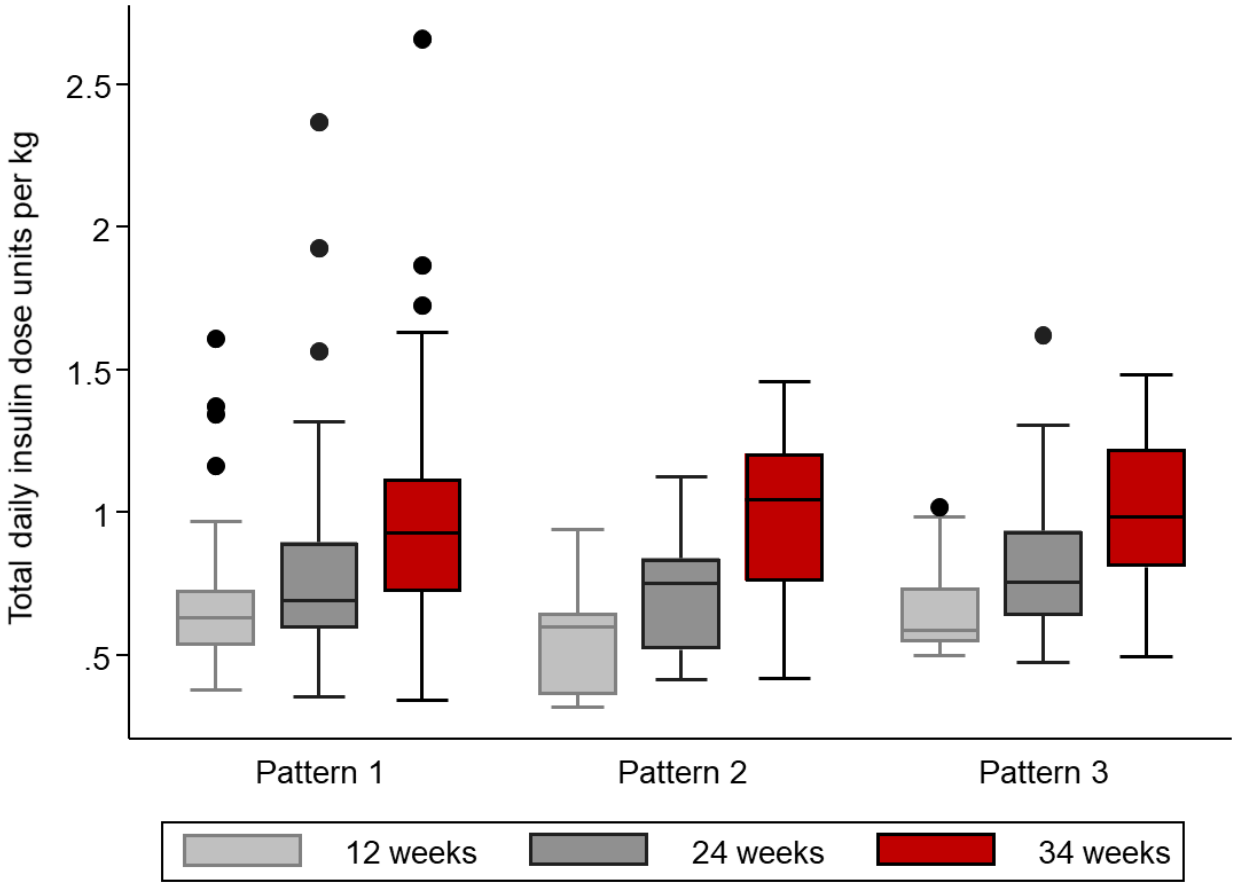
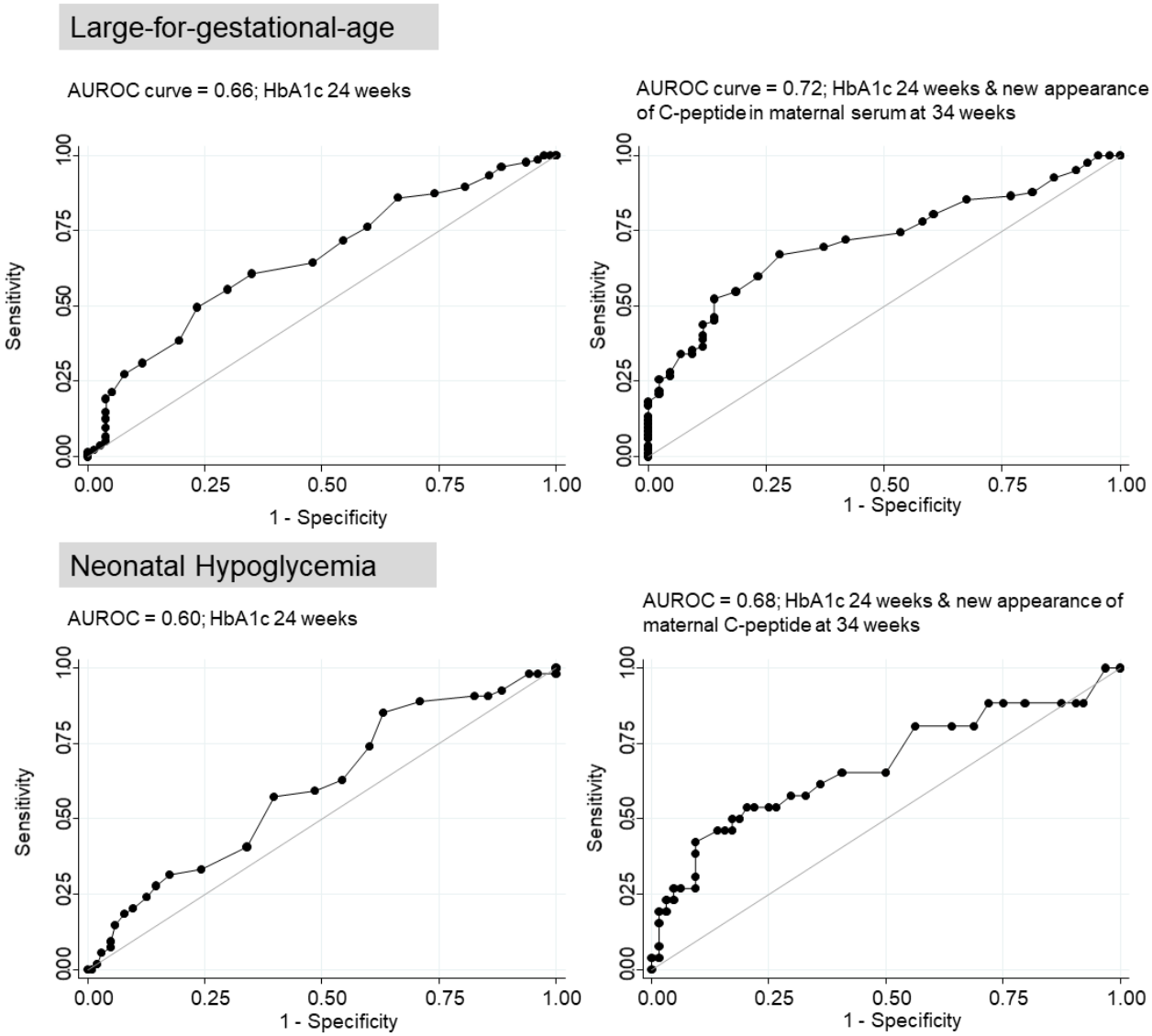


Figure S2: Area under the receiver operator curve (AUROC) for large-for-gestational age and neonatal hypoglycemia. Two models were compared for each outcome. The first model (on the left) used HbA1c at 24 weeks alone, while the second model (shown on the right) used new appearance of maternal serum C-peptide at 34 weeks and HbA1c at 24 weeks. Adding new appearance of maternal C-peptide increased the predictive capacity of the model.



Appendix 1 - CONCEPTT Collaborative Group (listed according to recruitment numbers):

Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK: Helen Murphy, Jeannie Grisoni, Carolyn Byrne, Sandra Neoh, Katy Davenport, (43); Alberta Health Services, University of Calgary, Calgary, Canada: Lois Donovan, Claire Gougeon, Carolyn Oldford, Catherine Young (39); King's College Hospital, London, UK: Stephanie Amiel, Katharine Hunt, Louisa Green, Helen Rogers, Benedetta Rossi (29); Mount Sinai Hospital, Toronto, Canada: Denice Feig, Barbara Cleave, Michelle Strom (22); Hospital de la Santa Creu i Sant Pau, Barcelona, Spain and CIBER-BBN, Zaragoza, Spain: Rosa Corcoy, Alberto de Leiva, Juan María Adelantado, Ana Isabel Chico, Diana Tundidor (22); The Ottawa Hospital General Campus, Ottawa, Canada: Erin Keely, Janine Malcolm, Kathy Henry (15); Ipswich Hospital NHS Trust, Ipswich, UK: Damian Morris, Gerry Rayman, Duncan Fowler, Susan Mitchell, Josephine Rosier (13); Norfolk and Norwich University Hospital, Norwich, UK: Rosemary Temple, Jeremy Turner, Gioia Canciani, Niranjala Hewapathirana, Leanne Piper (13); St. Joseph's Health Centre, London, Canada: Ruth McManus, Anne Kudirka, Margaret Watson (13); Niguarda ca' Granda Hospital, Milano, Italy: Matteo Bonomo, Basilio Pintaudi, Federico Bertuzzi, Giuseppina Daniela Corica, Elena Mion (12); Sunnybrook Health Sciences Centre, Toronto, Canada: Julia Lowe, Ilana Halperin, Anna Rogowsky, Sapida Adib (11); Glasgow Royal Infirmary, Glasgow, UK: Robert Lindsay, David Carty, Isobel Crawford, Fiona Mackenzie, Therese McSorley (10); McMaster University, Hamilton, Canada: John Booth, Natalia McInnes, Ada Smith, Irene Stanton, Tracy Tazzeo (8); Centre hospitalier universitaire de Québec, Quebec City, Canada: John Weisnagel (6); Queen's Medical Centre, Nottingham, UK: Peter Mansell, Nia Jones, Gayna Babington, Dawn Spick (6); Royal Victoria Infirmary, Newcastle Upon Tyne, Newcastle, UK: Malcolm MacDougall, Sharon Chilton, Terri Cutts, Michelle Perkins (6); Leeds Teaching Hospitals NHS Trust, Leeds, UK: Eleanor Scott, Del Endersby (6); Royal Infirmary of Edinburgh, Edinburgh, UK: Anna Dover, Frances Dougherty, Susan Johnston (6); Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK: Simon Heller, Peter Novodorsky, Sue Hudson, Chloe Nisbet (6); Izaak Walton Killam Health Sciences Centre (IWK), Halifax, Canada: Thomas Ransom, Jill Coolen, Darlene Baxendale (5); University Hospital Southampton NHS Foundation Trust, Southampton, UK: Richard Holt, Jane Forbes, Nicki Martin, Fiona Walbridge (6); Galway University Hospitals, Galway, Ireland: Fidelma Dunne, Sharon Conway, Aoife Egan, Collette Kirwin (4); Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK: Michael Maresh, Gretta Kearney, Juliet Morris, Susan Quinn (4); South Tees Hospitals, NHS Foundation Trust, Middlesbrough, UK: Rudy Bilous, Rasha Mukhtar (4); Centre de Recherche du Centre Hospitalier de Université de Montréal (CR-CHUM), Montreal, Canada: Ariane Godbout, Sylvie Daigle (3); The Dudley Group NHS FT, Russells Hall Hospital, Dudley, UK: Alexandra Lubina Solomon, Margaret Jackson, Emma Paul, Julie Taylor (3); Kingston General Hospital, Queen's University, Kingston, Canada: Robyn Houlden, Adriana Breen (3); Guys and St Thomas' NHS Foundation Trust, London, UK: Anita Banerjee, Anna Brackenridge, Annette Briley, Anna Reid, Claire Singh (2); Royal University Hospital, Saskatoon, Canada: Jill Newstead-Angel, Janet Baxter (2); Grampian Diabetes Centre, Aberdeen, UK: Sam Philip, Martyna Chlost, Lynne Murray (2); William Sansum Diabetes Center, Santa Barbara, USA: Kristin Castorino, Lois Jovanovic, Donna Frase (2). The Centre for Clinical Trial Support (CCTS) at the Sunnybrook Research Institute, Toronto, Canada: Sonya Mergler, Kathryn Mangoff, Johanna Sanchez, and Gail Klein. The Jaeb Center for Health Research, Tampa, USA: Katrina Ruedy and Craig Kollman. Juvenile Diabetes Research Foundation (non-clinical collaborators): Olivia Lou and Marlon Pragnell.

Appendix 2– Definitions of outcomes used in the CONCEPTT trial.

Large-for-gestational age (LGA):

Birth weight >90th centile using customised GROW centiles, calculated using version 8 (2017) of the GROW calculator using data about maternal self-reported ethnicity, parity, height, weight, gestational age at birth and neonatal sex [8].

Respiratory distress:

Respiratory difficulties requiring any positive pressure ventilation ≥ 24 hours beyond resuscitation period (10 minutes), and/or given surfactant within 72 hours after birth.

Neonatal hypoglycemia:

A plasma glucose <2.6 mmol/L on one or more occasions, starting at 30-60 minutes after birth, and necessitating intravenous dextrose, within the first 48 hours of life.

Admission to the neonatal intensive care unit (NICU):

Admission to NICU for at least 24 hours.

Hyperbilirubinemia:

Significant jaundice based on bilirubin levels requiring treatment with either phototherapy > 6 continuous hours, or an exchange transfusion, or receiving intravenous gamma globulin or requiring readmission into hospital during the first 7 days of life.

Appendix 3: Calculations related to possible C-peptide transfer across the placenta.

In order to ascertain if the volume of fetal C-peptide synthesis would be likely to result in measurable C-peptide concentrations in maternal serum, we aimed to provide an assessment of this mathematically. Unfortunately, as there are so many unknown variables, it is difficult to do an accurate volume of distribution calculation. However, we have done some provisional modelling suggesting that this is physiologically possible. While lots of important information is missing, the available information we have available suggests the transfer of C-peptide from the fetus could realistically result in measurable maternal plasma C-peptide concentrations in a mother with type 1 diabetes.

Calculations are as follows:

As pregnant women at term have a circulating volume of around 5L, this gives a clearance of 6.93 L/hr: 0.5hr = 0.693 x 5L/Clearance (Equation: t½ = 0.693 × Vd /CL)

As the fetus has a much smaller circulating volume, clearance would be lower at 0.44L/hr: 0.5hr = 0.693 x 0.32L/Clearance (Equation: t½ = 0.693 × Vd /CL)

For the fetus, the rate of production of C-peptide in order to provide a steady state within the blood (k0) is below (ignoring first pass hepatic metabolism). We have used a cord blood concentration of 1300 pmol/l as an example as this is around the median for group 3.

'Infusion' rate K0 = concentration in plasma / clearance. K0 = 1300pmol/l / 0.44 = 2954 pmol/hr

As rate in must equal rate out in order to maintain a steady state, we assume that 2954 pmol/hr could be theoretically available to enter the maternal circulation.

Plasma concentration in mothers (for a steady state infusion) C = k0 / CL = 2954 pmol/hr / 6.93L = 426 pmol/l

In practice, it is likely that transfer between fetus and mother may be 5-50% rather than 100% of available C-peptide in cord blood, resulting in feasible concentrations of 21– 213 pmol/l in the maternal circulation.

We have based our modelling on several assumptions:

- Adult C-peptide is cleared by the kidney and metabolised in the liver. For the fetus, we assumed that there are three potential routes to eliminate C-peptide – renal excretion into the amniotic fluid, hepatic metabolism and transfer to the maternal circulation.
- Previous work in adults suggests the half-life of C-peptide is around 30 minutes. It is unclear how this might change in pregnancy. It is also unclear how this might be different in a fetus. We assumed consistent half-lives of 30 minutes in both mother and fetus, but this is unlikely to be accurate.
- In terms of calculations, the situation is similar to receiving an intravenous infusion. The fetus receives a regular supply of C-peptide from beta cells into the circulation and potentially the mother receives a regular supply of C-peptide from the fetus. Unlike a drug which is injected into the

intravenous compartment, C-peptide will undergo first pass metabolism by the liver, removing a proportion. We have no way of estimating the proportion of secreted C-peptide which might be removed by a fetal liver and have therefore omitted this step, resulting in an unavoidable underestimation of fetal C-peptide production.

- In order to do a much more accurate assessment, further information is needed about the elimination of C-peptide from the fetus, distribution in fetal body fluids and amniotic fluid, clearance rates, and the proportion of peptide which might cross the placenta for accurate assessment of fetal to maternal transfer.

We conclude that while lots of important information is missing, the information we have available suggests the transfer of C-peptide from the fetus could realistically result in measurable maternal plasma C-peptide concentrations in a mother with type 1 diabetes.