BIRTH DEFECTS FROM IMPAIRED GLUCOSE METABOLISM

1. Meta Hypothesis

Among women without diabetes before pregnancy, impaired glucose metabolism during pregnancy is associated with risk of major congenital malformations of the heart, central nervous system, musculoskeletal system, and all birth defects combined.

2. Specific Hypotheses

1. Elevated maternal HgbA1C during the first trimester of gestation is associated with increased risk of birth defects.

2. Children of mothers with gestational diabetes have an increased risk for birth defects.

3. Mothers with impaired glucose metabolism who supplement with anti-oxidant vitamins or folic acid reduce the risk of having children born with birth defects.

3. Background and Justification

Among women who have type 1 or type 2 diabetes before pregnancy, the risk of congenital anomalies in offspring is increased, and animal models confirm the teratogenicity of impaired glucose metabolism. Whether women first diagnosed with diabetes during pregnancy (gestational diabetes) or those with lesser degrees of impaired glucose metabolism during pregnancy have offspring with increased frequency of birth defects has not been determined, though limited data suggest an association (Farrell, Neale, & Cundy, 2002; Schaefer et al., 1997; Anderson et al., 2005; Kanwar et al., 2005).

3.1 Public Health Importance

Prevalence/incidence

Impairments in glucose metabolism during pregnancy are associated with an increased risk of birth defects; thus, infants of women with type 1, type 2, and gestational diabetes are at greater risk of having a birth defect (Casson et al., 1997; Schaefer-Graf et al., 2000). The risk of birth defects among these infants is estimated at three to five times higher than for nondiabetic mothers and increases as glycemic control worsens (Kucera, 1971). Major congenital malformations of the heart, central nervous system, and musculoskeletal system, among the most common defects seen in the offspring of diabetic women, are induced before the seventh week of gestation (Mills, Baker, & Goldman, 1979). The prevalence in the general population of targeted birth defects is:

- 0.6 percent of births with major congenital malformations of the heart (Hoffman & Kaplan, 2002),
- 0.3 percent of births with central nervous system defects (Branum et al., 2003),
- 0.2 percent of births with musculoskeletal birth defects (Feuchtbaum, 1999), and
- 3-4 percent of births with major birth defects combined (Leppig, Werler, Cann, Cook, & Holmes, 1987; Lynberg & Edmonds, 1994).

The total prevalence of diabetes in women ages 20-39 is 1.1 percent and 4.4 percent among those ages 40-49 (Harris et al., 1998 as cited in Beckles & Thompson-Reid, 2001). Among reproductive-aged women with diabetes, 0.6 percent younger than 40 and about 1.6 percent of those 40 or older did not know they had the disease (Harris et al., 1998 as cited in Beckles & Thompson-Reid, 2001). The combined prevalence of diagnosed diabetes, undiagnosed diabetes, and impaired fasting glucose is 2.9 percent and 10.3 percent among white women ages 20-39 and ages 40-49, respectively. Among Mexican-American women ages 20-39 and ages 40-49, 5.6 percent and 22 percent, respectively, had diagnosed diabetes, undiagnosed diabetes, or impaired fasting glucose. Combined prevalence of the same for African-American women ages 20-39 and 40-49 were 5.8 percent and 17.2 percent, respectively. About 4-7 percent of pregnancies are complicated by gestational diabetes, possibly even higher numbers in various ethnic groups (Kjos & Buchanan, 1999).

There are two types of impaired glucose tolerance: impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). Depending on the test used to diagnose impaired glucose tolerance, prevalence estimates differ. In a cross-section of U.S. adults ages 40-74 tested from 1988 to 1994, 33.8 percent had IFG, 15.4 percent had IGT, and 40.1 percent had pre-diabetes (IGT or IFG or both). When these percentages of IGT and IFG are applied to the 2000 U.S. population, approximately 35 million adults ages 40-74 would have IFG, 16 million would have IGT, and 41 million would have pre-diabetes (National Diabetes Information Clearinghouse, 2004).

Based on data from the 1999-2000 National Health and Nutrition Examination Survey, approximately 2 percent of U.S. adults between 20 and 39 years old are affected by impaired fasting glucose, a type of impaired glucose metabolism (Centers for Disease Control and Prevention [CDC], 2003).

Economic and/or social burden

The estimated lifetime cost of birth defects among children born during one year in the United States is $2 billion for heart defects, central nervous defects, and musculoskeletal defects combined, and $8 billion (1992 dollars) for all birth defects combined (CDC, 1995).

3.2 Justification for a Large Prospective Longitudinal Study

The study of the broad hypotheses proposed in this document within a longitudinal study is justified for several reasons: (1) birth defects associated with poor glycemic control can be lethal or cause morbidity resulting in significant expenditures of health care resources; (2) preliminary studies suggest the effects of impaired glucose metabolism can be lessened by readily accessible therapies, providing a base for possible preventive public health recommendations; (3) the prevalence of obesity, impaired glucose metabolism, type 2, and gestational diabetes in women of childbearing age is increasing steadily; and (4) the longitudinal nature and potential scope of the National Children's Study (NCS) provide the most robust, and perhaps the only adequate, study design to understand the relationship between impaired glucose metabolism and birth defects.

3.3 Scientific Merit

The NCS can provide new information on the relationship between impaired glucose metabolism and birth defects. Researchers will be able to identify overt diabetes and occult forms of abnormal glucose metabolism, ideally before conception, and evaluate their effects on the developing fetus. Such comprehensive assessment is important because latent disorders of glucose metabolism are more prevalent than overt diabetes, therefore, many birth defect cases could be attributed to small increases in associated teratogenic risk. Because the effects of these metabolic abnormalities are poorly

understood relative to those associated with overt insulin-dependent diabetes, the longitudinal study will expand the knowledge base of preventive clinical and public health relevance.

3.4 Potential for Innovative Research

The NCS can study glucose metabolism prospectively using objective measurements. This is an improvement over the many retrospective (e.g., case-control) studies in which the ascertainment of disease is based solely on maternal self-reports. Where possible, measurements will begin before conception because glucose metabolism can change during pregnancy. Abnormalities of glucose metabolism might have multiple effects upon the fetus that manifest themselves during pregnancy (in fetal death), at birth (as a birth defect), or later (as childhood obesity). The longitudinal component of the study provides an opportunity to discover and assess the potential multiple effects of altered glucose metabolism during gestation on children over time.

3.5 Feasibility

The current literature concerning impaired glucose metabolism and birth defects suggests a number of well-established biomarkers as well as potential confounders and effect measure modifiers can be collected within the NCS. Some procedures may represent a slight burden to participants, but most samples and data should be easily obtainable.

Ascertainment of birth defects in the NCS will follow the approach used by existing birth-defects surveillance programs. There is extensive experience in the ascertainment of birth defects and collection of clinical information for classification purposes by population-based birth defects surveillance registries in various states around the country. Some of these registries have served as the basis for a number of large population-based case-control studies of birth defects such as the National Birth Defects Prevention Study (NBDPS) in which clinical information was reviewed by clinical geneticists for classification purposes. The Metropolitan Atlanta Congenital Defects Program has been successful in achieving high levels of case ascertainment with relatively high quality of the clinical information gathered (Correa et al., 2007).

Proper classification of cases will be somewhat challenging within a nationwide study such as the NCS, and experience gained by investigators during the NBDPS could be useful. The NCS conducted a workshop that included clinical geneticists, pediatric cardiologists, and birth defects experts from around the country to review and prioritize possible strategies and pilot studies for ascertainment and classification of birth defects in the NCS.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Maternal reports of pregestational and gestational diabetes
- Blood glucose and HgbA1C
- Serum lipid profiles

- Serum insulin or related samples (e.g., C-peptide)
- Serum inositol
- Genetic samples (for exploration of potentially relevant genes such as variable number tandem repeats [VNTR] insulin, glucokinase)

Primary/child

- Serum glucose
- Serum insulin or related samples (e.g., C-peptide)
- Genetic samples (for exploration of potentially relevant genes such as VNTR insulin, glucokinase)

4.2 Methods

Primary/maternal

- Blood samples
- Medical record reviews

Primary/child

- Blood samples
- Medical record reviews
- Physical exams

4.3 Life Stage

Primary/maternal

- Preconception, prenatal, postnatal (follow-up of maternal carbohydrate metabolism would be a valuable ancillary study)

Primary/child

- Birth

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

Assessment for birth defects:

- Standard physical examinations (done routinely on all newborns and infants)
- Review of medical records from birth through age 21
- Photographs of the face in infancy

5.2 Methods

Primary

- Direct observations by a medical professional, photographs of the face, or via medical record reviews
- Clinical information reviewed by experts for classification of birth defects

5.3 Life Stage

Primary

- Birth through age 21

6. Important Confounders, Mediators, and Effect Modifiers

- **Previous history of birth defects:** Mothers whose first child had a birth defect are 2.4 times more likely than other women to have a second infant with a birth defect. Most of the risk is accounted for by the same defect (Lie, Wilcox, & Skjaerven, 1994).
- **HgbA1C:** Low HgbA1C is associated with the reduced incidence of congenital abnormalities (Haire-Joshu, 1996).
- **Maternal obesity (body mass index):** Maternal obesity is associated with an increased risk of birth defects and with impaired glucose metabolism (Anderson et al., 2005).
- **Lipid profile:** Maternal fat-modified diets result in lower total and HDL cholesterol in infants and could be a suitable way to prevent cardiovascular disease among infants from the beginning of life (Fard, Mehrabian, Sarraf-Zadegan, & Sajadi, 2004).
- **Maternal serum inositol:** The mechanisms by which hyperglycemia leads to birth defects are not clear but are probably complex and could be related to oxidative stress, low inositol levels, and other metabolic abnormalities associated with hyperglycemia.

Studies in animals suggest low levels of inositol may be associated with an increased risk of NTDs and other defects (Cockroft, Brook, & Copp, 1992; Hashimoto et al., 1990; Baker, Piddington, Goldman, Egler, & Moehring, 1990; Green & Copp, 1997; Cogram et al., 2002). A recent case-control study of spina bifida also suggests that low maternal serum levels of inositol may be associated with an increased risk for spina bifida (Groenen et al., 2003). So in evaluating the role of hyperglycemia, it is important to take into account the inositol status of women early in pregnancy. Inositol, a sugar with important structural and functional properties, is part of the diet but is also synthesized by the body. Therefore, measurements of inositol status during pregnancy will need to be based on maternal serum levels as well as on interview data on dietary intake.

- **Insulin gene VNTR:** There is some conflicting information regarding this insulin gene. Most data suggest VNTR variations do not influence early growth, but some data suggest an increased risk of childhood obesity and insulin resistance (Bazaes et al., 2003; Bennett et al., 2004; Kraemer, Ratliff, Bartholdi, Brown & Longmire, 1989).

- **Glucokinase mutation:** The intrauterine environment is associated with insulin resistance in childhood. Birth weight is reduced if one parent has a glucokinase mutation (Hattersley et al., 1998).

- **Hormone levels such as cortisol:** Fetuses exposed to glucocorticosteroids in the first trimester had a lowed median birth weight and were born at an earlier gestational age, which increased the risk for being underweight or having birth defects resulting from underdevelopment, but did not exhibit an increased teratogenic risk (Gur, Diav-Citrin, Shechtman, Arnon, & Ornoy, 2004).

- **Smoking status:** The more a pregnant woman smokes, the greater the cumulative health risk for premature birth and birth defects for her baby. Nicotine and carbon monoxide play a role in causing adverse pregnancy outcomes (U.S. Department of Health and Human Services [HHS], 2004; Law et al., 2003; American College of Obstetricians and Gynecologists [ACOG], 2000; Wang et al., 2002; Little, Cardy, & Mungar, 2004).

- **Use of medication:** Anticonvulsants can cause serious problems, including mental retardation and slow growth, in the developing fetus. Other drugs associated with birth defects include antipsychotic and antianxiety agents and certain antibiotics (Jones, 1996; Hernandez-Diaz, Werler, Walker, & Mitchell, 2000; Hernandez-Diaz, Werler, Walker, & Mitchell, 2001).

- **Use of nutritional supplements:** Supplementation with folic acid attenuates the risk for neural tube defects (Berry, 1999; Bower & Stanley, 1989; Czeizel, 1993; Czeizel & Dudas, 1992; Daly, Kirke, Molloy, Weir, & Scott, 1995; Laurence, James, Miller, Tennant, & Campbell, 1981; Milunsky, 1989; MRC Vitamin Study Research Group, 1991), cardiac defects (Botto, Khoury, Mulinare, & Erickson, 1996; Botto, Mulinare, & Erickson, 2000; Czeizel, 1996, Czeizel, Toth, & Rockenbauer, 1996; Lewis, Van Dyke, Stumbo, & Berg, 1998; Shaw et al., 2002), oral clefts (Itikala, Watkins, Mulinare, Moore, & Liu, 2001; Lewis et al., 1998; Shaw, Lammer, Wasserman, O'Malley, & Tolarova, 1995; Tolarova & Harris, 1995; Yang, Khoury, Olney, & Mulinare, 1997), and urinary tract defects (Czeizel, 1996; Czeizel et al., 1996;

Lewis et al., 1998; Werler, Hayes, Louik, Shapiro, & Mitchell, 1999; Yang et al., 1997). Supplementation with antioxidant vitamins appears to attenuate risk for cardiac defects (Correa, Botto, Liu, Mulinare, & Erickson, 2003). For markers of oxidative stress, consider collecting first voided morning urine for acrolein-lysine adducts (ELISA), 8-hydroxy-2-deoxyguanosine (8-OHdG) (ELISA), and nitric oxide metabolites (colorimetric, non-enzymatic assay). These have been used as markers for oxidative stress in children with type 1 diabetes (Hata et al., 2006).

- **Gene-nutrient interaction:** Methylenetetrahydrafolate reductase (MTHFR) polymorphisms appear to be associated with increased risk of birth defects (Botto & Yang, 2000). Reduced folate carrier and MTHFR appear to interact with folic acid supplementation to modify risk of birth defects (Shaw et al., 2002; Shaw, Rozen, Finnell, Wasserman, & Lammer, 1998).

- **Family history:** About 20 percent of birth defects are hereditary, resulting from the interaction of genes from one or both parents plus environmental influences. Defects may include cleft lip and palate, spina bifida, and heart defects (Shaw, Rozen, Finnell, Wasserman, & Lammer, 1998; Lott, 1996).

- **Other factors:** Recreational drugs have been associated with arm and leg abnormalities and central nervous system problems (Jones, 1996).

7. Power and Sample Size

For women with and without diabetes prior to pregnancy and their offspring, assuming 100,000 infants are born into the Study, with a prevalence of impaired fasting glucose early in pregnancy of 2 percent, the power to detect relative risks in the range of 1.5-2.5 for selected major defects is as follows:

Birth defect group	Rate per 1,000 births	Relative Risk			
		1.5	1.75	2.0	2.5
Heart defects	6	47.4	72.3	87.8	98.3
Central nervous system	3	29.8	48.7	65.6	87.0
All major birth defects	30	96.3	99.9	99.99	99.99

The Study has more than adequate power to assess associations of maternal impaired fasting glucose and all major defects as a group. Statistical power to analyze subgroups of defects is less than that for the main defect categories. The power to detect these associations may be also reduced when controlling for confounders and evaluating effect measure modifiers. If, however, various defects are associated with maternal hyperglycemia as suggested by the literature on diabetes and birth defects, it may be possible to group them into a larger group and, thereby, increase the power of the Study to examine smaller risks and various interactions (e.g., with folic acid or antioxidants).

8. Other Design Issues

- **Drug/supplement classification:** Data collection concerning prescription and over-the-counter medications and herbal and multivitamin supplements must contain accurate information about the specific brand, product line, and dosage to be effectively used in evaluating confounders and effect measure modifiers.

- **Ethical/burden considerations:** Identification of clinically significant abnormalities in carbohydrate metabolism in preconceptional or early pregnant women will require notification of the woman's care provider.

- **Cost/complexity of data collection:** Diabetes status prior to pregnancy may be unknown for currently pregnant women who enter the sample. Postnatal assessment of diabetes status may serve as a surrogate.

9. References

American College of Obstetricians and Gynecologists. (2000). Smoking cessation during pregnancy. *ACOG Educational Bulletin*, 260.

Anderson, J.L., Waller, D.K., Canfield, M.A., Shaw, G.M., Watkins, M.L., & Werler, M.M. (2005). Maternal obesity, gestational diabetes, and central nervous system birth defects. *Epidemiology, 16*, 87-92.

Baker, L., Piddington, R., Goldman, A., Egler, J., & Moehring, J. (1990). Myo-inositol and prostaglandins reverse the glucose inhibition of neural tube fusion in cultured mouse embryos. *Diabetologia, 33*(10), 593-596.

Bazaes, R.A., Petry, C.J., Ong, K.K., Avila, A., Dunger, D.B., & Mericq, M.V. (2003). Insulin gene VNTR genotype is associated with insulin sensitivity and secretion in infancy. *Clinical Endocrinology, 59*(5), 599-603.

Bennett, A.J., Sovio, U., Ruokonen, A., Martikainen, H., Pouta, A., Taponen, S., et al. (2004). Variation at the insulin gene VNTR (variable number tandem repeat) polymorphism and early growth: Studies in a large Finnish birth cohort. *Diabetes, 53*(8), 2126-2131.

Berry, R.J., Li, Z., Erickson, J.D., Li, S., Moore, C.A., Wang, H., et al. (1999). Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *New England Journal of Medicine, 341*, 1485-1490.

Botto, L.D., & Yang, Q. (2000). 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: A HuGE review. *American Journal of Epidemiology, 151*, 862-877.

Botto, L.D., Khoury, M.J., Mulinare, J., & Erickson, J.D. (1996). Periconceptional multivitamin use and the occurrence of conotruncal heart defects: Results from a population-based, case-control study. *Pediatrics, 98*, 911-917.

Botto, L.D., Mulinare, J., & Erickson, J.D. (2000). Occurrence of congenital heart defects in relation to maternal mulitivitamin use. *American Journal of Epidemiology, 151*, 878-884.

Bower, C., & Stanley, F.J. (1989). Dietary folate as a risk factor for neural-tube defects: Evidence from a case-control study in Western Australia. *Medical Journal of Australia, 150*, 613-619.

Casson, I.F., Clarke, C.A., Howard, C.V., McKendrick, O., Pennycook, S., Pharoah, P.O., et al. (1997). Outcomes of pregnancy in insulin dependent diabetic women: Results of a five year population cohort study. *British Medical Journal, 315*, 275-278.

Centers for Disease Control and Prevention. (1995). Economic costs of birth defects and cerebral palsy–United States, 1992. *Morbidity and Mortality Weekly Report, 44*(37), 649-659.

Centers for Disease Control and Prevention. (2003). Prevalence of Diabetes and Impaired Fasting Glucose in Adults–United States, 1999-2000. *Morbidity and Mortality Weekly Report, 52*(35), 833-837.

Cockroft, D.L., Brook, F.A., & Copp, A.J. (1992). Inositol deficiency increases the susceptibility to neural tube defects of genetically predisposed (curly tail) mouse embryos in vitro. *Teratology, 45*(2), 223-232.

Cogram, P., Tesh, S., Tesh, J., Wade, A., Allan, G., Greene, N.D., et al. (2002). D-chiro-inositol is more effective than myo-inositol in preventing folate-resistant mouse neural tube defects. *Human Reproduction, 17*(9), 2451-2458.

Correa, A., Botto, L., Liu, Y., Mulinare, J., & Erickson, J.D. (2003). Do multivitamin supplements attenuate the risk for diabetes-associated birth defects? *Pediatrics, 111*, 1146-1151.

Correa, A., Cragan, J.D., Kucik, M.E., Alverson, C.J., Gilboa, S.M., Balakrishnan, R., et al. (2007). Metropolitan Atlanta Congenital Defects Program 40th Anniversary Edition Surveillance Report. *Birth Defects Research Part A, 79*, 65-186.

Czeizel, A.E. (1993). Prevention of congenital abnormalities by periconceptional multivitamin supplementation. *British Medical Journal, 306*, 1645-1648.

Czeizel, A.E. (1996). Reduction of urinary tract and cardiovascular defects by periconceptional multivitamin supplementation. *American Journal of Medical Genetics, 62*, 179-183.

Czeizel, A.E., & Dudas, I. (1992). Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *New England Journal of Medicine, 327*, 1832-1835.

Czeizel, A.E., Toth, M., & Rockenbauer, M. (1996). Population-based case control study of folic acid supplementation during pregnancy. *Teratology, 53*, 345-351.

Daly, L.E., Kirke, P.N., Molloy, A., Weir, D.G., & Scott, J.M. (1995). Folate levels and neural tube defects. Implications for prevention. *Journal of the American Medical Association, 274*, 1698-1702.

Fard, N.M., Mehrabian, F., Sarraf-Zadegan, N., & Sajadi, F. (2004). Fat-modified diets during pregnancy and lactation and serum lipids after birth. *The Indian Journal of Pediatrics, 71*(8), 683-687.

Farrell, T., Neale, L., & Cundy, T. (2002). Congenital anomalies in the offspring of women with type 1, type 2 and gestational diabetes. *Diabetic Medicine, 19*(4), 322-326.

Feuchtbaum, L.B., Currier, R.J., Riggle, S., Roberson, M., Lorey, F.W., Cunningham, G.C. (1999). Neural tube defect prevalence in California (1990-1994): Slicing patterns by type of defect and race/ethnicity. *Genetic Testing, 3*, 5-72.

Greene, N.D., & Copp, A.J. (1997). Inositol prevents folate-resistant neural tube defects in the mouse. *Nature Medicine, 3*(1), 60-66.

Groenen, P.M., Peer, P.G., Wevers, R.A., Swinkels, D.W., Franke, B., Mariman, E.C., et al. (2003). Maternal myo-inositol, glucose, and zinc status is associated with the risk of offspring with spina bifida. *American Journal of Obstetrics and Gynecology, 189*(6), 1713-1719.

Gur, C., Diav-Citrin, O., Shechtman, S., Arnon, J., & Ornoy, A. (2004). Pregnancy outcome after first trimester exposure to corticosteroids: A prospective controlled study. *Reproductive Toxicology, 18*(1), 93-101.

Haire-Joshu, D., (Ed.). (1996). *Management of diabetes mellitus: Perspectives of care across the life span* (p.14). St. Louis, MO: Washington University.

Beckles, G.L.A. & Thompson-Reid, P.E. (Eds.). (2001). *Diabetes and women's health across the life stages: A public health perspective*. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Division of Diabetes Translation.

Hashimoto, M., Akazawa, S., Akazawa, M., Akashi, M., Yamamoto, H., Maeda, Y., et al. (1990). Effects of hyperglycaemia on sorbitol and myo-inositol contents of cultured embryos: Treatment with aldose reductase inhibitor and myo-inositol supplementation. *Diabetologia, 33*(10), 597-602.

Hata, I., Kaji, M., Kirano, S., Shigematsu, Y., Tsukahara, H., & Mayumi, M. (2006). Urinary oxidative stress markers in young patients with type 1 diabetes. *Pediatrics International, 48*, 58-61.

Hattersley, A.T., Beards, F., Ballantyne, E., Appleton, M., Harvey, R., & Ellard, S. (1998). Mutations in the glucokinase gene of the fetus result in reduced birth weight. *Nature Genetics, 19*(3), 268-270.

Hernandez-Diaz, S., Werler, M.M., Walker, A.M., & Mitchell, A.A. (2000). Folic acid antagonists during pregnancy and the risk of birth defects. *New England Journal of Medicine, 343*, 1608-1614.

Hernandez-Diaz, S., Werler, M.M., Walker, A.M., & Mitchell, A.A. (2001). Neural tube defects in relation to use of folic acid antagonists during pregnancy. *American Journal of Epidemiology, 153*, 961-968.

Hoffman, J.I., & Kaplan, S. (2002). The incidence of congenital heart disease. *Journal of the American College of Cardiology, 39*(12), 1890-1900.

Itikala, P.R., Watkins, M.L., Mulinare, J., Moore, C.A., & Liu, Y. (2001). Maternal multivitamin use and orofacial clefts in offspring. *Teratology, 63*, 79-86.

Jones, K.L. (1996). Dysmorphology. In W.E. Nelson, R.E. Behrman, R.M. Kliegman, A.M. Arvin (Eds.). *Nelson Textbook of Pediatrics*, 15th edition. Philadelphia: W.B. Saunders.

Kanwar, Y.S., Nayak, B., Lin, S., Akagi, S., Xie, P., Wada, J., et al. (2005). Hyperglycemia: Its imminent effects on mammalian nephrogenesis. *Pediatric Nephrology, 20*, 858-866.

Kjos, S.L., & Buchanan, T.A. (1999). Gestational diabetes mellitus. *New England Journal of Medicine, 341*(23), 1749-1756.

Kraemer, P.M., Ratliff, R.L., Bartholdi, M.F., Brown, N.C., & Longmire, J.L. (1989). Use of variable number of tandem repeat (VNTR) sequences for monitoring chromosomal instability. *Progress in Nucleic Acid Research and Molecular Biology, 36*, 187-204.

Kucera, J. (1971). Rate and type of congenital anomalies among offspring of diabetic women. *Journal of Reproductive Medicine, 7*, 73-82.

Laurence, K.M., James, N., Miller, M.H., Tennant, G.B., & Campbell, H. (1981). Double-blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. *British Medical Journal (Clinical Research Edition), 282*, 1509-1511.

Law, K.L., Stroud, L.R., LaGasse, L.L., Niaura, R., Liu, J., & Lester, B.M. (2003). Smoking during pregnancy and newborn neurobehavior. *Pediatrics, 111*(6), 1318-1323.

Leppig, K.A., Werler, M.M., Cann, C.I., Cook, C.A., & Holmes, L.B. (1987). Predictive value of minor anomalies. I. Association with major malformations. *Journal of Pediatrics, 110*, 530-537.

Lewis, D.P., Van Dyke, D.C., Stumbo, P.J., & Berg, M.J. (1998). Drug and environmental factors associated with adverse pregnancy outcomes. Part I: Antiepileptic drugs, contraceptives, smoking, and folate. *Annals of Pharmacotherapy, 32*, 802-817.

Li, S., et al. (2000). Maternal periconceptional folic acid supplementation and reduced risk for congenital heart defects. *Reproductive Toxicology, 14*, 73-77.

Lie, R.T., Wilcox, A.J., & Skjaerven, R. (1994). A population-based study of the risk of recurrence of birth defects. *New England Journal of Medicine, 330*, 1-6.

Little, J., Cardy, A., & Mungar, R.G. (2004). Tobacco smoking and oral clefts: A meta-analysis. *Bulletin of the World Health Organization, 82*(3), 213-8.

Lott, J.W. (1996). Fetal development: Environmental influences and critical periods. In C. Kenner, J.W. Lott, & A.A. Flandermeyer (Eds.). (1998). *Comprehensive Neonatal Nursing*. Philadelphia: W.B. Saunders.

Lynberg, M.C., & Edmonds, L.D. (1994). State use of birth defects surveillance (pp. 217-226). In L.S. Wilcox & J.S. Marks (Eds.). *Data to action: CDC's public health surveillance for women, infants, and children.* Atlanta: Centers for Disease Control and Prevention, U.S. Department of Health and Human Services.

Mills, J.L., Baker, L., & Goldman, A.S. (1979). Malformations in infants of diabetic mothers occur before the seventh gestational week. Implications for treatment. *Diabetes, 28*, 292-293.

Milunsky, A., Jick, H., Jick, S.S., Bruell, C.L., MacLaughlin, D.S., Rothman, K.J., et al. (1989). Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *Journal of the American Medical Association, 262*, 2847-2852.

MRC Vitamin Study Research Group. (1991). Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study. *Lancet, 338*, 131-137.

National Diabetes Information Clearinghouse. (2004). National Diabetes Statistics [NIH Publication No. 04-3892]. Retrieved from http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.htm#3

Schaefer, U.M., Songster, G., Xiang, A., Berkowitz, K., Buchanan, T.A., & Kjos, S.L. (1997). Congenital malformations in offspring of women with hyperglycemia first detected during pregnancy. *American Journal of Obstetrics and Gynecology, 177*(5), 1165-1171.

Schaefer-Graf, U.M., Buchanan, T.A., Xiang, A., Songster, G., Montoro, M., & Kjos, S.L. (2000). Patterns of congenital anomalies and relationship to initial maternal fasting glucose levels in pregnancies complicated by type 2 and gestational diabetes. *American Journal of Obstetrics and Gynecology, 182*, 313-320.

Shaw G.M., Lammer, E.J., Zhu, H., Baker, M.W., Neri, E., & Finnell, R.H. (2002). Maternal periconceptional vitamin use, genetic variation of infant reduced folate carrier (A80G), and risk of spina bifida. *American Journal of Medical Genetics, 108*, 1-6.

Shaw, G.M., Lammer, E.J., Wasserman, C.R., O'Malley, C.D., & Tolarova, M.M. (1995). Risks of orofacial clefts in children born to women using multivitamins containing folic acid periconceptionally. *Lancet, 346*, 393-396.

Shaw, G.M., O'Malley, C.D., Wasserman, C.R., Tolarova, M.M., & Lammer, E.J. (1995). Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring. *American Journal of Medical Genetics, 59*, 536-545.

Shaw, G.M., Rozen, R., Finnell, R.H., Wasserman, C.R., & Lammer, E.J. (1998). Maternal vitamin use, genetic variation of infant methylenetetrahydrofolate reductase, and risk for spina bifida. *American Journal of Epidemiology, 148*, 30-37.

The National Children's Study Interagency Coordinating Committee. (2003). The National Children's Study of Environmental Effects on Child Health and Development. *Environmental Health Perspectives, 111*, 642-646.

Tolarova, M., & Harris, J. (1995). Reduced recurrence of orofacial clefts after periconceptional supplementation with high-dose folic acid and multivitamins. *Teratology, 51*, 71-78.

U.S. Department of Health and Human Services. (2004). *The health consequences of smoking: A report of the Surgeon General*. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

Wang, X., Zuckerman, B., Pearson, C., Kaufman, G., Chen, C., Wang, G., et al. (2002). Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. *Journal of the American Medical Association, 287*(2), 195-202.

Werler, M.M., Hayes, C., Louik, C., Shapiro, S., & Mitchell, A.A. (1999). Multivitamin supplementation and risk of birth defects. *American Journal of Epidemiology, 150*, 675-682.

Yang, Q., Khoury, M.J., Olney, R.S., & Mulinare, J. (1997). Does periconceptional multivitamin use reduce the risk for limb deficiency in offspring? *Epidemiology, 8*, 157-161.

INCREASED RISK OF PRETERM BIRTH FROM INTRAUTERINE EXPOSURE TO MEDIATORS OF INFLAMMATION

1. Meta Hypothesis

Intrauterine exposure to mediators of inflammation due to infection of either vaginal, cervical, or uterine sites or of more distal sites (e.g., periodontal disease) is associated with an increased risk of preterm birth.

2. Specific Hypotheses

1. Intrauterine infection, as indicated by histological evidence in the fetal membranes or placenta at the time of birth, is associated with increased risk of preterm birth (less than 37 weeks) and early preterm birth (less than 32 weeks).

2. Pro-inflammatory cytokines in the maternal circulation during pregnancy are biomarkers of systemic infection that places the fetus at risk for preterm birth.

3. Pro-inflammatory markers in cervical/vaginal secretions are biomarkers of local infection that increase the risk for preterm birth.

4. Genetic predisposition to an enhanced inflammatory response (e.g., increased production of tumor necrosis factor [TNF]) among women with evidence of vaginal or other infection during pregnancy is associated with an increased risk of spontaneous preterm birth.

3. Background and Justification

While the cause of most preterm births is unclear, evidence of placental or chorioamniotic infection is present in as many as 40 percent of all spontaneous preterm births and in up to 75 percent of those born before 32 weeks gestation (the infants at greatest risk of subsequent adverse outcome) (Romero, Espinoza, Chaiworapongsa, & Kalache, 2002; Andrews, Hauth, & Goldenberg, 2000). Numerous common organisms of generally low virulence (e.g., ureaplasma, mycoplasma, gardenerella) have been associated with spontaneous preterm birth, and multiple micro-organisms can be present concurrently. Chorioamniotic or vaginal markers of inflammation and infection have been associated with preterm birth in the absence of identifiable infectious agents. Most work has focused on the association between local infection in gestational tissues and the vagina and preterm birth (Goldenberg, Hauth, & Andrews, 2000). However, systemic infection or infection at sites remote from the fetus, for example, periodontal disease, have been associated with preterm birth (Offenbacher et al., 1998; Goepfert et al., 2004; Jeffcoat et al., 2001) although a recent meta-analysis reported that this association was weaker in high-quality studies than it was in studies of lower quality (Vergnes & Sixou, 2007). The latter findings suggest a systemic infection of the mother influences the fetus through the blood. At present, it cannot be ruled out that an association between periodontal disease and preterm birth is the result of confounding, for example, by socioeconomic status (Goldenberg et al., 2006).

Observation of higher prevalence of infection at birth for preterm births compared to normal gestation infants has led to suggestions that screening and treatment for infection has the potential to reduce the incidence of preterm birth. However, interventions intended to reduce risk of preterm birth by addressing infectious causes of inflammation have, to date, not provided consistent evidence of benefit, (Pararas, Skevaki, & Kafetzis, 2006). An example includes a randomized trial of antibiotic therapy during

the interpregnancy interval for mothers with a prior preterm birth in which no difference in preterm birth was found among 124 analyzable pregnancy outcomes (Andrews et al., 2006). Another trial randomized 823 women for treatment of periodontal disease (scaling and root planning) at 13-17 weeks of pregnancy but failed to find a resulting difference in proportion of preterm births (Michalowicz et al., 2006) despite promising results for this therapy in a small pilot study (Jeffcoat et al., 2003). Several explanations can be proposed for the lack of efficacy of interventions tested to date. First, the choice of an agent for antibiotic interventions or the timing of their administration (i.e., after infection became symptomatic, when it has been suggested infections that have a role in preterm delivery may have been present at conception) (Goldenberg et al., 2000) were not suitable to obtain the desired effect. Second, the presence of infection and inflammation at preterm birth may not be a case of cause-and-effect but of phenomena with a common risk factor. Certain research gaps regarding infection and inflammation in preterm birth are best addressed through observational studies: to more specifically identify the infectious agents and inflammatory processes most closely related to preterm birth; to obtain better evidence of a causal link between infection and preterm birth, including evaluating temporal sequence; to determine if infection causes preterm birth mainly among women or fetuses with variant inflammatory response genes; and to detect biomarkers for risk of preterm birth.

Observational studies are also needed to investigate genetic susceptibility to the influences of infection and inflammation on birth outcomes. A putative susceptibility allele of the proinflammatory cytokine TNF-alpha has been examined in several studies, but a pooled analysis revealed no overall association between the polymorphism and preterm birth (Menon et al., 2006). A woman with a history of preterm birth is at high risk of another preterm birth, and incidence varies by race. This implies factors intrinsic to the mother, possibly genetic, play an important role. There is continued interest in finding genetic factors that act in the pathway of infection and inflammation effects on preterm birth (Pararas et al., 2006).

The National Children's Study (NCS) is an ideal setting in which to address these issues, because it will enroll a representative sample of pregnant women. This will enable the determination of the contribution of inflammation to the burden of preterm birth among different ethnic groups. The large size of the cohort will allow for more definitive data on the presence and magnitude of an interaction between the TNF-alpha promoter mutation and the presence of bacterial vaginosis on the occurrence of preterm birth (Goldenberg et al., 2000).

In summary, it is likely inflammation and infection are intimately associated with preterm birth as well as with short- and long-term neonatal outcomes including neurologic impairment. The exact mechanism of these associations, including timing and mode of action, remain unclear. And while there are sufficient data to suggest certain populations are at increased risk (e.g., African-American women), there may be other predisposing facts such as single nucleotide polymorphisms in inflammatory genes or other genes also present in women or their infants who ultimately experience this complication. There are also likely specific pathogens and gene-environment interactions that result in preterm birth and poor neonatal outcomes.

3.1 Public Health Importance

Prevalence/incidence

Each year in the United States, approximately 12 percent of all births are preterm (less than 37 weeks gestation) and 1-2 percent are early preterm (less than 32 weeks) (Martin, Hamilton, Ventura, Menacker & Park, 2002). Two-thirds of all infant deaths in the United States occur among those born preterm; preterm birth is associated with substantial neonatal morbidity, a high risk of long-term neurodevelopmental deficits, and low academic achievement (Hack et al., 2002). In term pregnancies,

about 1-2 percent are affected by chorioamnionitis (intrauterine infection), but in pregnancies ending in preterm births, the prevalence of such infection is higher (Wu & Colford, 2000).

Economic and/or social burden

Hospital charges in 2003 for infants who were preterm or low birth weight totaled $18.1 billion in 2003, and these infants accounted for half of the hospital charges for all infants (March of Dimes, 2006). The estimate does not include the monetary value related to use of community health services, the increased educational costs, the costs of social services, nor the out-of-pocket expenses incurred by the guardians of these children (Petrou, Sach, & Davidson, 2001). This underestimates current costs because of increasing preterm birth rates and improved survival of preterm infants. Due to large socioeconomic and racial or ethnic disparities, the U.S. population does not evenly share the medical, educational, and economic costs of preterm births.

3.2-3.3 Justification and Scientific Merit for a Large Prospective Longitudinal Study

It is likely that inflammation and infection are intimately associated with preterm birth as well as with short- and long-term neonatal outcomes including neurologic impairment. The exact mechanism of these associations, including timing and mode of action, remain unclear and can only be elucidated within the context of a large prospective longitudinal study such as the NCS. The Study will enroll a representative sample of pregnant women. This will enable the determination of the contribution of inflammation to the burden of preterm births among different ethnic groups. In addition, the large size of the cohort will allow for more definitive data on the presence and magnitude of an interaction between the TNF-alpha promoter mutation and the presence of bacterial vaginosis on the occurrence of preterm births (Goldenberg et al., 2000).

3.4 Potential for Innovative Research

There is evidence for an association between preterm birth and infection/inflammation as outlined above, but important questions remain to be answered to develop appropriate screening and interventions. As a longitudinal study, the NCS can examine issues of causality (exposure preceding disease) and timing of exposure that cannot be studied in other settings. The study will have good feasibility and statistical power, as noted below, to address research questions about infection, inflammation, and preterm birth.

3.5 Feasibility

Given the present knowledge about infection and inflammation and their relationship to preterm birth, a number of specific infectious agents can by hypothesized to be risk factors and a number of proinflammatory cytokines can be hypothesized to be mediators in this pathway. Therefore, the list of potential biomarkers to be measured in a study is long and may change as knowledge advances. However, as long as suitable biological samples are obtained in appropriate quantities and stored in a manner that prevents degradation of DNA and proteins, researchers will have the opportunity to return to these samples and examine a number of specific biomarkers of exposure as the science develops.

In designing research to examine infection and preterm birth, there are questions about what tissue and what timing of sample collection will best capture the biologically relevant exposure. For example, a case-control study observed an association between symptomatic periodontal disease and preterm birth found periodontal disease was not reflected in local markers of infection (i.e., placental culture, cord plasma IL-6) (Goepfert et al., 2004). This is indicative of questions about measurement of local versus systemic infections and potential influence of each on the fetus. Table 1 summarizes the

types of samples and timing of their collection relevant for assessment of fetal exposures to infection and inflammation. The study protocol calls for collection of two of the most critical tissues for assessment of infection: the placenta (including membranes) and the umbilical cord. Amniotic fluid will not be obtained. The study protocol includes good coverage of maternal samples of interest (i.e., a blood draw and a vaginal swab at each trimester).

Periodontal disease as a risk factor for preterm birth has received attention in recent literature. Collection of dental records or physical exams under the study protocol to ascertain presence of periodontal disease in the mother will not be feasible in the NCS. It is possible several sites might propose an ancillary study of periodontal disease and preterm birth. Given that periodontal disease is found among approximately 25 percent of African-American and 15 percent of white and Mexican-American reproductive age women (Xiong, Buekens, Vastardis, & Wu, 2006), a study of approximately 6,000 women would be needed to detect a 2.4-fold increase of birth at less than 28 weeks of gestation to women with periodontal disease compared to women with normal periodontal health. Just such a relative risk was reported in a recent meta-analysis (Xiong et al., 2006). Adding periodontal examinations for this number of women may be feasible as an ancillary study. Alternatively, assessment of bacterial DNA specific to pathogens responsible for periodontal disease in maternal sera may provide a surrogate marker for periodontal disease itself. Biomarkers of infection in maternal serum are another method for the indirect assessment of periodontal disease.

Table 1. Types of biological samples useful for assessment of infection during pregnancy, and planned collection under the NCS protocol

Priority*	Type of sample	Timing of collection	Collected under current NCS protocol?	Biomarkers of interest
1	Placenta	At birth	Yes, B1	Histologic evidence of inflammation, biomarkers†
2	Membrane (amnion and chorion)	At birth	Yes, B1	Histologic evidence of inflammation, biomarkers†
3	Umbilical cord segment	At birth	Yes, B1	Histologic evidence of inflammation, biomarkers†
4	Vaginal secretions	Each trimester and at birth	Yes, vaginal swab at T1, T2/3	Biomarkers†, Gram stain
5	Maternal cervical fluid	To be decided	No	PCR for specific pathogens
6	Maternal urine	Each trimester and at birth	Yes, T1, T2, T3, B1	White cell count, PCR for specific pathogens
7	Fetal cord blood serum	At birth	Yes, (cord is collected) B1	Biomarkers†
8	Maternal serum	Each trimester and at birth	T1, T2, T3	Biomarkers†
‡	Mother and infant DNA	Any	Yes	Genetic polymorphisms

* Qualitative ranking of importance of this sample type for assessment of in utero exposure to infection.

† Biomarkers include PCR for specific pathogens, cytokine levels, specific gene expression, and glucose level. The former can feasibly be assessed retrospectively from stored samples.

‡ For evaluation of gene-environment interaction, not assessment of infection.

4. Exposure Measures

Primary/maternal

Maternal infection/inflammation:

- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers, metalloproteinases)
- Gram stain for bacterial vaginosis
- Medical history of fever and infection (medicine usage) during pregnancy
- Dental exams (potential adjunct study in subpopulation)

Primary/child

- Prenatal infection: Umbilical cord/placental histology
- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers, metalloproteinases)

4.1 Individuals Targeted for Measurement

The most efficient design would be to conduct detailed assessment of exposure for a case-cohort or nested case-control sample of participants.

4.2 Methods

Primary/maternal

- Blood samples
- Vaginal fluid swabs self-collected
- Interviews
- Medical record reviews

Primary/child

- Physical sampling at delivery
- Histologic exam of placenta, umbilical cord, and fetal membranes at birth
- Cord blood

4.3 Life Stage

Primary/maternal

- Prenatal

Primary/child

- Birth

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Preterm birth: Gestational age; birth weight

5.2 Methods

- Longitudinal data from prior study contacts, including prenatal ultrasounds
- Medical records

5.3 Life Stage

- Birth

6. Important Confounders, Mediators, and Effect Modifiers

- **Economic status:** Increased risk associated with low educational level; unmarried status (Centers for Disease Control and Prevention [CDC], 1990), and psychosocial stress (Hogue, Hoffman, & Hatch, 2001).
- **Race/ethnicity:** As a percent of live births, 17.6 percent among blacks are preterm, 11.4 percent among Hispanics, and 10.8 percent among whites (Division of Vital Statistics, National Center for Health Statistics, CDC, 2003).
- **Mother's medical history:** Increased risk associated with maternal smoking, alcohol consumption; older maternal age (indicated preterm births); younger maternal age (spontaneous preterm births); low or high parity; previous preterm birth or stillbirth (Heffner, Sherman, Speizer, & Weiss, 1993; Meis et al., 1995; Wisborg, Henriksen, Hedegaard, & Secher, 1996).
- **Others:** Increased risk; unwantedness of the pregnancy (Olsen et al., 1995; McDonald, Armstrong, & Sloan, 1992).
- **Potential effect modifiers:** Antibiotic treatment (which may decrease exposure to infectious agent-initiated processes of inflammation, especially if systemic, or may increase exposure through alteration of normal vaginal flora, allowing bacterial

vaginosis, invasion of inappropriate organisms, or through the dissemination of proinflammatory mediators after bacterial death).

- **Corticosteroids** (Goldenberg et al., 2006).

- **Genetics and gene-environment interactions:** Some (Macones et al., 2004), though not all (Menon et al., 2006), evidence suggests a specific polymorphism in the TNF gene leading to an exaggerated inflammatory response is associated with an increased risk of preterm birth among women with bacterial vaginosis. Variation in other proinflammatory genes (e.g., IL-1, IL-6) (Engel et al., 2005) may also be associated with increased risk of spontaneous preterm birth among women with infection during pregnancy.

- **Intermediate variables:** Data collection and analysis must be conducted with the awareness that certain conditions may fall in a causal pathway between infection and preterm birth (e.g., preeclampsia). Another issue is that factors other than infection can affect biomarker of inflammation. An example of this is that inflammation has been cited as possible mechanism of action of noninfectious exposures that contribute to risk of preterm birth (e.g., air pollutants) (Huynh, Woodruff, Parker, & Schoendorf, 2006).

7. Power and Sample Size

For a number of specific hypotheses, data analysis will be based on a case-cohort or case-control sample rather than the whole cohort. These designs are appropriate for exposures requiring expensive or time-consuming assays or data collection (e.g., cytokine assays, PCR for detection of microbial DNA, or retrospective chart review). Study power for specific hypothesis tests will vary depending on the outcome selected for analysis (e.g., preterm birth, defined as less than 37 weeks with an estimated prevalence of 12 percent, or early preterm birth, which may be a more specific endpoint with infection-associated inflammation but is much less frequent, estimated at 1-2 percent). The exposure of interest also affects power. Periodontal infection is very prevalent, as high as 30 percent among adults, whereas the percentage of intrauterine infections with any single specified microorganism will be quite low. Evaluation of study power should also take into account missing data. Given that missing data can be expected due to missed clinic visits, subject refusal for certain samples, laboratory assay failure, etc., it should be expected that not all subjects will have complete data for the exposure of interest and for covariates. Minimum detectable odds ratios for a range of scenarios are presented in Table 2.

Table 2. Minimum detectable odds ratios* (MDOR) for two preterm birth outcomes, by exposure prevalence

Outcome	Frequency	N cases	Percent with missing data	N cases with complete data	MDOR for each % exposure prevalence					
					0.5%	1%	2%	5%	10%	20%
Preterm birth	12%	12,000	10%	10,800	1.50	1.35	1.25	1.15	1.12	<1.10
< 37 weeks			25%	8,000	1.60	1.40	1.30	1.20	1.14	1.10
Early preterm birth	2%	2,000	10%	1,800	2.40	2.00	1.65	1.40	1.30	1.25
< 32 weeks			25%	1,500	2.60	2.10	1.70	1.45	1.35	1.25
Early preterm birth	1%	1,000	10%	900	>3.00	2.50	2.00	1.60	1.45	1.35
< 32 weeks			25%	750	>3.00	2.70	2.10	1.65	1.50	1.35

* At 80% power, alpha = 0.05, 1:1 case-control ratio; calculated using Stata software.

8. Other Design Issues

- **Changing biomarkers:** The list of potential biomarkers to be measured in a study is long and may change as knowledge advances. However, as long as suitable biological samples are obtained in appropriate quantities and stored in a manner that prevents degradation of DNA and proteins, researchers will have the opportunity to return to these samples and examine a number of specific biomarkers of exposure as the science develops.

- **Ideal tissues to collect:** In designing research to examine infection and preterm birth, there are questions about what tissue and what timing of sample collection will best capture the biologically relevant exposure. The study protocol calls for collection of two of the most critical tissues for assessment of infection: the placenta (including membranes) and the umbilical cord. Amniotic fluid will not be obtained. The study protocol includes good coverage of maternal samples of interest (i.e., a blood draw and a vaginal swab at each trimester).

- **Use of periodontal measures:** Periodontal disease as a risk factor for preterm birth has received attention in recent literature. Collection of dental records or physical exams under the study protocol to ascertain presence of periodontal disease in the mother will not be feasible in the NCS. It is possible several sites might propose an ancillary study of periodontal disease and preterm birth.

- **Reporting of findings:** Issues of reporting of findings to participants and parents of participants, dependent on what samples are collected and analyzed at what time, will require special attention.

9. References

Andrews, W.W., Goldenberg, R.L., Hauth, J.C., Cliver, S.P., Copper, R., & Conner, M. (2006). Interconceptional antibiotics to prevent spontaneous preterm birth: A randomized clinical trial. *American Journal of Obstetrics and Gynecology, 194*, 617-623.

Andrews, W.W., Hauth, J.C., & Goldenberg, R.L. (2000). Infection and preterm birth. *American Journal of Perinatology, 17*, 357-365.

Centers for Disease Control and Prevention. (1990). Effects of maternal cigarette smoking on birth weight and preterm birth—Ohio, 1989. *Morbidity and Mortality Weekly Report, 39*(38), 662-665.

Engel, S.A., Erichsen, H.C., Savitz, D.A., Thorp, J., Chanock, S.J., & Olshan, A.F. (2005). Risk of spontaneous preterm birth is associated with common proinflammatory cytokine polymorphisms. *Epidemiology, 16*, 469-477.

Goepfert, A.R., Jeffcoat, M.K., Andrews, W.W., Faye-Petersen, O., Cliver, S.P., Goldenberg, R.L., et al. (2004). Periodontal disease and upper genital tract inflammation in early spontaneous preterm birth. *Obstetrics and Gynecology, 104*, 777-783.

Goldenberg, R.L., & Culhane, J.F. (2006). Preterm birth and periodontal disease. *New England Journal of Medicine, 355*, 1925-1927.

Goldenberg, R.L., Andrews, W.W., Faye-Petersen, O.M., Cliver, S.P., Goepfert, A.R., & Hauth, J.C. (2006). The Alabama preterm birth study: Corticosteroids and neonatal outcomes in 23- to 32-week newborns with various markers of intrauterine infection. *American Journal of Obstetrics and Gynecology, 195*, 1020-1024.

Goldenberg, R.L., Hauth, J.C., & Andrews, W.W. (2000). Intrauterine infection and preterm delivery. *New England Journal of Medicine, 342*, 1500-1507.

Hack, M., Flannery, D.J., Schluchter, M., Cartar, L., Borawski, E., & Klein, N. (2002). Outcomes in young adulthood for very-low-birth-weight infants. *New England Journal of Medicine, 346*, 149-157.

Heffner, L.J., Sherman, C.B., Speizer, F.E., & Weiss, S.T. (1993). Clinical and environmental predictors of preterm labor. *Obstetrics and Gynecology, 81*(5, Pt. 1), 750-757.

Hogue, C., Hoffman, S., & Hatch, M. (2001). Stress and preterm delivery: A conceptual framework. *Paediatric Perinatal Epidemiology, 15*(Suppl. 2), 30-40

Huynh, M., Woodruff, T.J., Parker, J.D., & Schoendorf, K.C. (2006). Relationships between air pollution and preterm birth in California. *Paediatric Perinatal Epidemiology, 20*, 454-461.

Jeffcoat, M.K., Geurs, N.C., Reddy, M.S., Cliver, S.P., Goldenberg, R.L., & Hauth, J.C. (2001). Periodontal infection and preterm birth: Results of a prospective study. *Journal of the American Dental Association, 132*, 875-880.

Jeffcoat, M.K., Hauth, J.C., Geurs, N.C., Reddy, M.S., Cliver, S.P., Hodgkins, P.M., et al. (2003). Periodontal disease and preterm birth: Results of a pilot intervention study. *Journal of Periodontology, 74*, 1214-1218.

Macones, G.A., Parry, S., Elkousy, M., Clothier, B., Ural, S.H., & Strauss, J.F., 3rd. (2004). A polymorphism in the promoter region of TNF and bacterial vaginosis: Preliminary evidence of gene-environment interaction in the etiology of spontaneous preterm birth. *American Journal of Obstetrics and Gynecology, 190*, 1504-1508.

March of Dimes. (2006). Compendium on Preterm Birth [Slide presentation]. Accessed March 31, 2007, at http://www.marchofdimes.com/prematurity/21329_21509.asp

Martin, J.A., Hamilton, B.E., Ventura, S.J., Menacker, F., & Park, M.M. (2002). Births: Final data for 2000. *National Vital Statistics Reports*, Vol. 50, No 5. Hyattsville, MD: National Center for Health Statistics.

McDonald, A.D., Armstrong, B., & Sloan, M. (1992). Cigarette, alcohol, and coffee consumption and prematurity. *American Journal of Public Health, 82*(1), 87-90.

Meis, P.J., Michielutte, R., Peters, T.J., Wells, H.B., Sands, R.E., Coles, E.C., et al. (1995). Factors associated with preterm birth in Cardiff, Wales. II. Indicated and spontaneous preterm birth. *American Journal of Obstetrics and Gynecology, 173*(2), 597-602.

Menon, R., Merialdi, M., Betran, A.P., Dolan, S., Jiang, L., Fortunato, S.J., et al. (2006). Analysis of association between maternal tumor necrosis factor-alpha promoter polymorphism (-308), tumor necrosis factor concentration, and preterm birth. *American Journal of Obstetrics and Gynecology, 195*, 1240-1248.

Michalowicz, B.S., Hodges, J.S., DiAngelis, A.J., Lupo, V.R., Novak, M.J., Ferguson, J.E., et al. (2006). Treatment of periodontal disease and the risk of preterm birth. *New England Journal of Medicine, 355*, 1885-1894.

Offenbacher, S., Jarod, H.L., O'Reilly, P.G. , Wells, S.R., Salvi, G.E., Lawrence, H.P., et al. (1998). Potential pathogenic mechanisms of periodontitis associated pregnancy complications. *Annals of Periodontalogy, 3*, 233-250.

Olsen, P., Laara, E., Rantakallio, P., Jarvelin, M.R., Sarpola, A., & Hartikainen, A.L. (1995). Epidemiology of preterm delivery in two birth cohorts with an interval of 20 years. *American Journal of Epidemiology, 142*(11), 1184-1193.

Pararas, M.V., Skevaki, C.L., & Kafetzis, D.A. (2006). Preterm birth due to maternal infection: Causative pathogens and modes of prevention. *European Journal of Clinical Microbiology and Infectious Disease, 25*, 562-569.

Petrou, S., Sach, T., & Davidson, L. (2001). The long-term costs of preterm birth and low birth weight: Results of a systematic review. *Child Care, Health and Development, 27*, 97-115.

Romero, R., Espinoza, J., Chaiworapongsa, T., & Kalache, K. (2002). Infection and prematurity and the role of preventive strategies. *Seminars in Neonatology, 7*, 259-274.

Vergnes, J.N., & Sixou, M. (2007). Preterm low birth weight and maternal periodontal status: A meta-analysis. *American Journal of Obstetrics and Gynecology, 196*(135), e1-e7.

Wisborg, K., Henriksen, T.B., Hedegaard, M., & Secher, N.J. (1996). Smoking during pregnancy and preterm birth. *British Journal of Obstetrics and Gynaecology, 103*(8), 800-805.

Wu, Y.W., & Colford, J.M., Jr. (2000). Chorioamnionitis as a risk factor for cerebral palsy: A meta-analysis. *Journal of the American Medical Association, 284*(11), 1417-1424.

Xiong, X., Buekens, P., Vastardis, S., & Wu, T. (2006). Periodontal disease as one possible explanation for the Mexican paradox. *Medical Hypotheses, 67,* 1348-1354.

INCREASED RISK OF FETAL GROWTH RESTRICTION, PRETERM BIRTH, BIRTH DEFECTS, AND DEVELOPMENTAL DISABILITIES IN CHILDREN BORN THROUGH ASSISTED REPRODUCTIVE TECHNOLOGY

1. Meta Hypothesis

Children whose conceptions were aided by assisted reproductive technology (ART) are at increased risk of fetal growth restriction, birth defects, and developmental disabilities in comparison to children who were conceived without ART.

2. Specific Hypotheses

1. Singleton fetuses resulting from ART treatment will have higher rates of fetal growth restriction as compared to singleton fetuses from the general population and also as compared to singleton fetuses from couples with subfertility who did not undergo ART.

2. Women with singleton pregnancies resulting from ART treatment will have higher rates of preterm delivery as compared to women with singleton pregnancies from the general population and also as compared to women with singleton pregnancies arising from couples with subfertility who did not undergo ART.

3. Infants born from singleton pregnancies resulting from ART treatment will have higher rates of any birth defects as compared to infants born from singleton pregnancies from the general population and also as compared to infants born from singleton pregnancies arising from couples with subfertility who did not undergo ART.

4. Children born from singleton pregnancies resulting from ART treatment will have higher rates of developmental disability as compared to children born from singleton pregnancies from the general population and also as compared to children born from singleton pregnancies from couples with subfertility who did not undergo ART.

3. Background and Justification

ART is infertility treatments in which both oocytes and sperm are handled in the laboratory. These treatments include in vitro fertilization (IVF) with transcervical embryo transfer (common), IVF with intracytoplasmic sperm injection (ICSI) and transcervical embryo transfer (common), gamete and zygote intrafallopian transfer (gametes or zygotes transferred into fallopian tubes rather than uterus, which is rare), frozen embryo transfer (less common), and donor embryo transfer (less common). ART is recognized as an important contributor to the U.S. low birth weight rate because of the known association between the use of ART and multiple births (Centers for Disease Control and Prevention [CDC], 2004b; CDC, 2000) and between multiple births and low birth weight (Martin & Park, 1999). It has been suggested that concerns about adverse outcomes of ART could be mitigated by single embryo transfer, avoiding the major complications associated with multiparity (Wennerholm et al., 2000; Catt, Wood, Henman & Jansen, 2003; Davis, 2004). However, as reviewed below, there is substantial evidence suggesting increased risk for singleton pregnancies from ART. This hypothesis therefore focuses on the outcomes for singletons.

Fetal growth restriction

Many retrospective studies have found rates of low birth weight and preterm birth are increased among singleton infants conceived with ART as compared with naturally conceived singleton infants or population-based rates (MRC Working Party on Children Conceived by In Vitro Fertilisation, 1990; FIVNAT, 1995; Gissler, Malin Silverio, & Hemminki, 1995; Verlaenen, Cammu, Derde, & Amy, 1995; Bergh, Ericson, Hillensjo, Nygren & Wennerholm, 1999; Dhont, De Sutter, Ruyssinck, Martens, & Bekaert, 1999; Westergaard, Johansen, Erb, & Andersen, 1999; Schieve et al., 2002; Schieve et al., 2002; Jackson, Gibson, Wu, & Croughan, 2004; Schieve, Ferre, et al., 2004; Poikkeus, Gissler, Unkila-Kallio, Hyden-Granskog, & Tiitenen, 2007). The question remains whether the observed risks for singletons conceived with ART is a direct effect of the procedure or reflects another factor related to the underlying infertility of the couples who conceive using these procedures (Westergaard et al., 1999; Buck Louis, Schisterman, Dukic, & Schieve, 2005). Subfertile controls (i.e., trying for more than one year) who conceive spontaneously are necessary to address this question. A recent large population-based study from the Netherlands using subfertile couples conceiving naturally as controls suggests ART (or some component of it) may have independent adverse effects for low birth weight and preterm birth (Kapeiteijn et al., 2006). In addition, studies to date have been limited in their ability to evaluate low birth weight due to intrauterine growth restriction separately from low birth weight due to preterm delivery. This is an important distinction for understanding etiology and developing prevention strategies. No study has monitored fetal growth changes prospectively. Rapidly changing ART treatments (most notably, a rapid increase in the use of ICSI in the past five years) result in a need to re-examine the association with precise data about treatments (Schultz & Williams, 2002).

Birth defects

Mixed results exist regarding the association between ART and birth defects in the offspring, with some studies finding no increased risk (MRC Working Party on Children Conceived by In Vitro Fertilisation, 1990; Friedler, Mashiach, & Laufer, 1992; FIVNAT, 1990; Westergaard et al., 1999), and other studies finding an increased risk for all birth defects and/or some conditions (Bergh et al., 1999; Wennerholm et al., 2000; Ericson & Kallen, 2001; Hansen, Kurinczuk, Bower, & Webb, 2002; Bonduelle et al., 2005; Kallen, Finnstrom, Nygren, & Olausson, 2005; Klemetti et al., 2005; Merlob, Sapir, Sulkes, & Fisch, 2005; Olson et al., 2005). A recent meta-analysis concluded ART carried a 30-40 percent increase in the risk of all birth defects as compared with naturally conceived infants (Hansen, Bower, Milne, de Klerk, & Kurinczuk, 2005). Prior studies have suffered from methodological problems, most notably low statistical power, and differential case ascertainment and coding schemes for infants conceived using ART vs. infants conceived naturally (Schieve, Rasmussen, & Reefhuis, 2005). Nearly all studies have relied on retrospective registry data. Most studies of birth defects have not analyzed singleton infants separately from infants from multiple gestations, and multiple gestation is a risk factor for birth defects. Analogous to low birth weight, the question remains as to whether increased risk is due to the treatment or the underlying infertility (Schieve, Rasmussen, et al., 2004; Kallen et al., 2005; Olson et al., 2005; Schieve et al., 2005). Work in progress from Iowa suggests underlying ovulation disorders are themselves a risk factor for low birth weight, regardless of infertility treatment received (ART or other treatment) (VanVoorhis et al., 2006). A large population-based cohort from Denmark found that when compared to spontaneously conceiving subfertile controls, the incidence of all genital malformations was increased fourfold although the incidence of birth defects overall was not increased (Zhu, Basso, Obel, Bille, & Olsen, 2006). (This study is discussed further below.) Retrospective studies will substantially underestimate the incidence of birth defects because some result in spontaneous abortion or in some cases, elective abortion, so they are not identified in retrospective cohorts based on births. If such early terminations of pregnancy were more common in ART pregnancies, then retrospective studies based on births would underestimate the risk.

Developmental disabilities

The study of longer-term outcomes such as developmental disabilities among ART children has been hampered by inadequate sample sizes and follow-up. Cross-sectional studies have generally not found increased developmental problems for children conceived by ART (Olivennes et al., 2002; Ponjaert-Kristoffersen et al., 2005). A retrospective population-based study from Sweden reported an increased risk for cerebral palsy (odds ratio 2.8, 95 percent confidence interval 1.3-5.8), and a nonsignificant trend for suspected developmental delay (odds ratio 2.0, 95 percent confidence interval 0.7-5.4) among singleton children conceived with ART (Stromberg et al., 2002). The study suffered from a number of methodological drawbacks, including a reliance on registry data and a lack of standardized neurodevelopmental evaluation. There have been no longitudinal prospective studies with thorough follow-up and standardized assessment across children conceived with and without ART.

Epigenetic effects have been shown with ART techniques applied to animals (Khosla, Dean, Brown, Reik & Feil, 2001, Young et al., 2001) and this may be a possible mechanism for some adverse outcomes with ART in humans (Schultz, 2005). The rare imprinting disorders Angelman Syndrome and Beckwith-Wiedemann Syndrome have been linked to ART, though the absolute risk is small (Cox et al.., 2002; DeBaun, Niemitz, & Feinberg, 2003; Chang, Moley, Wangler, Feinberg, & Debaun, 2005; Maher, 2005; Sutcliffe et al., 2006). Nevertheless, this highlights the potential value of investigating potential genetic and epigenetic mechanisms for adverse outcomes of ART within a large cohort study (Bjornsson, Fallin & Feinberg, 2004).

3.1 Public Health Importance

Prevalence/incidence

In the United States and worldwide, the use of ART to overcome infertility is increasing rapidly. In 2004, the most recent year for which U.S. population-based data are available, more than 128,000 ART procedures were performed, resulting in more than 49,000 live-born infants (CDC, 2004b). These infants are estimated to represent slightly more than 1 percent of the total infants born in the United States in 1999. However, ART treatments represent only a fraction of the infertility treatments currently used. Results from the population-based National Survey of Family Growth suggest that in 2002, treatment with ovulation stimulation medications without ART was about 15 times more frequent than the use of ART, and artificial (or assisted) insemination was about five times more frequent (Chandra, Martinez, Mosher, Abma, & Jones, 2005). In addition, 15.1 percent of married women of reproductive age reported some type of subfertility, but only 36 percent of women with subfertility ever received any form of infertility service (of which about half received medical advice only), and only 1 percent ever received ART. This represents a large pool of subfertile women who may have spontaneous conceptions and also a large growth potential for increased use of ART and other infertility treatments as accessibility improves. In a representative sample of births based on 2004's Pregnancy Risk Assessment Monitoring System (PRAMS) in Utah, 7 percent of intended births without infertility treatment had a time to pregnancy of greater than one year (representing spontaneous conceptions in subfertile women) (Stanford, Ellis, Simonsen, & Baksh, 2007).

In the United States, the incidence of fetal growth restriction is approximately 5 percent (Peleg, Kennedy, & Hunter, 1998). The preterm birth rate has been rising and is 12.5 percent of all births as of 2004 (Committee on Understanding Premature Birth and Assuring Health Outcomes, 2006). Major birth defects occur in approximately 3 percent of children born in this country (CDC, 2005; Boyle & Cordero, 2005). The prevalence of serious developmental delay is estimated from 2-3 percent in national surveys (Simpson, Colpe, & Greenspan, 2003; Van Naarden Braun, Autry, & Boyle, 2005), while the presence of any developmental impairment, including mild impairment, is estimated to be 17 percent

(Rice, Schendel, Cunniff, & Doernberg, 2004; Boyle & Cordero, 2005). Cerebral palsy affects approximately 0.2 percent of children (Kuban & Leviton, 1994), and autism affects about 0.3 percent (Yeargin-Allsopp et al., 2003).

Economic and/or social burden

Children with birth defects and those born too small contribute disproportionately to infant and pediatric health care costs (Sandhu, Stevenson, Cooke, & Pharoah, 1986; Lynberg & Edmonds, 1994; Committee on Understanding Premature Birth and Assuring Health Outcomes, 2006). An investigation conducted in the United States in 2003 concluded that the proportion of preterm and low-birth-weight infants has been growing. Ten billion dollars was spent on care for preterm births in the form of acute care, education, and child care costs (Cuevas, Silver, Brooten, Youngblut, & Bobo, 2005). A report estimated an aggregate incremental cost of $26 billion in 2005 associated with preterm birth. That was $20 billion in direct costs for health care, early intervention, and education and $6 billion in lost productivity due to the increased risk of developmental disabilities (Institute of Medicine Report, in press). The lifetime costs for persons born in 2000 were estimated at $51.2 billion for those with mental retardation, $11.5 billion for those with cerebral palsy, $2.1 billion for those with hearing loss, and $2.5 billion for those with impaired vision (in 2003 dollars) (CDC, 2004a). More than 25 percent of pediatric hospital admissions are estimated to occur among children with birth defects (Lynberg & Edmonds, 1994). Children with developmental disabilities require special education services, medical services, and supportive care.

3.2 Justification for a Large Prospective Longitudinal Study

There is strong scientific consensus about the importance of understanding the health outcomes of ART, including longitudinal studies to identify concerns about child development (President's Council on Bioethics, 2004). Although a large body of literature suggests an association between ART and the adverse outcomes discussed above for children from singleton pregnancies, critical questions remain that can be answered only by a large prospective study that includes appropriate measures (Schieve, Rasmussen, et al., 2004; Schieve et al., 2005). These questions include parsing whether the associations are due to the ART or are due to the underlying condition of infertility itself, estimating the potential relative contribution of different types of ART treatment (most notably, ICSI), the relative contribution of ART to fetal growth restriction and preterm delivery (important for understanding underlying etiologic pathways and possible routes for prevention of low birth weight), and identifying the true magnitude of the association based on standardized prospective identification of outcomes. Prospective identification of outcomes is essential for adequate ascertainment of fetal growth restriction, birth defects, and developmental disabilities.

3.3 Scientific Merit

One previous study that has similar design characteristics and is of a similar size to the National Children's Study (NCS) is the Danish national birth cohort. This study enrolled subjects during the first or second trimester of pregnancy from 1997 to 2003 (Zhu et al., 2006). This study included 50,897 singleton births and 1,366 twin births born to normally fertile women, 5,764 singletons and 100 twins born spontaneously to subfertile couples, and 4,588 singletons and 1,690 twins born to subfertile couples with treatment. Exposure data for treatment were obtained by questionnaires, and pregnancy and child outcomes were obtained by linking to the national hospital register and medical birth register. As noted, this study found an increase in genital organ malformations among singletons conceived with the aid of infertility treatment compared to subfertile couples who conceived naturally. When broken out by treatment class, there was some evidence this might be most related to undergoing ICSI. There was also an increase of all malformations among spontaneously conceiving couples with longer times to pregnancy

(adjusted hazard ratio 1.3 for couples taking longer than 12 months to conceive compared to couples taking two months to conceive). Thus, this study found some evidence suggesting both connections to adverse outcomes both for underlying infertility and for infertility treatment.

The NCS could examine these questions:

- **Exposure:** Retrospective assessment through questionnaires of the type of infertility treatment, including different types of ART and other treatments. Prospective data from maternal reports may be available from women participating in the prepregnancy aspects of the Study.

- **Outcome:** Fetal growth trajectories assessed by ultrasound in utero. Standardized clinical examinations for malformations at birth. Capture of most early pregnancy outcomes, including early miscarriage or abortion. Standardized sequential neurodevelopmental evaluations for childhood.

- **Extended outcomes:** Longer term follow-up for developmental issues in adolescence, including pubertal transitions.

- **Mechanisms:** Genetic and epigenetic investigations of both parents and of the children.

- **Potential confounders:** Detailed data on environmental and chemical exposures, dietary exposures, as well as data on treatments received during pregnancy.

3.4 Potential for Innovative Research

As described immediately above, the NCS would be able to examine these hypotheses more rigorously than any study. Perhaps the greatest potential for innovation in relation to these hypotheses comes from the opportunity to investigate the potential genetic and epigenetic mechanisms for adverse outcomes of ART within a large cohort study (Bjornsson et al., 2004). In addition, structuring the study to test these hypotheses will result in a cohort able to answer questions about other specific child health outcomes from subfertility and ART that may arise in the future.

3.5 Feasibility

Most of the measurements necessary for this hypothesis are already incorporated into the study protocol or else can be easily added with minimal change of burden for the participants. The follow-up schedule is adequate for the study. The major alteration that would be required in the study to address this hypothesis would be to expand the sampling frame to include a targeted subsample of couples undergoing ART as described under “Power and Sample Size.” Addressing these hypotheses with the study protocol is feasible.

4. Exposure Measures

The following lists include measures relevant to a robust exploration of the hypotheses, including exploring possible mechanisms and confounders:

4.1 Individuals Targeted for Measurement

Primary/maternal

- Maternal reports of reproductive health/infertility.
- Genetic information.
- Hormones.

Primary/paternal

- Infertility.
- Genetic information.

4.2 Methods

Primary/maternal

- Questionnaire materials: Age; prior menstrual and pregnancy history; history of any infertility evaluation and treatment for index or expected pregnancy; time "at risk" of pregnancy at time of study entry and again at time of diagnosis of pregnancy; smoking; alcohol; and socioeconomic status.
- Desirable questionnaire measures: Daily diaries of bleeding, coitus, and vaginal mucus discharge for at least one of the cycles leading to the index pregnancy performed and recorded by the ART center. For women not completing daily diaries, usual frequency of sexual intercourse will be queried upon entry into the study. Prior gynecologic history, including the history of sexually transmitted infection. Family planning history (especially use of hormonal methods). History of infertility evaluation and treatment in past. Time to pregnancy question for past pregnancies. Prior history of periods of noncontracepted sexual intercourse and family planning use.
- Anthropomentric measures: Height and weight.
- Exact infertility diagnoses and details of treatments, including ART (IVF and ICSI), and other evaluations or treatments.
- Prenatal treatments (such as progesterone) for index pregnancy. (These cannot be obtained reliably from the couple by questionnaires alone).
- Basal serum FSH and estradiol levels if available from the ART Center.
- DNA of both parents.

Primary/paternal

- Essential questionnaire materials: Age; history of infertility evaluation or treatment for index pregnancy; smoking; alcohol; and socioeconomic status.

- Exact infertility diagnoses and details of treatments, including ART (IVF and ICSI), and other evaluations or treatments.
- Prior andrologic history, including sexually transmitted infection, pregnancies caused, and prior history of infertility treatments.
- Anthropomentric measures: Height and weight.
- DNA of both parents.

4.3 Life Stage

Primary/maternal

- Preconception/periconception and following birth (depending on contact availability)

Primary/paternal

- Preconception/periconception and following birth (depending on contact availability)

5. Outcome Measures

The following lists include measures relevant to a robust exploration of the hypotheses, including exploring possible mechanisms and confounders:

5.1 Outcomes Targeted for Measurement in Child

Primary

- Preterm birth.
- Congenital malformations.
- Developmental disorders.

5.2 Methods

Essential questionnaire measures

- Child developmental measures: Screening questionnaires regarding the child periodically in childhood; assessment of social environment for the child in relation to potential impacts on development.
- Standardized physical examination of all neonates and infants with attention to the ascertainment of birth defects.
- Abstraction of relevant clinical information on birth defects from medical records.

- Reviews of clinical information for classification of birth defects by clinical geneticists.
- Standardized psychoneurologic assessment for developmental milestones periodically through childhood.
- Neonatal complications; evaluations or treatments in relation to possible birth defects or developmental delay.
- Desirable measures from medical records (ideally prospectively or perhaps retrospectively): School records.
- Biologic measures: DNA of infant/child.
- Fetal biometric measurements from ultrasound.
- Radiology: Neonatal cardiac ultrasound, if available (for structural defects).

5.3 Life stage

Prenatal, birth, and childhood through early adulthood.

6. Important Confounders, Mediators and Effect Modifiers

- Maternal age at conception
- Parity
- Previous obstetric outcome
- Ethnicity
- Socioeconomic status
- Genetic carrier states of parents

7. Power and Sample Size

The study design must ensure the sample selected is appropriate to study the effects of ART on various outcomes for singleton births separately. Because a high proportion of ART births are multiple, this must be factored into sample size estimations. It may be desirable to inflate the ART sample in order to have full power to assess major subtypes of ART (e.g., IVF with and without ICSI). Based on the following table, a minimum of 3,000 and a maximum of 16,000 ART-exposed pregnancies will be needed to adequately address the hypotheses, depending on whether power is sought for specific conditions of low incidence, and whether full power is desired to analyze two subgroups of ART. By comparison, the Danish national birth cohort included 4,588 singleton infants exposed to infertility treatment of which only a subgroup of unreported size actually had ART (Zhu et al, 2006). Within the existing study sample frame, approximately 1,000 infants will be ART-exposed, meaning that targeted oversampling for participants with ART will be necessary for sufficient power for the hypotheses. Of the

approximately 85,000 non-ART pregnancies available for the nonexposed comparison group, approximately 7 percent (5,950) can be expected to be pregnancies resulting from spontaneous conceptions to subfertile couples, allowing for somewhere between a 1:1 and 10:1 ratio of ART-exposed to unexposed pregnancies resulting from spontaneous conceptions from subfertile couples.

Table 1. Sample Size Calculation

1 Outcome of Interest	2 Prevalence in general population	3 Minimum expected increase in ART singleton population	4 Sample size of singleton births needed in ART group	5 Number of ART live birth deliveries needed to obtain number of singletons in column 4	6 Number of ART pregnancies needed to obtain number of deliveries in column 5
Intrauterine growth restriction (term infants)	5%	1.5 fold (7.5%)	925	1,423	1,674
Preterm delivery	10.0%	1.5 fold (15.0%)	434	668	809
Birth defects (all)	3.0%	1.5 fold (4.5%)	1,578	2,428	2,856
Serious developmental disabilities (all)	2.0%	1.5 fold (3.0%)	2,395	3,685	4,335
Mild-serious developmental disabilities (all)	17.0%	1.5 fold (25.5%)	233	358	421
Specific birth defect or developmental disability	1.0%	2.0 fold (2.0%)	1,445	2,223	2,615
Specific birth defect or developmental disability	0.5%	2.0 fold (1.0%)	2,909	4,475	5,265
Specific birth defect or developmental disability	0.3%	2.0 fold (0.6%)	4,861	7,478	8,798

Assumptions: Column 4: At least 4:1 ratio non-ART (nonexposed) to ART infants; alpha = 0.05 (two-sided); Beta = 0.2. Column 5: 65% of deliveries will be singletons. Column 6: 15% fetal loss rate.

8. Other Design Issues

- **Ethical/burden considerations:** Couples seeking ART are already undergoing intensive and time-consuming medical testing and treatments. Preconception data collection could be structured to coincide with their regular visits to an infertility clinic. While it would be ideal to recruit all ART-exposed women preconceptionally,

it would also be possible to recruit women and their partners at the time of conception. For many couples, infertility is an emotionally charged condition, and it may turn out that willingness to participate is more likely once conception has occurred.

- **Cost/complexity of data collection:** A sampling strategy that incorporates selected recruitment at infertility treatment centers will be required. These centers should be located at or near the study sites to maximize comparability of the women recruited. Arrangements will need to be made with infertility clinics to facilitate coordination of data collection, including review of records of diagnosis and treatment.

9. References

Bergh, T., Ericson, A., Hillensjo, T., Nygren, K.G., & Wennerholm, U.B. (1999). Deliveries and children born after in-vitro fertilisation in Sweden 1982-95: A retrospective cohort study. *Lancet, 354*(9190), 1579-1585.

Bjornsson, H.T., Fallin, M.D., & Feinberg, A.P. (2004). An integrated epigenetic and genetic approach to common human disease. *Trends in Genetics, 20*(8), 350-358.

Bonduelle, M., Wennerholm, U.B., Loft, A., Tarlatzis, B.C., Peters, C., Henriet, S., et al. (2005). A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. *Human Reproduction, 20*(2), 413-9.

Boyle, C. & Cordero, J. (2005). Birth defects and disabilities: A public health issue for the 21st century. *American Journal of Public Health, 95*(11), 1884-1886.

Buck Louis, G.M., Schisterman, E.F., Dukic V.M., & Schieve, L.A. (2005). Research hurdles complicating the analysis of infertility treatment and child health. *Human Reproduction, 20*(1), 12-18.

Catt, J., Wood, T., Henman, M., & Jansen, R. (2003). Single embryo transfer in IVF to prevent multiple pregnancies. *Twin Research, 6*(6), 536-539.

Centers for Disease Control and Prevention, National Center on Birth Defects and Developmental Disabilities. (2005). Birth defects: Frequently asked questions. Retrieved May 11, 2007, from http://www.cdc.gov/ncbddd/bd/facts.htm

Centers for Disease Control and Prevention. (2000). Contribution of assisted reproductive technology and ovulation-inducing drugs to triplet and higher-order multiple births—United States, 1980-1997. *Morbidity and Mortality Weekly Report, 49*(24), 535-538.

Centers for Disease Control and Prevention. (2004). Economic costs associated with mental retardation, cerebral palsy, hearing loss, and vision impairment—United States, 2003. *Morbidity and Mortality Weekly Report, 53*(3), 57-59.

Centers for Disease Control and Prevention, (2004). Assisted reproductive technology success rates: National summary and fertility clinic reports. Atlanta: Author.

Centers for Disease Control and Prevention. (2006). 2004 Assisted reproductive technology success rates: National summary and fertility clinic reports. Division of Reproductive Health. Atlanta: Author.

Chandra, A., Martinez, G., Mosher, W.D., Abma, J.C., & Jones, J. (2005). Fertility, family planning, and reproductive health of U.S. women: Data from the 2002 National Survey of Family Growth. *National Center for Health Statistics Vital Health Statistics 23*(25).

Chang, A.S., Moley, K.H., Wangler, M., Feinberg, A.P., & Debaun, M.R. (2005). Association between Beckwith-Wiedemann syndrome and assisted reproductive technology: A case series of 19 patients. *Fertility and Sterility, 83*(2), 349-54.

Committee on Understanding Premature Birth and Assuring Health Outcomes, Board on Health Sciences Policy, Behrman, R.E., & Butler, A.S. (Eds.). (2006). Preterm birth: Causes, consequences, and prevention. Washington, DC: National Academies Press.

Cox, G. F., J. Burger, Lip, V., Mau, U.A., Sperling, K., Wu, B.L., et al. (2002). Intracytoplasmic sperm injection may increase the risk of imprinting defects. *American Journal of Human Genetics, 71*(1), 162-164.

Cuevas, K.D., Silver, D.R., Brooten, D., Youngblut, J.M., & Bobo, C.M. (2005). The cost of prematurity: Hospital charges at birth and frequency of rehospitalizations and acute care visits over the first year of life: A comparison by gestational age and birth weight. *American Journal of Nursing, 105*, 56-64.

Davis, O. K. (2004). Elective single-embryo transfer—has its time arrived? *New England Journal of Medicine, 351*(23), 2440-2442.

DeBaun, M.R., Niemitz, E.L., & Feinberg, A.P. (2003). Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *American Journal of Human Genetics, 72*(1), 156-160.

Dhont, M., De Sutter, P., Ruyssinck, G., Martens, G., & Bekaert, A. (1999). Perinatal outcome of pregnancies after assisted reproduction: A case-control study. *American Journal of Obstetrics and Gynecology, 181*(3), 688-695.

Ericson, A. & Kallen, B. (2001). Congenital malformations in infants born after IVF: A population-based study. *Human Reproduction, 16*(3), 504-509.

FIVNAT (French In Vitro National). (1995). Pregnancies and births resulting from in vitro fertilization: French national registry, analysis of data 1986 to 1990. *Fertility and Sterility, 64*(4), 746-756.

Friedler, S., Mashiach, S., & Laufer, N. (1992). Births in Israel resulting from in-vitro fertilization/embryo transfer, 1982-1989: National Registry of the Israeli Association for Fertility Research. *Human Reproduction, 7*(8): 1159-1163.

Gissler, M., Malin Silverio, M., Hemminki, E. (1995). In-vitro fertilization pregnancies and perinatal health in Finland 1991-1993. *Human Reproduction, 10*(7), 1856-1861.

Hansen, M., Bower, C., Milne, E., de Klerk, N., & Kurinczuk, J.J. (2005). Assisted reproductive technologies and the risk of birth defects—A systematic review. *Human Reproduction, 20*(2): 328-338.

Hansen, M., Kurinczuk, J.J., Bower, C., & Webb, S. (2002). The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *New England Journal of Medicine, 346*(10): 725-30.

Helmerhorst, F.M., Perquin, D.A., Donker, D., & Keirse, M.J. (2004). Perinatal outcome of singletons and twins after assisted conception: A systematic review of controlled studies. *British Medical Journal, 328*(7434), 261.

Jackson, R.A., Gibson, K.A., Wu, Y.W., & Croughan, M.S. (2004). Perinatal outcomes in singletons following in vitro fertilization: A meta-analysis. *Obstetrics and Gynecology, 103*(3), 551-563.

Kallen, B., Finnstrom, O., Nygren, K.G., & Olausson, P.O. (2005). In vitro fertilization (IVF) in Sweden: Risk for congenital malformations after different IVF methods. *Birth Defects Research Part A: Clinical and Molecular Teratology, 73*(3), 162-169.

Kapiteijn, K., de Bruijn, C.S., de Boer, E., de Craen, A.J., Burger, C.W., van Leeuwen, F.E., et al. (2006). Does subfertility explain the risk of poor perinatal outcome after IVF and ovarian hyperstimulation? *Human Reproduction, 21*(12), 3228-3234.

Khosla, S., Dean, W., Brown, D., Reik, W., & Feil, R.. (2001). Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biology of Reproduction, 64*(3), 918-926.

Klemetti, R., Gissler, M., Sevon, T., Koivurova, S., Ritvanen, A., & Hemminki, E. (2005). Children born after assisted fertilization have an increased rate of major congenital anomalies. *Fertility and Sterility, 84*(5): 1300-1307.

Kuban, K.C., & Leviton A. (1994). Cerebral palsy. *New England Journal of Medicine, 330*(3): 188-95.

Lynberg, M.C., & L.D. Edmonds. (1994). State use of birth defects surveillance (pp. 217-230). In L.S. Wilcox & J.S. Marks (Eds.). Data to Action: CDC's Public Health Surveillance for Women, Infants, and Children. Atlanta: Centers for Disease Control and Prevention.

Maher, E.R. (2005). Imprinting and assisted reproductive technology. *Human Molecular Genetics 14 Special No. 1*, R133-R138.

Martin, J.A., & Park, M. (1999). Trends in twin and triplet births:1980-97. *National Vital Statistics Reports, 47*(24).

Merlob, P., Sapir, O., Sulkes, J., & Fisch, B. (2005). The prevalence of major congenital malformations during two periods of time, 1986-1994 and 1995-2002 in newborns conceived by assisted reproduction technology. *European Journal of Medical Genetics, 48*(1), 5-11.

MRC Working Party on Children Conceived by In Vitro Fertilisation. (1990). Births in Great Britain resulting from assisted conception, 1978-87. *British Medical Journal, 300*(6734), 1229-1233.

Olivennes, F., Fanchin, R., Ledee, N., Righini, C., Kadoch, I.J., & Frydman, R. (2002). Perinatal outcome and developmental studies on children born after IVF. *Human Reproduction Update, 8*(2), 117-128.

Olson, C. K., Keppler-Noreuil, K.M., Romitti, P.A., Budelier, W.T., Ryan, G., Sparks, A.E., et al. (2005). In vitro fertilization is associated with an increase in major birth defects. *Fertility and Sterility, 84*(5), 1308-1315.

Peleg, D., Kennedy, C.M., & Hunter, S.K. (1998). Intrauterine growth restriction: Identification and management. *American Family Physician, 58*(2), 453-460, 466-467.

Poikkeus, P., Gissler, M., Unkila-Kallio, L., Hyden-Granskog, C., & Tiitinen, A. (2007). Obstetric and neonatal outcome after single embryo transfer. *Human Reproduction, 22*(4), 1073-1079.

Ponjaert-Kristoffersen, I., Bonduelle, M., Barnes, J., Nekkebroeck, J., Loft, A., Wennerholm, U.B., et al. (2005). International collaborative study of intracytoplasmic sperm injection-conceived, in vitro fertilization-conceived, and naturally conceived 5-year-old child outcomes: Cognitive and motor assessments. *Pediatrics, 115*(3): e283-e289.

President's Council on Bioethics. (2004). Reproduction and Responsibility: The Regulation of New Biotechnologies. Washington, DC: Government Printing Office.

Rice, C., Schendel, D., Cunniff, C., & Doernberg, N. (2004). Public health monitoring of developmental disabilities with a focus on the autism spectrum disorders. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics, 125C*(1), 22-27.

Sandhu, B., Stevenson, R.C., Cooke, R.W., & Pharoah, P.O. (1986). Cost of neonatal intensive care for very-low-birthweight infants. *Lancet, 1*(8481): 600-3.

Schieve, L.A., Ferre, C., Peterson, H.B., Macaluso, M., Reynolds, M.A., & Wright, V.C. (2004). Perinatal outcome among singleton infants conceived through assisted reproductive technology in the United States. *Obstetrics and Gynecology, 103*(6), 1144-1153.

Schieve, L.A., Rasmussen, S.A., & Reefhuis, J. (2005). Risk of birth defects among children conceived with assisted reproductive technology: Providing an epidemiologic context to the data. *Fertility and Sterility, 84*, 1320-1324; discussion 1327.

Schieve, L.A., Meikle, S.F., Ferre, C., Peterson, H.B., Jeng, G., & Wilcox, L.S.. (2002). Low and very low birth weight in infants conceived with use of assisted reproductive technology. *New England Journal of Medicine, 346*(10), 731-737.

Schieve, L.A., Rasmussen, S.A., Buck, G.M., Schendel, D.E., Reynolds, M.A., & Wright, VC. (2004). Are children born after assisted reproductive technology at increased risk for adverse health outcomes? *Obstetrics and Gynecology, 103*(6), 1154-1163.

Schultz, R. M. (2005). From egg to embryo: A peripatetic journey. *Reproduction, 130*, 825-828.

Schultz, R.M. & Williams, C.J. (2002). The science of ART. *Science, 296*, 2188-2190.

Simpson, G.A., Colpe, L., & Greenspan, S. (2003). Measuring functional developmental delay in infants and young children: Prevalence rates from the NHIS-D. *Paediatric and Perinatal Epidemiology, 17*(1): 68-80.

Stanford, J., Ellis Simonsen, S., & Baksh, L. (2007). Infertility in Utah, 2004-2005. *Utah's Health: An Annual Review, 12*(Suppl. Women's Health in Utah), 153-157.

Stromberg, B., Dahlquist, G., Ericson, A., Finnstrom, O., Koster, M., & Stjernqvist, K. (2002). Neurological sequelae in children born after in-vitro fertilisation: A population-based study. *Lancet, 359*(9305), 461-465.

Sutcliffe, A.G., Peters, C.J., Bowdin, S., Temple, K., Reardon, W., Wilson, L., et al. (2006). Assisted reproductive therapies and imprinting disorders—A preliminary British survey. *Human Reproduction, 21*(4), 1009-1011.

Van Naarden Braun, K., Autry, A., & Boyle, C. (2005). A population-based study of the recurrence of developmental disabilities—Metropolitan Atlanta Developmental Disabilities Surveillance Program, 1991-94. *Paediatric and Perinatal Epidemiology, 19*(1): 69-79.

VanVoorhis, B., Sparks, A., Syrop, C.H., Sipe, C.S., Ryan, G.L., & Dokras, A. (2006). *Anovulation may be a risk factor for major birth defects in children born after infertility treatment*. Concurrent session conducted at the American Society of Reproductive Medicine 2006 Annual Meeting, New Orleans, LA.

Verlaenen, H., Cammu, H., Derde, M.P., & Amy, J.J. (1995). Singleton pregnancy after in vitro fertilization: Expectations and outcome. *Obstetrics and Gynecology, 86*(6), 906-910.

Wennerholm, U.B., Bergh, C., Hamberger, L., Lundin, K., Nilsson, L., Wikland, M., et al. (2000). Incidence of congenital malformations in children born after ICSI. *Human Reproduction 15*(4), 944-948.

Westergaard, H.B., Johansen, A.M., Erb, K., & Andersen, A.N. (1999). Danish National In-Vitro Fertilization Registry 1994 and 1995: A controlled study of births, malformations and cytogenetic findings. *Human Reproduction, 14*(7), 1896-1902.

Yeargin-Allsopp, M., Rice, C., Karapurkar, T., Doernberg, N., Boyle, C., & Murphy, C. (2003). Prevalence of autism in a U.S. metropolitan area. *Journal of the American Medical Association, 289*(1): 49-55.

Young, L.E., Fernandes, K., McEvoy, T.G., Butterwith, S.C., Gutierrez, C.G., Carolan, C., et al. (2001). Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. *Nature Genetics, 27*(2): 153-4.

Zhu, J.L., Basso, O., Obel, C., Bille, C., & Olsen, J. (2006). Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. *British Medical Journal, 333*, 679.

MATERNAL SUBCLINICAL HYPOTHYROIDISM AND NEURODEVELOPMENTAL DISABILITIES/ADVERSE PREGNANCY OUTCOMES

1. Meta Hypothesis

Maternal subclinical hypothyroidism is associated with adverse pregnancy outcomes and neurodevelopmental disabilities.

2. Specific Hypotheses

1. Maternal subclinical hypothyroidism during pregnancy results in changes in neurodevelopmental trajectories in the offspring.

2. Maternal/fetal environmental exposures during gestation result in changes in neurodevelopmental trajectories in children, at least in part, via disruption of the maternal thyroid system.

3. Maternal subclinical hypothyroidism during gestation results in adverse pregnancy outcomes, specifically preterm birth (delivery earlier than 37 weeks) and preeclampsia.

4. Maternal hypothyroidism in the first trimester of pregnancy before the fetus can produce its own thyroxine is more damaging to childhood neurodevelopment than hypothyroidism later in pregnancy (American Thyroid Association [ATA], 2004).

3. Background and Justification

Epidemiologic data show suboptimal thyroid function in pregnancy is associated with impaired intellectual development. Two studies have shown an association between low thyroid hormone concentrations in early gestation and significant IQ decrements in children at 7 years and 10 months of age, respectively (Klein et al., 2001; Pop et al., 1999) although both studies are single-center studies with small sample sizes. Further evidence for this association comes from a recent study showing an inverse correlation between the severity of maternal hypothyroidism and IQ of the offspring (Klein et al., 2001) and from a study suggesting maternal hypothyroidism in the first trimester of pregnancy before the fetus can produce its own thyroxine is more damaging to childhood neurodevelopment than hypothyroidism later in pregnancy (Pop et al., 2003).

In 2004, a statement from the ATA and the American Association of Clinical Endocrinologists workshop "Impact of Maternal Thyroid Status on Pregnancy and Fetal and Childhood Development" concluded although hypothyroidism from iodine deficiency in the United States has been addressed, "there are still additional adverse outcomes for maternal health, maintenance of pregnancy, and child development that may occur as a result of overt maternal hypothyroidism, as well as subclinical hypothyroidism (normal serum thyroxine concentration and elevated serum thyroid-stimulating hormone concentration), maternal hypothyroxinemia (depressed serum free thyroxine concentration), and the presence of thyroid autoantibodies (Pop et al., 2003)." The ATA highlighted several recent research findings, among which two are particularly relevant to this proposed hypothesis and need further data with regards to "magnitude:"

- Pregnant mothers with overt or subclinical hypothyroidism are at an increased risk for premature delivery.

- The offspring of mothers with (subclinical) thyroid hormone deficiency or thyroid-stimulating hormone elevation during pregnancy may be at risk of mild impairment in their intellectual function and motor skills (Casey, 2005).

Autoimmune disorders are considered to be the most common cause of hypothyroidism in reproductive-age women (Brucker-Davis, 1998). However, endocrine disruption as a mode of action for the toxicity of chemicals has been of increasing interest and concern during the past decade. Of particular interest are chemicals demonstrated to alter thyroid hormone function in laboratory animals and wildlife. There is much less information from studies that assess thyroid effects in humans (Howdeshell, 2002). A recent review listed 116 chemicals that could interact with thyroid hormone status (Landrigan, Garg, & Droller, 2003). Although the potential of environmental chemicals to interfere with thyroid function is well established, the role of endocrine disruption in hypothyroidism at the population level is not known.

While the literature suggests variations of endocrine parameters, such as thyroid dysfunction in utero, appear to be associated with developmental disabilities in the resulting child, the threshold for manifestation of these effects is currently undefined. There are information gaps regarding the potential effects of hormonally active agents in the environment on the developing fetus via maternal endocrine disruption. There also are potential direct interactions with the fetus for agents that can cross the placental barrier. A recent conference that focused on evaluation of endocrine disruption within the National Children's Study (NCS) investigated the issue of thyroid hormone function as well as other endocrine effects. Scientists who participated in this workshop highlighted the potential for chemical exposures to cause subclinical hypothyroidism or subclinical hypothyroxinemia and thus increase the risk for adverse health and developmental outcomes in children as an important priority for the Study (Longnecker et al., 2003; Chung, Lau, Yip, Chiu, & Lee, 2001).

Maternal depression affects fetal health (Van den Bergh, Mulder, Mennes, & Glover, 2005; Chrousos, Torpy, & Gold, 1998). Although psychological and biological explanations have been researched, hypotheses focused on hormonal exposures have received more attention. Depression is known to affect thyroid and pituitary function and is associated with hypothalamo-pituitary-adrenal (HPA) axis hyperactivity. Maternal stress, anxiety, or depression (factors regulated by peptides derived from the activated HPA axis) each affect birth outcomes (Paarlberg, Vingerhoets, Passchier, Dekker, & van Geijn, 1995; Sandman et al., 1994; Sandman, Wadhwa, Chicz-DeMet, Dunkel-Schetter, & Porto, 1997; Smith et al., 1990; Wadhwa, Dunkel-Schetter, Chicz-DeMet, Porto, & Sandman, 1996). Thus, increased HPA-axis activity, including subclinical hypothyroidism and hypothyroxinemia, through psychological influences such as depression, may directly affect maternal and fetal function, fetal growth, and ex utero growth and function.

Clinical maternal hypothyroidism also has been linked to adverse pregnancy outcomes, including infertility, preeclampsia, placental abruption, postpartum hemorrhage, and associated perinatal morbidity and mortality with a high frequency of low birth weight and fetal death (Davis, Leveno, & Cunningham, 1988; Lao, Chin, & Swaminathan, 1988). Subclinical hypothyroidism has been linked to eclampsia, preeclampsia, and gestational hypertension (Leung, Millar, Koonings, Montoro, & Mestman, 1993). Thyroid hormone status (decreased free T4 and increased thyroid-stimulation hormone) also has been found to be related to severity of preeclampsia and degree of low birth weight (Basbug et al., 1999). Preterm babies born to mothers with preeclampsia and hypothyroxinemia show lower thyroid hormone levels in utero and at birth (Belet, Imdat, Yanik, & Kucukoduk, 2003; Chan, Chiu, & Lau, 2003). Evidence supports the premise that clinical hypothyroidism is associated with preeclampsia, preterm birth, and associated adverse outcomes in infants. Subclinical hypothyroidism and hypothyroxinemia may be implicated as well, but it has not been adequately studied.

3.1 Public Health Importance

Prevalence/incidence

The prevalence of subclinical hypothyroidism during pregnancy in the United States is estimated at 2.4 percent (Casey et al., 2005). No data exist to define the proportion of these cases resulting, either in part or in total, from environmental exposures. While preeclampsia and spontaneous preterm birth remain major contributors to adverse long-term outcomes for children, it is not clear what proportion of these cases are from subclinical hypothyroidism, environmental exposures, or the interaction between environmental exposures and resultant thyroid dysfunction.

Economic and/or social burden

No studies have precisely calculated the costs associated with thyroid dysfunction during pregnancy. Although newborn screening for congenital hypothyroidism already occurs in all states, maternal screening for thyroid dysfunction during pregnancy is not yet considered an obstetric standard of care. The screening of all million pregnant women delivering live-born infants each year in the United States could lead to several billion dollars annually in screening costs alone just for detection of subclinical hypothyroidism or hypothyroxinemia. The costs of treatment would add substantial additional expenditures. All these costs should be adequately justified in a prospective fashion.

3.2 Justification for a Large Prospective Longitudinal Study

No data exist to guide clinical obstetric decision making regarding appropriate interventions for subclinical hypothyroidism or hypothyroxinemia during pregnancy. Although a randomized clinical trial of thyroxine replacement vs. placebo for such women is registered with clinicaltrials.gov (NCT 00388297), results will not be available until at least 2015, and the study is not addressing environmental influences on laboratory testing or outcomes. In addition, this randomized clinical trial will follow subjects only to age 5.

As a result, a large prospective longitudinal study that collects information both about maternal thyroid function and environmental exposures and correlates these data with truly adequate long-term outcomes is needed in order to guide future interventions and public policy.

3.3 Scientific Merit

Such a study has obvious clinical applicability and scientific merit. All of the dependent and independent variables described below are validated and accepted as appropriate clinical markers capable of answering the relevant study hypotheses. The potential clinical implications are tremendous.

3.4 Potential for Innovative Research

The evolving disciplines of genomics, proteomics, and epigenetics will offer multiple opportunities to assess the interactions between maternal and fetal thyroid genetic regulation and environmental exposure.

3.5 Feasibility

This study is likely to be accomplished within the NCS framework. The independent variable samples will be collected within the basic framework of the study (maternal and cord blood, environmental exposures, long-term function of children). No extra interventions are required beyond

those already outlined in the Study's basic framework. Information will also be available on all newborns from newborn metabolic screening.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Maternal thyroid status, including L-thyroxine (T4), free thyroxine (free T4), L-triiodothyronine (T3), free T3, thyroid stimulating hormone, thyroid gland enlargement, and serologic markers of thyroid autoimmunity (anti-thyroid peroxidase, anti-thyroglobulin, thyroid-stimulating, and TSH receptor-binding inhibitory antibodies), prepregnancy or in the first trimester, as well as in the third trimester.

- Maternal stress during pregnancy as assessed by stress hormones (e.g., cortisol) and report of stressful situations, anxiety, or depression.

Primary/child

- Neonatal thyroid status as assessed by L-thyroxine (T4), free thyroxine (free T4), L-triiodothyronine (T3), and thyroid stimulating hormones.

4.2 Methods

Primary/maternal

- Biological specimens: Blood, urine, saliva, breast milk

- Examination by a medical professional

- Interview(s)

Primary/child

- Umbilical cord blood culture/pathology

- Blood samples/newborn blood spots

4.3 Life Stage

Primary/maternal

- Repeated measures: Preconception, first through third trimesters, birth, and during nursing period

Primary/child

- Repeated measures at birth, during infancy

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

- Neurodevelopmental trajectories via neurocognitive and developmental tests including IQ, domain-specific evaluations of attention/concentration, executive function, learning, memory and motor skills
- Preterm birth: Gestational age, birth weight

Secondary

- School performance

5.2 Methods

Primary

- Study personnel will include a neurological examination and clinical and observational tests, including Bayley Scales of Infant Development III, with subtests for cognition, motor skills, and language. Future neurological exams will be age-appropriate.

Secondary

- School record examination for grades/performance

5.3 Life Stage

- Primary/child: 1, 6, 12, and 18 months; 3, 5, 7, 9, 12, 16, and 20 years
- Secondary/child: Follow-up in year 7, 9, 12, 16, and 20

6. Important Confounders, Mediators, and Effect Modifiers

- **Child's exposure to neurotoxicants (e.g., lead) during childhood:** Increased exposure to other neurotoxins would increase adverse neurodevelopment behavior (Steenland et al., 1994; Calvert et al., 1998).
- **Family history:** Twin and family studies have suggested a genetic link, which may be shown through family histories, to neurodevelopmental disorders (Muhle, Trentacoste, & Rapin, 2004).
- **Mother's medical and obstetrical histories:** Particularly in cases without a family history, various obstetric complications are often cited as possible causes for fetal neurodevelopmental disruption and consequent disorders (Nelson & Willoughby, 2000).

- **Economic status:** Increased risk of preterm birth is associated with low educational level; unmarried status (Centers for Disease Control and Prevention, 1990).

- **Race/ethnicity:** As a percent of live births, 17.6 percent among blacks are preterm, 11.4 percent among Hispanics, and 10.8 percent among whites (U.S. Department of Health and Human Services, 2003.).

- **Mother's medical history:** Increased risk of preterm birth is associated with maternal smoking, alcohol consumption; older maternal age (indicated preterm births); younger maternal age (spontaneous preterm births); low or high parity; previous stillbirth (Heffner, Sherman, Speizer, & Weiss, 1993; Meis et al., 1995; Wisborg, Henriksen, Hedegaard, & Secher, 1996).

- **Function of endocrine disruptors:** The role that endocrine disruptors play in the relationship between maternal hypothyroidism and child neurodevelopment will be investigated. Measurements of endocrine disruptors will be made available through data collected for other NCS hypotheses (in particular, Hypothesis 5) and will allow for the examination of whether endocrine disruptors have an impact on maternal thyroid status during pregnancy and on neurodevelopment of the offspring.

- **Others:** Unwantedness of the pregnancy (Olsen et al., 1995; McDonald, Armstrong, & Sloan, 1992).

7. Power and Sample Size

Assuming 100,000 infants born into the Study with an exposure prevalence of 2 percent, a type I error of 0.05, and power of 0.8, the smallest detectable relative risk would be, for cerebral palsy, 2.8, and for autism, 2.4. Adjusted odds ratios for Bayley PDI are less than 85 for infants of women with free T4 values less than the tenth percentile at 12 weeks' gestation have been reported as 5.8 (95 percent confidence interval 1.3-12.6) (Pop et al., 1999). In Maine, 15 percent of children born to women with hypothyroidism measured in the second trimester scored below 85 on WISC full-scale IQ at a mean age of 8 years, compared to 5 percent of children of women who were euthyroid (Haddow et al., 1999). These studies, which suggest three-fold or greater risks of intellectual disabilities in the offspring of mothers with hypothyroidism indicate the Study will have the power to detect similar relative risks.

8. Other Design Issues

- **Cost/complexity of data collection:** Since the Study will collect maternal serum in the first and third trimesters of pregnancy and the children will be routinely followed for their cognitive and neurological development, this hypothesis could be addressed through data and specimens already included in the study plan.

- **Cost of sample analysis:** Analyses to assay TSH in maternal serum are inexpensive and can be automated. Free T4, however, is somewhat more complex and expensive to assay.

9. References

American Thyroid Association (ATA). (2004). Statement on Early Maternal Thyroidal Insufficiency: Recognition, Clinical Management and Research Directions. (ATA Public Health Statement). Falls Church, VA: Author.

Basbug, M., Aygen, E., Tayyar, M., Tutus, A., Kaya, E., & Oktem, O. (1999). Correlation between maternal thyroid function tests and endothelin in preeclampsia-eclampsia. *Obstetrics and Gynecology, 94*(4), 551-555.

Belet, N., Imdat, H., Yanik, F., & Kucukoduk, S. (2003). Thyroid function tests in preterm infants born to preeclamptic mothers with placental insufficiency. *Journal of Pediatric Endocrinology and Metabolism, 16*(8), 1131-1135.

Brucker-Davis, F. (1998). Effects of environmental synthetic chemicals on thyroid function. *Thyroid 8*(9), 827-856.

Calvert, G.M., Mueller, C.A., Fajen, J.M., Chrislip, D.W., Russo, J., Briggle, T., et al. (1998). Health effects associated with sulfuryl fluoride and methyl bromide exposure among structural fumigation workers. *American Journal of Public Health, 88*(12), 1774-1780.

Casey, B. (2005). Hypothyroidism. (2005). In J.T. Queenan, J.C. Hobbins, & C.Y. Spong CY (Eds). *Protocols for high-risk pregnancies* (4th ed., pp.235-238). Malden, MA: Blackwell.

Casey, B.M., Dashe, J.S., Wells, C.E., McIntire, D.D., Byrd, W., Leveno, K.J., et al. (2005). Subclinical hypothyroidism and pregnancy outcomes. *Obstetrics and Gynecology, 105*(2), 239-245.

Centers for Disease Control and Prevention. (1990). Effects of maternal cigarette smoking on birth weight and preterm birth—Ohio, 1989. *Morbidity and Mortality Weekly Report, 39*(38), 662-665.

Chan, L.Y., Chiu, P.Y., & Lau, T.K. (2003). Cord blood thyroid-stimulating hormone level in high-risk pregnancies. *European Journal of Obstetrics and Gynecology and Reproductive Biology, 108*(2), 142-145.

Chrousos, G.P., Torpy, D.J., & Gold, P.W. (1998). Interactions between the hypothalamicpituitary-adrenal axis and the female reproductive system: Clinical implications. *Annals of Internal Medicine, 129*(3), 229–240.

Chung, T.K., Lau, T.K., Yip, A.S., Chiu, H.F., & Lee, D.T. (2001). Antepartum depressive symptomatology is associated with adverse obstetric and neonatal outcomes. *Psychosomatic Medicine, 63*(5), 830–834.

Davis, L.E., Leveno, K.J., & Cunningham, F.G. (1988). Hypothyroidism complicating pregnancy. *Obstetrics and Gynecology, 72*(1), 108-112.

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Division of Vital Statistics, National Center for Health Statistics. (2003). VitalStats. Retrieved May 17, 2007, from http://www.cdc.gov/nchs/datawh/vitalstats/VitalStatsbirths.htm

Haddow, J.E., Palomaki, G.E., Allan, W.C., Williams, J.R., Knight, G.J., Gagnon, J., et al. (1999). Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *New England Journal of Medicine, 341*(8), 549-555.

Heffner, L.J., Sherman, C.B., Speizer, F.E., & Weiss, S.T. (1993). Clinical and environmental predictors of preterm labor. *Obstetrics and Gynecology, 81*(5, Pt. 1), 750-757.

Howdeshell, K.L. (2002). A model of the development of the brain as a construct of the thyroid system. *Environmental Health Perspectives, 110*(Suppl. 3), 337-348.

Klein, R.Z., Sargent, J.D., Larsen, P.R., Waisbren, S.E., Haddow, J.E., & Mitchell, M.L. (2001). Related Articles, Relation of severity of maternal hypothyroidism to cognitive development of offspring. *Journal of Medical Screening, 8*(1), 18-20.

Landrigan, P., Garg, A., & Droller, D.B. (2003). Assessing the effects of endocrine disruptors in the National Children's Study. *Environmental Health Perspectives, 111*(13), 1678-1682.

Lao, T.T., Chin, R.K., & Swaminathan, R. (1988). Thyroid function in pre-eclampsia. *British Journal of Obstetrics and Gynaecology, 95*(9), 880-883.

Leung, A.S., Millar, L.K., Koonings, P.P., Montoro, M., & Mestman, J.H. (1993). Perinatal outcome in hypothyroid pregnancies. *Obstetrics and Gynecology, 81*(3), 349-353.

Longnecker, M.P., Bellinger, D.C., Crews, D., Eskenazi, B., Silbergeld, E.K., Woodruff, T.J., et al. (2003). An approach to assessment of endocrine disruption in the National Children's Study. *Environmental Health Perspectives, 111*(13), 1691-1697.

McDonald, A.D., Armstrong, B., & Sloan, M. (1992). Cigarette, alcohol, and coffee consumption and prematurity. *American Journal of Public Health, 82*(1), 87-90.

Meis, P.J., Michielutte, R., Peters, T.J., Wells, H.B., Sands, R.E., Coles, E.C., et al. (1995). Factors associated with preterm birth in Cardiff, Wales. II. Indicated and spontaneous preterm birth. *American Journal of Obstetrics and Gynecology, 173*(2):597-602.

Muhle, R., Trentacoste, S.V, & Rapin, I. (2004). The Genetics of Autism. *Pediatrics, 113*(5), e472-e486

Nelson, K.B., & Willoughby, R.E. (2000). Overview: Infection during pregnancy and neurological outcome in the child. *Mental Retardation and Developmental Disabilities Research Reviews, 8*(1), 1-2.

Olsen, P., Laara, E., Rantakallio, P., Jarvelin, M.R., Sarpola, A., & Hartikainen, A.L. (1995). Epidemiology of preterm delivery in two birth cohorts with an interval of 20 years. *American Journal of Epidemiology, 142*(11), 1184-1193.

Paarlberg, K.M., Vingerhoets, A.J., Passchier, J., Dekker, G.A., & van Geijn, H.P. (1995). Psychosocial factors and pregnancy outcome: A review with emphasis on methodological issues. *Journal of Psychosomatic Research, 39*(5), 563–595.

Pop, V.J., Brouwers, E.P., Vader, H.L., Vulsma, T., Van Baar, A.L., & deVijlder, J.J. (2003). Maternal hypothyroxinemia during early pregnancy and subsequent child development: A 3-year follow-up study. *Clinical Endocrinology, 59*(3), 282-288.

Pop, V.J., Kuijpens, J.L., van Baar, A.L., Verkerk, G., van Son, M.M., de Vijlder, J.J., et al. (1999). Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. *Clinical Endocrinology, 50*(2), 149-155.

Sandman, C.A., Wadhwa, P.D., Chicz-DeMet, A., Dunkel-Schetter, C., & Porto, M. (1997). Maternal stress, HPA activity, and fetal/infant outcome. *Annals of the New York Academy of Sciences, 814*, 266–275.

Sandman, C.A., Wadhwa, P.D., Dunkel-Schetter, C., Chicz-DeMet, A., Belman, J., Porto, M., et al. (1994). Psychobiological influences of stress and HPA regulation on the human fetus and infant birth outcomes. *Annals of the New York Academy of Sciences, 739*, 198– 210.

Smith, R., Cubis, J., Brinsmead, M., Lewin, T., Singh, B., Owens, P., et al. (1990). Mood changes, obstetric experience and alterations in plasma cortisol, beta-endorphin and corticotrophin releasing hormone during pregnancy and the puerperium. *Journal of Psychosomatic Research, 34*(1), 53–69.

Steenland, K., Jenkins, B., Ames, R.G., O'Malley, M., Chrislip, D., & Russo, J. (1994). Chronic neurological sequelae to organophosphate pesticide poisoning. *American Journal of Public Health, 84*(5), 731-736.

Van den Bergh, B.R., Mulder, E.J., Mennes, M., & Glover, V. (2005). Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: Links and possible mechanisms. A review. *Neuroscience and Biobehavioral Reviews, 29*(2), 237-258.

Wadhwa, P.D., Dunkel-Schetter, C., Chicz-DeMet, A., Porto, M., & Sandman, C.A. (1996). Prenatal psychosocial factors and the neuroendocrine axis in human pregnancy. *Psychosomatic Medicine, 58*(5), 432–446.

Wisborg, K., Henriksen, T.B., Hedegaard, M., & Secher, N.J. (1996). Smoking during pregnancy and preterm birth. *British Journal of Obstetrics and Gynaecology, 103*(8), 800-805.

Vuguin, P., Linder, B., Rosenfeld, R.G., Saenger, P., & DiMartino-Nardi, J. (1999). Roles of insulin sensitivity, insulin-like growth factor I (IGF-I), and IGF-binding protein-1 and -3 in the hyperandrogenism of African-American and Caribbean Hispanic girls with premature adrenarche. *Journal of Clinical Endocrinology and Metabolism, 84*(6), 2027-2042.

Wang, Y. (2002). Is obesity associated with early sexual maturation? A comparison of the association in American boys versus girls. *Pediatrics, 110*(5), 903-910.

Windham, G.C., Bottomley, C., Birner, C., & Fenster, L. (2004) Age at menarche in relation to maternal use of tobacco, alcohol, coffee, and tea during pregnancy. *American Journal of Epidemiology, 159*(9), 862-871.

Wolf, C.J., Ostby, J.S., & Gray, L.E., Jr. (1999). Gestational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) severely alters reproductive function of female hamster offspring. *Toxicological Science 51*(2), 259-264.

Wu, T., Buck, G.M., & Mendola, P. (2003). Blood lead levels and sexual maturation in U.S. girls: The Third National Health and Nutrition Examination Study, 1988-94. *Environmental Health Perspectives, 111*(5), 737-741.

Yao, P.L., Lin, Y.C., Sawhney, P., & Richburg, J.H. (2007). Transcriptional regulation of FasL expression and participation of sTNF-alpha in response to sertoli cell injury [Electronic version]. *Journal of Biological Chemistry, 282*(8), 5420-5431.

Yu, W.J., Lee, B.J., Nam, S.Y., Ahn, B., Hong, J.T., Do, J.C., et al. (2004). Reproductive disorders in pubertal and adult phase of the male rats exposed to vinclozolin during puberty. *Journal of Veterinary Medical Science, 66*(7), 847-853.