Seroconversion to islet autoantibodies between early pregnancy and delivery in non-diabetic mothers.

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Running title: Islet autoantibodies in non-diabetic pregnancy

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Abstract

Islet autoantibodies at diagnosis are currently used to classify type 1 diabetes as they reflect the autoimmune pathogenesis of the disease. The presence of maternal autoantibodies reactive with pancreatic islet antigens is thought to increase the risk for type 1 diabetes in the offspring. The objective of this study was to determine seroconversion to islet autoantibodies in non-diabetic mothers during pregnancy. Screening of 33,682 mothers between September 2000 and August 2004 in the Diabetes Prediction in Skåne (DiPiS) study showed that 242 non-diabetic mothers at delivery had increased islet autoantibody titers of either glutamic acid decarboxylase (GADA), islet antigen-2 (IA-2A) or insulin (IAA), alone or in combination. Inclusion criteria for control mothers were islet autoantibody negative mothers at delivery. Control mothers (n=1419) were randomly selected and matched by age, parity and pregnancy sampling date. Mothers positive for GADA (92%), IA-2A (84%) or IAA (65%) at delivery had increased titers already in early pregnancy. Titers declined for GADA ($p<0.0001$), IA-2A ($p<0.0001$) and IAA ($p<0.0001$). Seroconversion during pregnancy was observed for GADA in 10 (8%), IA-2A in 3 (16%) and IAA in 37 (35%) mothers. It is concluded that non-diabetic mothers during pregnancy with islet autoantibodies at delivery had significantly higher titers during early pregnancy than delivery. As the statistical power in the seroconverting mothers was insufficient, further studies are needed to determine if the risk for type 1 diabetes in the offspring differs between mothers who already had increased titers of islet autoantibodies during early pregnancy or acquired them during pregnancy.

Keywords: Autoimmunity, pregnancy, seroconversion, glutamic acid decarboxylase autoantibody, islet antigen-2 autoantibody, insulin autoantibody

1. Introduction

Several studies suggest that gestational events are important to type 1 diabetes in the offspring, such as enterovirus infections (Dahlquist et al., 1995, Hyoty et al., 1995), blood group incompatibility (AB0) (Dahlquist and Kallen, 1992), preeclampsia (Jones et al., 1998) and high intake of nitrosamine compounds (Helgason and Jonasson, 1981). Even though previous studies have found that these events are associated with increased risk for type 1 diabetes, other studies have shown inconsistent results (Viskari et al., 2002, Stene et al., 2003). Islet autoantibodies at diagnosis are currently used to classify type 1 diabetes as they reflect the autoimmune pathogenesis of the disease (Alberti and Zimmet, 1998, ADA, 2003). Incomplete and sometimes complete beta cell failure has been shown in islet antibody positive patients, whereas a lack of GADA or IA-2A in low titers indicated preservation of beta cell function (Borg et al., 2002).

It is controversial whether islet autoantibodies against glutamic acid decarboxylase (GADA), islet antigen-2 (IA-2A), or insulin (IAA) in cord blood increase the risk for type 1 diabetes (Lindberg et al., 1999, Elfving et al., 2008, Koczwara et al., 2004). As cord blood islet autoantibodies primarily emanate from the mother (Lynch et al., 2008), it was speculated that non-genetic factors may contribute to the development of these islet autoantibodies (Lynch et al., 2008). Alternatively, the mother may have been islet autoantibody positive already when she became pregnant. To answer this question it would be necessary to analyze blood samples obtained from early pregnancy. There is limited information about GADA, IA-2A and, to our knowledge, none about IAA in non-diabetic mothers during pregnancy. A previous study on seroconversion of ICA, GADA and IA-2A during pregnancy showed a tendency of lower titers of all detected islet autoantibodies in samples at delivery compared to the samples from the early pregnancy, but none of the differences were statistically significant (Hamalainen et al., 2001). An additional study investigating patterns of GADA and IA-2A during type 1 diabetes pregnancy illustrated stability in islet autoantibody titers throughout pregnancy (Novak et al., 2000). Contradictory results on autoantibody titers in other autoimmune diseases during pregnancy have been reported as well. Thyroid antibodies were shown to decrease (D'Armiento et al., 1980), SLE associated autoantibodies to increase (Levy, 1982) while other autoantibodies did not change with gestational age (El-Roeiy and Shoenfeld, 1985). In the Diabetes Prediction in Skåne (DiPiS) study (Larsson et al., 2005), cord blood samples as well as serum samples from the mother were obtained at delivery (Lynch et al., 2008) from 35,683 mothers. In the present study, it was possible to obtain early pregnancy samples from islet autoantibody positive mothers along with matched control mothers from the Southern Sweden Microbiological Biobank (SSM-Biobank) (Ryding et al., 2008).

The objective of this study was to utilize samples from the SSM-biobank to test the hypothesis that mothers with islet autoantibodies at delivery developed these during

pregnancy. We therefore determined the end-point titers of GADA, IA-2A and IAA in serum samples from early pregnancy (gestational weeks 10-16) and at delivery from non-diabetic mothers who gave birth to children born with islet autoantibodies and compared them to islet autoantibody negative matched control mothers.

2. Materials and methods

2.1 Study Population

The DiPiS-biobank has emerged from the population-based study Diabetes Prediction in Skåne and consists of serum samples collected September 2000 to August 2004 and stored at -20 C. Serum samples from this study were thawed and an aliquot removed to be analyzed immediately for islet autoantibodies (Lynch et al., 2008, Larsson et al., 2005).

In parallel to the DiPiS effort, serum samples were collected from all pregnant women in Region Skåne at their first visit to their Maternity Care Center during early pregnancy (gestational weeks 12-16). The serum samples from this public health test were used to screen for antibodies against rubella, hepatitis, HIV and syphilis. Leftover serum samples were stored at -20^{o}C in the SSM-biobank (Ryding et al., 2008). As with the serum samples in the DiPiS-biobank, the samples from SSM-biobank were thawed and an aliquot removed to be analyzed for islet autoantibodies.

Out of the 48,058 recorded live births, cord blood and serum samples were obtained from 35,683 mothers at the time of delivery (Figure 1). The selection criteria for islet autoantibody positive mothers at delivery have been detailed elsewhere (Lynch et al., 2008). A total of 2001 mothers were excluded because they had diabetes (gestational, type 1, type 2, unknown type or uncertain diagnosis). The delivery sample from the non-diabetic mothers was also analyzed once the cord blood was positive for any of the three islet autoantibodies (Lynch et al., 2008, Larsson et al., 2005). As the cord blood islet autoantibody results were published previously (Lynch et al., 2008, Larsson et al., 2005) these results are not reported in the present study. A total of 532 mothers gave birth to children with cord blood islet autoantibodies (Figure 1). Due to the fact that mothers' serum sample at delivery was missing from 103 mothers, a total of 429 islet autoantibody positive mothers were available (Figure 1). However, in the SSM-biobank, 187 samples were missing, hence it was possible to analyze a total of 242 early pregnancy samples (gestational week 10-16) (Figure 1).

The mean gestational age of autoantibody positive mothers was 39.6 ± 12.3 (SD) weeks (range 27.5-43) and the mean birth weight was 3651 ± 681 (SD) g (range 1245-5745). Out of the 32,294 islet autoantibody negative mothers we initially selected 1716 (four controls per islet autoantibody positive mother) control mothers at random, matched for age, parity, and sampling date during early pregnancy ± 1 week (Figure 1). As serum samples from 297 control mothers weren't accessible from early pregnancy, our final number of mothers

included as controls were 1419 islet autoantibody negative control mothers. For the control mothers the mean gestational age was 39.6 ± 11.4 (SD) weeks (range 28-43) and the mean birth weight was 3616 ± 538 (SD) g (range 1215-5950). All end-point titers were corrected for a 45% increase in maternal plasma volume expansion in the mother at delivery.

The Regional Ethical Review Board in Lund, Sweden approved this study. All mothers gave informed consent to participate in the SSM-Biobank and the DiPiS-biobank.

2.2 Antibody analyses

2.2.1 Glutamic acid decarboxylase 65 autoantibodies (GADA) and islet antigen-2 autoantibodies (IA-2A)

GADA and IA-2A antibody titers were determined in radioligand binding assays described previously (Falorni et al., 1995). We previously defined GADA and IA-2A titers in the serum samples obtained at delivery from the mothers to compare with titers in the cord blood serum (Lynch et al., 2008). In the present study, the mothers were selected based on the results from their delivery serum sample, not the cord blood analyses. The titers of islet autoantibodies in the early pregnancy samples were determined from the international WHO standard for GADA and IA-2A, which defines titers in Units/mL (U/mL) (Williams et al., 1997). The standard was diluted in serum from GADA and IA-2A negative healthy subjects. The cut-off limit for positivity at delivery was previously reported (Lynch et al., 2008). The early pregnancy samples of the control mothers were used to define a cut-off at the 99th percentile for GADA and 99.8th percentile for IA-2A. Our laboratory is participating in the Diabetes Autoantibody Standardization Program (DASP) (Torn et al., 2008). In the DASP 2009 workshop our workshop specificity and sensitivity for GADA was 68% and 99%, respectively and for IA-2A it was 60% and 99%, respectively.

2.2.2 Insulin autoantibodies (IAA)

IAA antibody titers were determined in a competitive radioligand assay previously described (Williams et al., 1997) with minor alterations (Lynch et al., 2008). The early pregnancy and the corresponding delivery sample of each mother were run on the same microtiter plate. All samples above the 99th percentile were re-analyzed in duplicate wells using 8 U/mL cold insulin to block non-specific binding. Results were expressed in arbitrary units. The cut-off limit for positivity at delivery was previously reported (Lynch et al., 2008). The early pregnancy samples of the control mothers were used to define a cut-off at the 99.8th percentile for IAA. In the competitive assay, the inter-assay and intra-assay coefficient of variations were 8% and 6%, respectively. In the DASP 2009 workshop our workshop specificity and sensitivity for IAA was 20% and 89%, respectively.

2.3 Statistical Analysis

Data were analyzed using standard software SPSS (version 16.0 www.spss.com). GADA, IA-2A and IAA titers were not normally distributed and were therefore logarithmically transformed. Differences in titers of islet autoantibodies between control- and case mothers were assessed by unpaired Student t-test (Table 1)

3. Results

Presented results at delivery were corrected for a 45% increase in maternal plasma volume expansion in the mother.

3.1 Occurrence of single islet autoantibodies

3.1.1 Glutamic acid decarboxylase 65 autoantibodies (GADA)

The frequency of mothers positive for GADA both in early pregnancy and at delivery was 92% (123/133) indicating that 8% (10/133) seroconverted to GADA during pregnancy (Figure 2). The GADA titers (U/mL) in these mothers increased significantly between early pregnancy and delivery (Table 1) corresponding to a mean increase (data not shown) of 409% ($p<0.0001$).

In the remaining group of 123 mothers already positive during early pregnancy only 4% (5/123) showed a minor increase in GADA titers (U/mL) (Figure 2). The difference in titers was not statistically significant (Table 1).

The major group, 96% (118/123), showed a significant decrease in GADA titers during pregnancy ($p<0.0001$) (Figure 2 and Table 1). The GADA titers (U/mL) in these mothers decreased significantly between early pregnancy and delivery (Table 1) corresponding to a mean decrease (data not shown) of 69% ($p<0.0001$).

Among the control mothers 15/1419 (1%) were GADA positive in early pregnancy (Table 1).

3.1.2 Islet antigen-2 autoantibodies (IA-2A)

There were three (3/19) mothers who seroconverted to IA-2A between early pregnancy and delivery. Seroconversion from negativity to positivity in IA-2A titers (U/mL) were low and close to the cut-off limit at delivery. The difference in titers in these three mothers was not statistically significant (Table 1).

Similar to GADA, a majority, 84% (16/19) showed a significant decrease in IA-2A titers during pregnancy ($p<0.0001$) (Figure 3 and Table 1). The IA-2A titers (U/mL) in these mothers decreased significantly (Table 1) corresponding to a mean decrease (data not shown) of 83% ($p<0.0001$).

Among the control mothers 3/1419 (0.2%) were IA-2A positive during early pregnancy (Table 1).

3.1.3 Insulin autoantibodies (IAA)

The frequency of IAA positive mothers both at early pregnancy and at delivery was 65% (69/106) indicating that as many as 35% (37/106) of the mothers seroconverted to IAA during pregnancy (Figure 4 and Table 1). The IAA titers (RU) in these mothers increased significantly (Table 1) corresponding to a mean increase (data not shown) of 1962% ($p<0.0001$). Of the remaining 69 mothers already IAA positive in early pregnancy, 36% (25/69) showed a significant increase in titers (RU) between early pregnancy and delivery ($p<0.0001$) (Figure 4). The mean increase (data not shown) amounted to 192% ($p<0.0001$) (Table 1).

As many as 44/69 (64%) of the mothers with IAA during early pregnancy demonstrated a decrease in IAA titers (RU) between the early pregnancy and delivery (Figure 4 and Table 1). The mean decrease (data not shown) was 39% ($p<0.0001$).

Among the control mothers 4/1419 (0.3%) were found to be IAA positive during early pregnancy.

3.2 Occurrence of multiple islet autoantibodies

We next analyzed mothers with multiple islet autoantibodies comparing early pregnancy with delivery (Table 2). One mother had GADA and IAA both at early pregnancy and at delivery. Of the 13 mothers with both GADA and IA-2A at delivery, only two turned double positive as they both developed IA-2A during pregnancy. Only one mother seroconverted to triple positivity at delivery. As she was IAA positive at early pregnancy, she developed both GADA and IA-2A. None of the controls had multiple autoantibodies during early pregnancy.

4. Discussion

We initially asked whether non-diabetic mothers during pregnancy who were islet autoantibody positive at the time of delivery could have developed these during pregnancy or that they were islet autoantibody positive already in early pregnancy. This group of mothers is important to study as 97% of newly diagnosed type 1 diabetes children do not have a mother with type 1 diabetes. Furthermore, type 1 diabetes mothers often have GADA or IA-2A at the time of pregnancy and since they have been subjected to insulin therapy the ensuing insulin antibodies precludes measurements of insulin autoantibodies. It is also controversial to what extent islet autoantibody positive mothers affect the risk for type 1 diabetes in the offspring

(Lindberg et al., 1999, Elfving et al., 2008, Koczwara et al., 2004, Hamalainen et al., 2001) as well as in the mother herself (Ivarsson et al., 1997).

By contrasting the islet autoantibody titers in early pregnancy with delivery, three patterns were detected. The dominating pattern was that mothers showed a significant and easily detectable decrease in titers. The second pattern was that a subgroup of mothers showed an increase and the third pattern displayed ten mothers who developed GADA, three IA-2A and 37 mothers IAA. The description of these patterns was done without immediately considering the underlying etiopathogenesis.

The strength of the present investigation was the availability of 35,683 mothers participating in the Diabetes Prediction in Skåne (DiPiS) study (Larsson et al., 2005, Lernmark et al., 2004). Out of the 35,683 mothers we selected early pregnancy samples from the 242 non-diabetic mothers, representing about 1% of the population of non-diabetic mothers during pregnancy who we previously reported had GADA, IA-2A or IAA at the time of delivery (Lynch et al., 2008).

An additional strength of our study design was the possibility to determine whether titers of islet autoantibodies increased or decreased during pregnancy in each mother.

In studies of pregnant women it is necessary to correct for the increase in plasma volume. Although the increase in maternal plasma volume during pregnancy varies between individual women there is a general agreement of approximately 45% increase (Brinkman, 1984, Chesley, 1972, Peck and Arias, 1979, Pritchard, 1965). The increase begins at 6 to 10 weeks of gestation, rises sharply through the second trimester before beginning to stabilize at 32 weeks (Blackburn and Loper, 1992). A reduced concentration of islet autoantibodies at the time of delivery because of an increased plasma volume would amplify the differences in the mothers who showed either increased titers or seroconversion. We matched for parity and there was no difference in high birth weight or decreased fetal growth, neither between the mothers who increased, the ones who decreased nor antibody positive mothers and matched controls. Parity (Campbell and MacGillivray, 1972), high birth weight (Pirani et al., 1973, Hytten and Paintin, 1963), increased BMI during pregnancy (Dagher et al., 1965) and decreased fetal growth e.g. preeclampsia (Dagher et al., 1965) are all related to plasma volume expansion. A potential weakness to our study is the lack of information about the mothers BMI during the early pregnancy.

The maternal immune system is suppressed (Wilder, 1998) to prevent fetal allograft rejection during pregnancy. Steroid hormones such as progesterone are thought to contribute to the immune suppression (Wyle and Kent, 1977, Schindler, 1999). Factors linked with hormones such as age, gender and reproductive status also regulate numerous autoimmune diseases as well. For instance, the menopause is strongly associated with the age at onset in autoimmune thyroid disease (ATD) and rheumatoid arthritis (RA). While these diseases tend to ameliorate

during pregnancy, they commonly flares postpartum (Wilder, 1998). Women suffering from systemic lupus erythematosus (SLE) experience the opposite. The age at onset is highest during the reproductive years and usually declines around menopause. In contrast to ATD and RA, the immune complex glomerulonephritis which is considered to be the cause of the disease process in SLE patients has the tendency to worsen during pregnancy but improves postpartum. Female/male ratios are 19:1 in ATD, 3-4:1 in RA and 9:1 in SLE (Wilder, 1998). These observations evidently imply that pregnancy hormones are involved in the pathogenesis of these autoimmune diseases although the exact mechanisms require further investigation. Our study suggests that maternal immune suppression may also apply to subclinical islet autoimmunity.

Previous investigations have shown that gestational events are important to the development of type 1 diabetes in the offspring (Dahlquist et al., 1995, Dahlquist and Kallen, 1992, Jones et al., 1998). Of particular interest to our observation that some mothers did develop IAA or GADA are the reports that enterovirus infections in early pregnancy increase the risk for type 1 diabetes in the offspring, particularly among the very young (Dahlquist et al., 1995, Hyoty et al., 1995). Children born to islet autoantibody positive non-diabetic (Lindberg et al., 1999) but not necessarily to type 1 diabetes (Koczwara et al., 2004) mothers may have an increased risk for type 1 diabetes. Also, type 1 diabetes children who were born to mothers with islet autoantibodies at delivery tend to be islet autoantibody negative at clinical diagnosis (Elfving et al., 2008). In a previous study where seroconversion was reported, the level differences between early pregnancy and delivery were only shown in mothers with low titers and close to the cut-off limit (Hamalainen et al., 2001). In our study this was only true for IA-2A seroconverting mothers.

Conclusion

Non-diabetic mothers during pregnancy with islet autoantibodies at delivery showed three patterns of islet autoantibodies. The major pattern was a marked decrease in islet autoantibody titers. Titers were also reduced among the control mothers as 1% had GADA or IAA in early pregnancy but negative at delivery. This observation is consistent with other autoimmune conditions (D'Armiento et al., 1980, Wilder, 1998) known to be associated with reduced titers of autoantibodies during pregnancy.

One of the minor patterns was that some mothers seroconverted to GADA or IAA. It cannot be excluded therefore that mothers may seroconvert to islet autoimmunity during pregnancy. Longitudinal studies will be necessary to determine if the risk for type 1 diabetes in the offspring differs between mothers who already had islet autoantibodies in early pregnancy or acquired them during pregnancy.

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Table 1. Single autoantibody distribution of GADA (n 133), IA-2A (n 19) and IAA (n 106) at early pregnancy and at the time of delivery in islet autoantibody positive mothers and controls.

	GADA n/Total (%)	IA-2A n/Total (%)	IAA n/Total (%)
Mothers autoantibody positive at delivery	133/242 (55)	19/242 (8)	106 (44)
Mothers autoantibody positive at early pregnancy	123/133 (92)	16/19 (84)	69/106 (65)
Seroconverting mothers	10/133 (8)	3/19 (16)	37/106 (35)
Difference in titer (mean + SEM)*	0.66 ± 0.08	0.32 ± 0.11	0.52 ± 0.05
***p* value**	<0.0001	0.107	<0.0001
Mothers with increased autoantibodies	5/123 (4)	-	25/69 (36)
Difference in titer (mean + SEM)*	0.15 ± 0.10	-	0.23 ± 0.04
***p* value**	0.217	-	<0.0001
Mothers with decreased autoantibodies	118/123 (96)	16/16 (100)	44/69 (64)
Difference in titer (mean + SEM)*	-0.60 ± 0.03	- 0.85 ± 0.086	-0.25 ± 0.03
***p* value**	<0.0001	<0.0001	<0.0001
Control mothers at delivery	0/1419 (0)	0/1419 (0)	0/1419 (0)
Control mothers at early pregnancy	15/1419 (1)	3/1419 (0.2)	4/1419 (0.3)

* Mean difference in titer (mean + SEM)* between early pregnancy and delivery is shown in U/mL for GADA and IA-2A and in RU for IAA.

Table 2. Single- and multiple islet autoantibody distribution of GADA, IA-2A and IAA at early pregnancy and at the time of delivery in islet autoantibody positive mothers.

Mothers autoantibody positive at delivery			Early pregnancy	Delivery
GADA	**IA-2A**	**IAA**	**n**	**n (%)**
+	-	-	107 (56)	118 (49)
+	+	-	11 (5.7)	13 (5.4)
+	+	+	0 (0)	1 (0.4)
+	-	+	1 (0.5)	1 (0.4)
-	+	-	5 (2.6)	5 (2,1)
-	-	+	67 (35.2)	104 (42.7)
-	+	+	0 (0)	0
All autoantibody positive mothers at delivery			191	242

LEGENDS TO THE FIGURES

Figure 1. Study design of mothers participating in the DiPiS study is shown along with those whose early pregnancy samples were accessible in the Southern Sweden Microbiology (SSM)-Biobank. The 187 "not accessible" were lost to follow-up because the mother visited a maternity clinic outside of Skåne or that the sample contained less than 500 µl serum. Missing data analysis showed that the mother's age, parity or sampling date in the 187 not accessible serum samples did not differ from the 242 mothers who eventually were followed.

Figure 2. GADA titers (U/mL) in mothers GADA positive at delivery compared to the titers at early pregnancy divided in groups of seroconverting, increasing, decreasing titers. Titers of GADA in matched control mothers selected because they were islet autoantibody negative at delivery but positive during early pregnancy are also shown.

Figure 3. IA-2A titers (U/mL) in mothers IA-2A positive at delivery compared to the titers at early pregnancy divided in groups of seroconverting and decreasing titers. Titers of IA-2A in matched control mothers selected because they were islet autoantibody negative at delivery but positive during early pregnancy are also shown.

Figure 4. IAA titers (RU) in mothers IAA positive at delivery compared to the titers at early pregnancy divided in groups of seroconverting, increasing, decreasing titers. Titers of IAA in matched control mothers selected because they were islet autoantibody negative at delivery but positive during early pregnancy are also shown.

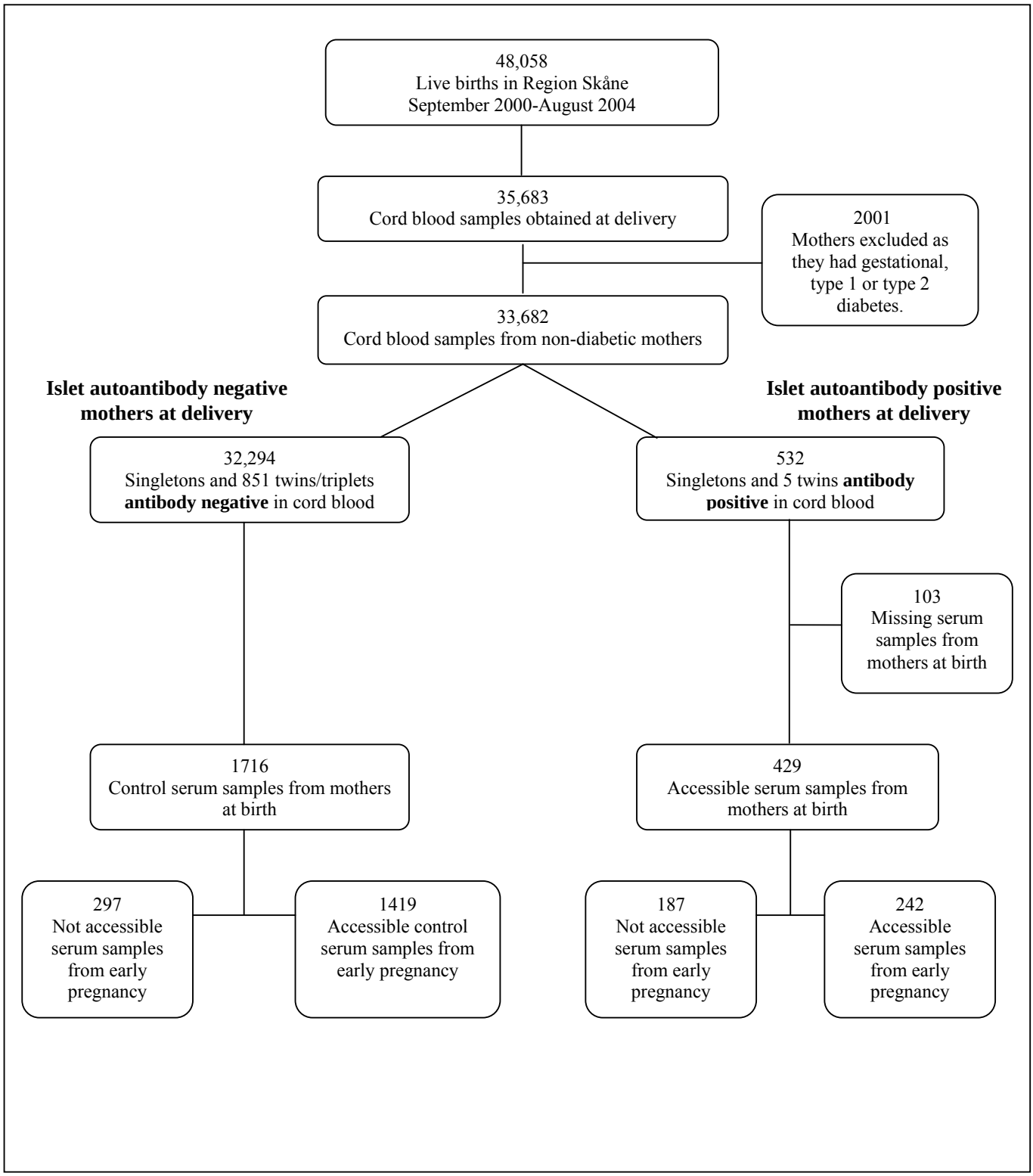
48,058
Live births in Region Skåne
September 2000-August 2004
35,683
Cord blood samples obtained at delivery
2001
Mothers excluded as they had gestational, type 1 or type 2 diabetes.
33,682
Cord blood samples from non-diabetic mothers
Islet autoantibody negative mothers at delivery
Islet autoantibody positive mothers at delivery
32,294
Singletons and 851 twins/triplets **antibody negative** in cord blood
532
Singletons and 5 twins **antibody positive** in cord blood
103
Missing serum samples from mothers at birth
1716
Control serum samples from mothers at birth
429
Accessible serum samples from mothers at birth
297
Not accessible serum samples from early pregnancy
1419
Accessible control serum samples from early pregnancy
187
Not accessible serum samples from early pregnancy
242
Accessible serum samples from early pregnancy

Figure 1

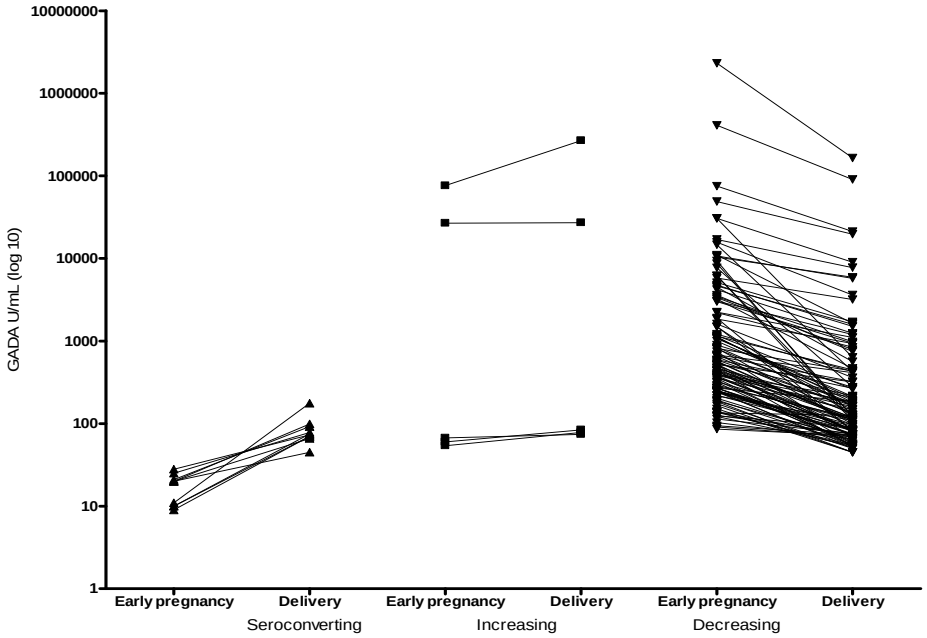

Figure 2

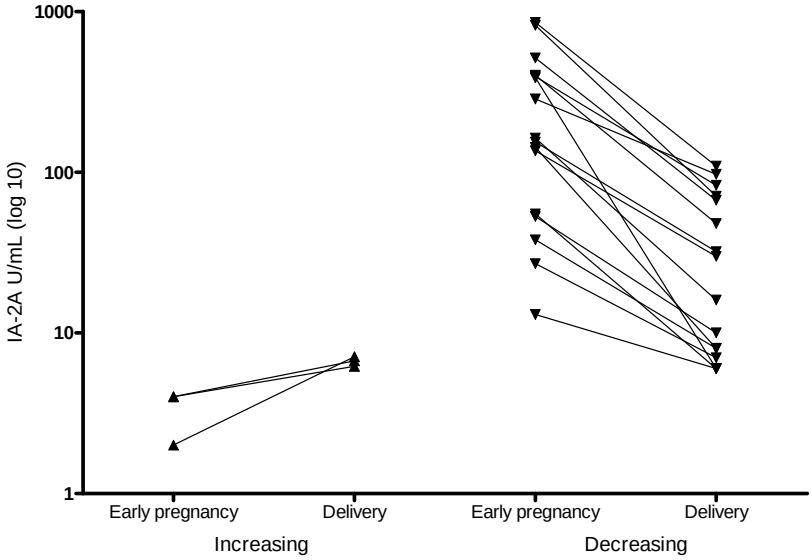

Figure 3

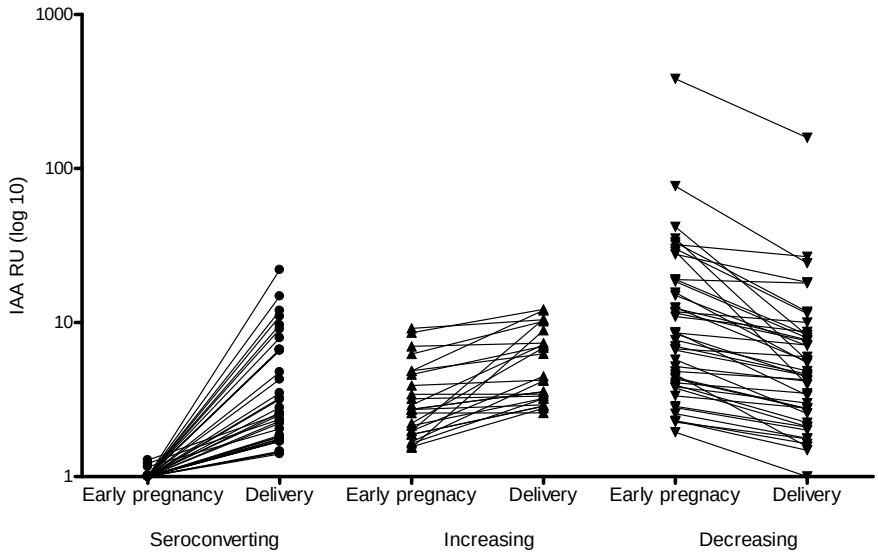
1000
100
10
1
IAA RU (log 10)
Early pregnancy
Delivery
Early pregnacy
Delivery
Early pregnacy
Delivery
Seroconverting
Increasing
Decreasing

Figure 4