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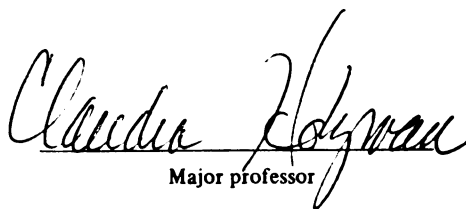
LEVELS OF MATERNAL SERUM FERRITIN  
IN MID-PREGNANCY AND FETAL GROWTH

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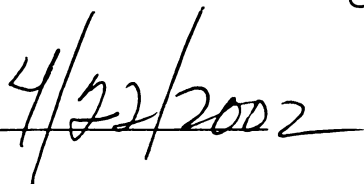
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**LEVELS OF MATERNAL SERUM FERRITIN  
IN MID-PREGNANCY AND FETAL GROWTH**

**By**

**Lisa Ann Peters**

**A THESIS**

**Submitted to  
Michigan State University  
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## ABSTRACT

### LEVELS OF MATERNAL SERUM FERRITIN IN MID-PREGNANCY AND FETAL GROWTH

By

Lisa Ann Peters

Early detection and management of poor fetal growth could allow for medical intervention to help prevent associated morbidity and mortality. It was hypothesized that extremely low or high levels of maternal serum ferritin in mid-pregnancy are associated with the delivery of small-for-gestational-age infants. This association was analyzed separately for women with normal levels and women with high levels of maternal serum alpha-fetoprotein (MSAFP). Women were sampled from a large prenatal screening program at Michigan State University from November 1, 1998 through March 1, 1992. All women were screened for MSAFP and were retrospectively screened for maternal serum ferritin. The women were stratified into two groups based upon normal (0.5-1.5 MoM) and high ( $\geq 2$  MoM) MSAFP levels and divided into four quartiles based upon maternal serum ferritin levels. High maternal serum ferritin levels measured at 15-19 weeks gestation were associated with more than a two-fold increased risk for the delivery of small-for-gestational-age infants. The increased risk occurred among women in the highest ferritin quartile ( $>98$  ng/ml) for women with normal MSAFP levels and in the highest two ferritin quartiles ( $>52$  ng/ml) for women with high MSAFP levels. This may indicate that women with high MSAFP levels have additional risk factors for poor fetal growth.

**To my husband**

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## INTRODUCTION

Small-for-gestational-age (SGA) infants have an increased risk for morbidity and mortality (1,2). Timely detection and management of underlying problems could allow for medical intervention to help prevent perinatal morbidity and mortality associated with poor fetal growth (3). Recent studies have found that one marker of small-for-gestational-age infants may be elevated maternal serum ferritin levels (4,5,6,7,8). It is speculated that elevated levels of maternal serum ferritin do not slow fetal growth, but rather serve as a biomarker for inflammatory processes or lack of plasma volume expansion. In some cases, maternal serum ferritin levels may appear elevated due to an overestimation of gestational age (9).

Studies have reported an association between elevated maternal serum ferritin levels and the risk of delivering small-for-gestational-age infants (4,5,6,7,8). However, the relationship of elevated maternal serum ferritin and poor fetal growth with respect to maternal serum alpha-fetoprotein (MSAFP) levels has not been examined. Elevated maternal serum alpha-fetoprotein is clinically used to detect fetal neural tube defects and other congenital abnormalities (10). In the absence of congenital abnormalities, elevated maternal serum alpha-fetoprotein levels may indicate fetal growth retardation or other pregnancy complications (10,11,12). In the present study, it was hypothesized that extremely low or high levels of maternal serum ferritin levels in mid-pregnancy may be associated with the delivery of small-for-gestational-age infants. Since women were sampled based upon MSAFP levels, this association

was analyzed separately for women with normal levels and women with high levels of maternal serum alpha-fetoprotein. In addition, it was speculated that women with high MSAFP levels might have other risk factors that make them more prone to poor fetal growth.

## **CHAPTER 1**

### **LITERATURE REVIEW**

#### **Serum ferritin**

Ferritin is the primary intracellular iron storage protein (13). Ferritin can be found in the spleen, liver, placenta, heart, bone marrow and serum (13,14,15). It contains a reserve of iron that, if needed, could be used for heme synthesis in the formation of hemoglobin (13). Up to 4,000 iron atoms (ferric hydroxyphosphate) can reside in a central cavity surrounded by a shell of apoferritin. Iron may enter the central cavity after oxidation or leave after reduction via six channels (13,14).

Serum ferritin concentration, while present in small quantities, is usually reflective of total body iron stores (14). The normal concentration of serum ferritin ranges from 15 µg/L to 300 µg/L (13). In virtually all cases, serum ferritin less than 15µg/L indicates iron storage depletion. However, values over 300 µg/L do not usually indicate iron overload. More commonly, infections or inflammation cause increased ferritin synthesis without relation to iron stores (13,16).

The average adult has a total iron content of approximately four and a half grams and the average loss of iron is only about one milligram per day. However, menstrual bleeding, pregnancy and lactation will increase the amount of iron removed from the body (13). Circulating hemoglobin contains

approximately 65% of body iron. The remaining iron is found in ferritin (15%), hemosiderin (8%), myoglobin (8%) and various enzymes (4%) (13).

### **Medical conditions and serum ferritin levels**

#### *Low serum ferritin*

Rarely, decreased ferritin synthesis can occur from ascorbate deficiency and hypothyroidism (13). However, decreased levels of serum ferritin are commonly associated with iron deficiency. The main causes for iron deficiency are malnutrition, increased physiological iron requirements (i.e. pregnancy), blood loss and malabsorption. However, in the presence of infection or inflammation, serum ferritin levels are elevated even in the presence of co-existing iron deficiency (13).

Kuvibidila et al (1994) studied the ability of serum ferritin to determine iron deficiency in 186 lactating and 27 non-lactating Zairean women of childbearing age. This study found that the mean of log serum ferritin levels were higher for lactating women with inflammation (1.9) than lactating women without inflammation (1.7) ( $p < 0.05$ ). The presence of inflammation was determined by measurement of  $\alpha$ 1-acid glycoprotein and C-reactive protein (both acute phase reactant proteins). In addition, there was a high prevalence (66-70%) of anemia (hemoglobin  $< 12\text{g dl}^{-1}$ ), but not a high prevalence (1 out of 180) of low serum ferritin ( $< 12\mu\text{g/L}$ ). It was hypothesized that the elevation of serum ferritin was due to the high level of chronic (43%) and mild (35%) inflammation in this population.



### *High serum ferritin*

Serum ferritin can act as an acute phase reactant (levels increase in response to inflammatory stimulus) in inflammatory processes (13, 18). During inflammatory processes, serum iron and the iron binding capacity are decreased. In addition, the level of hemoglobin and packed red cell are decreased while serum ferritin is increased. Iron absorption from the gut and release of iron from tissue are decreased while tissue iron stores are increased (14).

The human body has developed these iron-withholding mechanisms during infectious and inflammatory processes as a form of non-specific immunity (19). Microorganisms require iron and have mechanisms to obtain the needed iron from their host. During infection, the body withholds iron from microorganisms as a defense mechanism. During inflammation, the body probably additionally withholds iron to avoid tissue damage, since iron may cause tissue damage by participating in free radical generation during inflammation (14).

There are other conditions besides infection or inflammation that can lead to the elevation of serum ferritin. First, there are conditions that increase body iron stores such as idiopathic hemochromatosis and iron-loading anemias such as thalassemia (13). Next, redistribution of body iron by anemia not due to iron deficiency or blood loss such as megaloblastic or hypoplastic anemia can increase serum ferritin levels. Third, the release of tissue ferritin by cell necrosis in conditions such as hepatic necrosis, chronic liver disease, spleen or bone

marrow infarction can increase serum ferritin levels. Finally, increased ferritin synthesis can occur from hyperthyroidism or malignancy (13).

In a study by Lipschitz et al (1974), serum ferritin was measured in 250 patients who either had anemia or a possible disorder in iron metabolism. The patients were placed into categories as follows: uncomplicated iron deficiency, iron overload, inflammation, liver disease, renal disease and anemia with increased red cell turnover. It was found that inflammation, liver disease and increased red-cell turnover elevated serum ferritin levels without relation to iron stores. Lee et al (1995) found that for patients without conditions associated with iron overload, serum ferritin levels above 1,000 ng/ml could be used as a marker for infectious and neoplastic diseases.

### **Maternal serum ferritin levels during pregnancy**

Maternal serum ferritin levels change throughout pregnancy. Kaufer and Casanueva (1990), Milman et al (1995), Knight et al (1994), van Buul et al (1995) and Scholl et al (1998) were longitudinal studies that examined the levels of maternal serum ferritin at various intervals throughout pregnancy. Ho et al (1987) determined maternal serum ferritin levels at delivery. Most studies showed a maternal serum ferritin level of approximately 40 µg/L at 12 weeks gestation. This decreased to approximately 25 µg/L at 32 weeks gestation. At delivery, the maternal serum ferritin level was approximately 23.5 µg/L and one day postpartum it increased slightly to approximately 24.5 µg/L (21,22,23,24,25,26). However, Kaufer and Casanueva (1990) found that if the

women initially had inadequate maternal serum ferritin levels ( $<12\mu\text{g/L}$ ) then the level of maternal serum ferritin decreased only slightly and remained at low levels throughout pregnancy (Table 1).

**TABLE 1**  
**Maternal Serum Ferritin Levels during Pregnancy**

STUDY	1 <sup>ST</sup> TRIMESTER (1-13 WEEKS)	2 <sup>ND</sup> TRIMESTER (14-26 WEEKS)	3 <sup>RD</sup> TRIMESTER ( $\geq 27$ WEEKS)	DELIVERY OR LATER
Scholl, 1998	N/A*	39.2-71.0 $\mu\text{g/L}$ 50 <sup>th</sup> -75 <sup>th</sup> percentile at 15 weeks)	15.1-25.7 $\mu\text{g/L}$ (50 <sup>th</sup> -75 <sup>th</sup> percentile at 28 weeks)	N/A*
van Buul, 1995	40 $\mu\text{g/L}$ (12 weeks)	N/A*	13 $\mu\text{g/L}$ (32 weeks)	24 $\mu\text{g/L}$ (1 day after birth)
Milman, 1995	N/A*	43 $\mu\text{g/L}$ (19-22 weeks)	17 $\mu\text{g/L}$ (31-34 weeks)	24 $\mu\text{g/L}$ (1 day after birth)
Knight, 1994	33.3 ng/ml	28.2 ng/ml	19.3 ng/ml	23.5 ng/ml (delivery)
Kaufer, 1990				
Prepreg $<12\mu\text{g/l}$	10 $\mu\text{g/L}$ (8 weeks)	4 $\mu\text{g/L}$ (24 weeks)	5 $\mu\text{g/L}$ (32 weeks)	10 $\mu\text{g/L}$ (4 days after delivery)
Prepreg $\geq 20\mu\text{g/l}$	40 $\mu\text{g/L}$ (8 weeks)	21 $\mu\text{g/L}$ (24 weeks)	24 $\mu\text{g/L}$ (32 weeks)	28 $\mu\text{g/L}$ (at 4 days after delivery)
Ho, 1987	N/A*	N/A*	N/A*	23.1 ng/ml (during labor)

\*N/A Not available

## **Problems with measurement of maternal serum ferritin**

### *Commercial kits used for measuring maternal serum ferritin*

Immunoassays are commonly used to measure levels of serum ferritin. There are two basic groups of immunoassays. In one type, the antigen is labeled and allowed to bind to a fixed amount of antibody. Radioactive isotopes (radioimmunoassay), enzymes (enzymimmunoassay) or fluorescent compounds (fluoroimmunoassay) can be used to label the antigen (27). The other type of immunoassay is referred to as an immunometric assay. In immunometric assays, the antibody is labeled instead of the antigen (27). Two-site immunoradiometric assays (IRMA) are able to detect smaller quantities of ferritin than RIAs.

The ability of commercial kits to accurately measure the concentration of ferritin varies between kits. For example, two-site immunoradiometric assays are able to detect smaller concentrations of ferritin than radioimmunoassays (28). In rare cases, there can be a low-dose 'hook' effect where measurements are found to be greater than those in a sample containing no ligand (the substance measured) (27). Furthermore, there can be a high-dose 'hook' effect with high concentrations of ferritin (27,28). This is particularly a problem with two-site immunoradiometric assays and other labeled antibody assays. Once a threshold concentration of ferritin is reached, then there is a reversal of the standard curve (27). In other words, the assay will begin to report a lower concentration of ferritin in spite of an actual increase in ferritin concentration (27). One study

found that the high-dose 'hook' effect began at concentrations above 10,000 µg/L (29).

Studies of preterm labor and the delivery of SGA infants commonly used radioimmunoassays (RIA) to measure levels of maternal serum ferritin. In addition, immunoradiometric assay (IRMA), Micro-ELISA (enzyme linked immunosorbent assay) technique, immunoenzymetric assay and Enzym test were used. The types of kits used to measure maternal serum ferritin are summarized in Tables 2 and 3.

**TABLE 2**  
**Measurement of Maternal Serum Ferritin**  
**in Studies on Preterm Labor**

<b>REFERENCE</b>	<b>KIT USED</b>
Goepel, 1988	RIA (GammaDab, Clinical Assays, Cambridges)
Ulmer and Goepel, 1988	Enzym test (Boehringer, Mannheim)
Scholl, 1992	RIA (Micromedics Systems, Inc, Horsham, PA)
Tamura, 1996	MAGIC Ferritin Radioimmunoassay kit (Ciba-Corning Diagnostic, Irving, CA)
Goldenberg, 1996	MAGIC Ferritin [ <sup>125</sup> I] Radioassay kit (Ciba-Corning Diagnostic, Irvine, Calif.)
Scholl, 1998	IRMA (Bio-Rad, Hercules, CA)

**TABLE 3**  
**Measurement of Maternal Serum Ferritin**  
**in Studies on the Delivery of SGA Infants**

REFERENCE	KIT USED
Bhargava, 1991	Micro-ELISA technique using the modified double antibody sandwich technique
Abbas, 1994	RIA kit (Biorad Laboratories, Hemel Hempstead, UK)
Rondo, 1997	Immunoenzymetric assay (Ferritin Serozyme; Serono Diagnostics, Coinsins, Switzerland)
Rondo, 1999	Immunoenzymetric assay (Ferritin Serozyme; Serono Diagnostics, Coinsins, Switzerland)
Hou et al, 2000	MAGIC Ferritin [ <sup>125</sup> I] (Radioimmunoassay kit, Ciba-Corning Diagnostic, Irvine, Calif)

The accuracy or the ability to determine the true concentration of maternal serum ferritin in a sample will vary based upon laboratory protocol and the type of commercial kit used (27). Sometimes different laboratories will find a wide range of results for the same sample (27,30,31,32). For example, Grail et al (1982) found that there were substantial variations in the estimation of serum ferritin between seven immunoassay kits. The reference serum ferritin value was 950 ng/ml, but the kits underestimated the true value. The measurement of serum ferritin by the kits varied from 215 ng/ml to 680 ng/ml. The basic types of

kits used in this study were four radioimmunoassay (RIA) kits, two immunoradiometric assay (IRMA) kits and one ELISA kit.

Most assays are satisfactory for precision therefore; commercial kits should have reproducible results (28). However, commercial kits may not get accurate measures of serum ferritin levels. This would result in non-differential misclassification of serum ferritin levels. However, if the kit has a high-dose 'hook' effect then elevated serum ferritin levels could be underestimated and result in differential misclassification of serum ferritin levels. Since low-dose and high-dose 'hook' effects occur at the extremes ( $0\text{ }\mu\text{g/L}$  and  $10,000\text{ }\mu\text{g/L}$ ) of ferritin levels, most likely 'hook' effects did not bias the study results. In order to help insure accuracy, laboratories should calibrate their assay system in order to establish their own normal range. To help reduce inter-kit and inter-laboratory variations, a reference preparation could be used to calibrate the assay (27,28,32).

### *Measurement of gestational age*

When assessing maternal serum ferritin levels in pregnancy, it is important to have accurate measures of gestational age. An inaccurate gestational age could bias the results of the study. The methods used to assess gestational age include physician assessment at birth, maternal reported last menstrual period (LMP) and prenatal ultrasound.

Examples of physician assessment include the Ballard scoring system and the Capurro method. The Ballard scoring system uses six neuromuscular and

six physical maturity criteria to determine gestational age (33). The Capurro method uses five somatic and two neuromuscular findings (34). Studies have found that the Ballard scoring system and the Capurro method tend to overestimate gestational age by about two weeks in preterm and SGA infants based when compared to ultrasound (35,36,37,38). In addition, those methods underestimate gestational age by about two weeks in postterm infants when compared to ultrasound (35,36,37,38).

Some studies determine gestational age by LMP and early ultrasound. If the discrepancy between the LMP and ultrasound is greater than ten to 14 days then the gestational age is determined solely by ultrasound. Estimation of gestational age by early ultrasound is currently the most accurate and practical method available.

Gestational age determination by accurate LMP should be comparable to the gestational age determined by ultrasound since formulas for determining gestational age by ultrasound were derived from women with regular menstrual cycles and accurate LMPs (39). However, inaccurate recall or biological factors may have an effect on the estimation of gestational age by LMP. In 1987 California birth certificates, the numbers 1, 5, 10, 15, 20, 25 and 28 were the preferred (recorded more frequently than expected) dates cited for LMP (40). Women who cited non-preferred numbers for their LMP had closer agreement (discrepancies of 0-3 days) with gestational age based upon early ultrasound (44.5% vs 40.4%  $p < 0.002$ ) than women who cited preferred numbers. Differences of 15 days or more between LMP and early ultrasound occurred in



62.2% of women reporting non-preferred numbers. The high percentage of women who reported a non-preferred number suggests that most discrepancies greater than 15 days are based upon biological factors, not poor recall.

Inaccurate estimation of gestational age by LMP can be due to biological factors. For example, infants can be misclassified as younger due to vaginal bleeding in early pregnancy mistaken as menses (41). In addition, infants can be misclassified as older due to delayed ovulation (39,42). Gardosi et al 2000 found that 29.1% of 21,069 pregnancies had more than seven days discrepancy between LMP and ultrasound dates. LMP overestimated the true (ultrasound) gestational age of the infants in 23.3% of all pregnancies, while the gestational age was underestimated in only 5.7% of all pregnancies. (43). Therefore, gestational age is more likely to be overestimated by LMP as compared to the true (ultrasound) gestational age (40,43,44).

Early ultrasound (before 16-20 weeks) is commonly regarded as the gold standard for assessing gestational age because it is subject to little measurement error (45). Ultrasound allows for estimation of gestational age when the LMP is unknown or is incorrect. In addition, ultrasound can be used to assess fetal growth and weight throughout pregnancy (46).

The estimate of gestational age by ultrasound is based upon an assumed growth rate, but deviation from the assumed growth rate may result in incorrect estimation of gestational age (46). Early ultrasound (usually before 16-20 weeks gestation) gives the most accurate gestational age because measurements are taken at a time when there is little or no variation in the size of the fetus.

However, if there is a biological variation in the size of the fetus it is likely that this will result in regression towards the mean gestational age for the size of the infant (47). In other words, a smaller fetus would be assigned an earlier estimated gestational age and a larger fetus would be assigned a later gestational age (39).

The method used to estimate gestational age in studies relating levels of maternal serum ferritin to preterm delivery or the delivery of SGA infants are listed in Table 4. Five studies used LMP and early ultrasound to determine gestational ages (8,25,50,51,52). Three other studies used the Capurro method or LMP and Ballard scoring (4,6,7). Three studies did not specify which method was used to determine gestational age (5,48,49).

**TABLE 4**  
**Methods Used to Determine Gestational Age**

<b>METHOD</b>	<b>STUDY</b>
<b>Studies relating low maternal serum ferritin to preterm labor</b>	
Not specified	Goepel, 1998 Ulmer and Goepel, 1998
LMP and early ultrasound	Scholl, 1992
<b>Studies relating high maternal serum ferritin to preterm labor</b>	
LMP and early ultrasound	Tamura, 1992 Goldenberg, 1996 Scholl, 1998
<b>Studies relating high maternal serum ferritin to the delivery of SGA infants</b>	
Not specified	Abbas, 1994
Capurro method	Rondo, 1997 Rondo, 1999
LMP and Ballard scoring	Bhargava, 1991
LMP and early ultrasound	Hou, 2000

First, the consequence of inaccurate gestational age estimation will be evaluated for the studies relating low or high levels of maternal serum ferritin to preterm delivery. Studies that used physician assessment could overestimate gestational age by about two weeks in preterm infants when compared to ultrasound (35,36,37,38). Misclassifying preterm infants as older than what is true (ultrasound) would underestimate the number of preterm infants. Since maternal serum ferritin levels decrease throughout pregnancy, infants that are labeled preterm (but have an overestimated gestational age) would have the appearance of higher levels of serum ferritin. This differential misclassification would create a false association between elevated levels maternal serum ferritin and preterm delivery.

Studies that used LMP could overestimate gestational age and assign a higher gestational age to the infants. However, the overestimation of gestational age would occur to both preterm and term infants. This non-differential misclassification could attenuate the association between low or high maternal serum ferritin levels and preterm delivery. Occasionally, LMP could underestimate gestational age and assign a lower gestational age to the infants. Gestational age would most likely be underestimated if there were vaginal bleeding mistaken as menses in early pregnancy. Vaginal bleeding could be a marker for preterm delivery, therefore, the underestimation of gestational age would occur in preterm infants (53). This differential misclassification could create an erroneous association between low maternal serum ferritin and preterm delivery.

Preterm infants that are an appropriate size for their gestational age should be assigned an accurate gestational age by ultrasound. However, infants that are smaller or larger for their gestational age will be given a lower or higher gestational age, respectively. However, the underestimation or overestimation of gestational age would occur to both preterm and term infants. This non-differential misclassification could attenuate the association between low or high maternal serum ferritin levels and preterm delivery.

Next, the consequence of inaccurate gestational age estimation will be evaluated for studies relating high levels of maternal serum ferritin to the delivery of SGA infants. Studies that used physician assessment could misclassify some SGA infants as older than what is the true (ultrasound) gestational age. Since maternal serum ferritin levels decrease throughout pregnancy, misclassifying SGA infants as older than what is true could create the appearance that these infants have high levels of maternal serum ferritin. The differential misclassification would create a false association between elevated levels maternal serum ferritin and the delivery of SGA infants.

Studies that used LMP could assign a higher gestational age to infants. This bias would lead to some term AGA infants inaccurately labeled SGA and preterm. In addition, some term SGA infants may inaccurately be labeled preterm SGA infants. Since the overestimation of gestational age would occur to both AGA and SGA infants, this non-differential misclassification could attenuate the association between high maternal serum ferritin and the delivery of SGA infants. Occasionally, LMP could underestimate gestational age and assign a

lower gestational age to the infants. Gestational age would most likely be underestimated if there were vaginal bleeding mistaken as menses in early pregnancy. Vaginal bleeding could be a marker for SGA infants, therefore, the underestimation of gestational age would commonly occur in SGA infants (Kurjak). This differential misclassification could create an erroneous association between low maternal serum ferritin and the delivery of SGA infants.

Studies that used ultrasound could assign a lower gestational age to SGA infants and label them as SGA and preterm or AGA and preterm. Small-for-gestational-age infants assigned a lower gestational age during ultrasound have two potential growth patterns. First, if the SGA infant remains on a steady normal growth trajectory then at birth the infant will be labeled AGA, but incorrectly labeled preterm due to the original underestimation of gestational age. The second option is that the growth trajectory continues to slow. At birth the infant will be incorrectly labeled preterm and correctly labeled SGA, but the extent of poor fetal growth will be underestimated due to the inaccurate estimation of gestational age. Since maternal serum ferritin levels decrease throughout pregnancy, misclassifying SGA infants as preterm would create the appearance that these infants have lower levels of maternal serum ferritin. This differential misclassification would create a false association between low maternal serum ferritin and the delivery of SGA infants.

## **Maternal serum alpha-fetoprotein**

Alpha-fetoprotein (AFP) is a fetal protein (12). It is produced at four to eight weeks by the fetal yolk sac and the fetal liver (12). At 11.5 weeks the yolk sac involutes and the fetal liver becomes the main source of AFP production for the duration of pregnancy. AFP passes into maternal circulation across the placenta or fetal membranes. Maternal concentration of alpha-fetoprotein rises throughout pregnancy until it peaks at 32 weeks gestation (12). Levels decrease throughout the remainder of the pregnancy (12).

High maternal serum alpha-fetoprotein is used to screen for neural tube defects and congenital abnormalities (10). However, after a high resolution ultrasound or amniocentesis rules out anomalies, high MSAFP levels are associated with a variety of adverse pregnancy outcomes. For example, unexplained high maternal serum alpha-fetoprotein levels are associated with the delivery of SGA infants (11,54,55,56). In addition, high levels of MSAFP are associated with prematurity and preeclampsia (11). It is speculated that high serum levels of maternal alpha-fetoprotein may be caused by an increased transfer of MSAFP across the placenta probably due to uteroplacental disease (11).

## **Small-for-gestational-age infants**

SGA infants are at increased risk for fetal mortality (OR 2.8, 95% CI 2.1-3.6) and neonatal mortality (OR 2.8, 95% CI 2.3-3.3) as compared to AGA infants (1,2). In addition, SGA infants are at an increased risk for morbidity including

neurological impairment, necrotizing enterocolitis, respiratory distress syndrome, intrapartum asphyxia, meconium aspiration, hypoglycemia, hypothermia, seizures and sepsis (2,57,58,59).

Small-for-gestational-age (SGA) infants are, by definition, born at a low weight for their gestational age (3,60,61). Usually small-for-gestational-age infants are defined as those infants less than the 10<sup>th</sup> percentile of birthweight for gestational age based on a reference population (3,47,61,62). However, studies also have defined SGA infants as those infants that are less than the 5<sup>th</sup> percentile, less than the 15<sup>th</sup> percentile or the second standard deviation below the mean of a birthweight distribution curve (3,47,61,62).

The purpose of classifying infants as small-for-gestational-age is to define a subset of infants that are at an increased risk for morbidity and mortality. Since SGA infants are defined by a cross-sectional measurement of birthweight for gestational age, the “small-for-gestational-age” label should not be viewed as a diagnosis of IUGR. IUGR infants should be diagnosed by following the growth trajectory of the infants throughout pregnancy (3).

Small-for-gestational-age infants can be divided into two categories. The first category consists of those infants who may have normal growth trajectory, yet are constitutionally small (3,47). Women who are single, younger, primiparous, African-American, reside at an altitude above 5,000 feet, have a low body mass index (BMI), rely on government aid to pay for delivery and have a low level of education often deliver constitutionally small infants (9,60,61,63,64,65,66).

The second category consists of intrauterine growth retarded (IUGR) infants. IUGR infants do not attain their full growth potential due to some inhibiting factor and can be characterized by a slowing in their growth trajectory that is usually diagnosed by serial ultrasounds (3,47). Maternal behavioral factors related to fetal growth retardation include poor nutrition, cigarettes, alcohol use and illicit drug use (60,61,67). Maternal medical conditions related to poor fetal growth include chronic hypertension, pregnancy induced hypertension, preeclampsia, diabetes mellitus, systemic lupus erythematosus, chronic renal disease, inflammatory bowel disease, anemia, chronic asthma and severe hypoxic lung disease (9,60,61,62). Infectious processes related to IUGR infants include syphilis, cytomegalovirus, toxoplasmosis, rubella, parvovirus B 19, hepatitis A and B, herpes simplex types 1 and 2, human immunodeficiency virus (HIV) and malaria (9,61,62). Uteroplacental insufficiency due to infections, maternal hypertension or unknown causes can result in poor fetal growth (3). In addition, fetal chromosomal abnormalities (trisomy 13, 18, or 21 and Turner's syndrome), congenital malformations and irradiation of the fetus have been connected poor fetal growth (9,60,61).

#### *Internal and External validity issues with SGA definitions*

There is a general lack of consensus in the scientific community as to the classification of small-for-gestational-age infants. In some instances, studies label SGA infants as IUGR without following their growth trajectory over time. Some SGA infants may have fetal growth impairment, however there is not



substantial information to make the diagnosis of IUGR based upon a cross-sectional measurement of birthweight for gestational age (3,61,62).

In other instances, there is lack of comparability between studies due to the varying definitions of SGA infants. Small-for-gestational-age infants have been defined as those of infants less than the 5<sup>th</sup>, 10<sup>th</sup> or 15<sup>th</sup> percentile of birthweight for gestational age based on a reference population curve (3,47,61,62). Whereas, other studies have used two standard deviations below the mean of a birthweight distribution curve to define SGA infants (3,47,61,62).

Five studies have examined the association between high levels of maternal serum ferritin and the delivery of SGA infants (Table 5). These studies have varying definitions for SGA infants and some labeled infants as IUGR without following their growth trajectory over time. Bhargava et al (1991) compared the birthweight and gestational age between women with and without anemia. In the study by Abbas et al (1994), IUGR was assessed by fetal abdominal circumference and birthweight below the 5<sup>th</sup> percentile for gestational age based on a reference birthweight standard by Yudkin et al (1987). In the studies by Rondo et al (1997 and 1999) infants were labeled IUGR if their birthweight was less than the 10<sup>th</sup> percentile of the Lubchenco sex-specific birthweight for the gestational age standard. Hou et al (2000) determined fetal growth retardation (FGR) by birth weight <15<sup>th</sup> percentile for gestational age based on the Alabama standards stratified by sex, race and parity.

**TABLE 5****SGA Classifications in Studies of Elevated Maternal Serum Ferritin and the Delivery of SGA Infants**

<b>STUDY</b>	<b>TERM USED TO DEFINE SGA INFANTS</b>	<b>DEFINITION OF SGA INFANTS</b>
Bhargava, 1991	None	Lower birth weight for gestational age
Abbas, 1994	Intrauterine growth retardation (IUGR)	fetal abdominal circumference and birthweight below the 5 <sup>th</sup> percentile for gestational age by Yudkin et al
Rondo, 1997	Intrauterine growth retardation (IUGR)	<10 <sup>th</sup> percentile of Lubchenco sex-specific birthweight of gestational age standard
Rondo, 1997	Intrauterine growth retardation (IUGR)	<10 <sup>th</sup> percentile of Lubchenco sex-specific birthweight of gestational age standard
Hou, 1997	Fetal growth retardation (FGR)	Birthweight < 15 <sup>th</sup> percentile for gestational age based on the Alabama standards for sex, race and parity

***Low maternal plasma volume expansion and SGA infants***

Maternal plasma volume expansion is related to the birthweight of the infant and to the number of fetuses (3,69). Therefore, plasma volume increases about 50% in appropriate-for-gestational-age (AGA) pregnancies, but in SGA pregnancies the increase in plasma volume may only be 25% (9,70,71). In fact,

healthy women in a first pregnancy should increase their plasma volume from about 2600 ml to 3850 ml. Plasma volume expansion is usually greater in second pregnancies and the plasma volume in healthy women increases from approximately 2600 ml to 4100 ml (72). The mean pre-pregnancy plasma volume was 2373 ml in the study by Whittaker et al (1996). Compared to pre-pregnancy levels, by week 20 of gestation the plasma volume had increased approximately 23% and by week 36 it had increased by 35% (Table 6).

As plasma volume increases during pregnancy, levels of hemoglobin (Hb) and ferritin (Ft) decrease by dilution (Table 7). The mean hemoglobin level decreases from 140 g/L prior to pregnancy to 132 g/L at 20 weeks. After 20 weeks, the mean level of hemoglobin rises slightly and is 134 g/L at 36 weeks. The median level of serum ferritin decreases from 32 µg/L prior to pregnancy to 22 µg/L at 28 weeks gestation and rises slightly to 27 µg/L at 36 weeks gestation.

**TABLE 6**  
**Maternal Plasma Volume Expansion during Pregnancy**

	PRE-PREG	12 WEEKS	20 WEEKS	28 WEEKS	36 WEEKS
Pvol (ml)	2373	2667	3093	3517	3623

(Whittaker, 1996)

**TABLE 7****Maternal Levels of Hemoglobin and Serum Ferritin during Pregnancy**

	PRE-PREG	12 WEEKS	20 WEEKS	28 WEEKS	36 WEEKS
Hemoglobin (g/L)	140	135	132	133	134
Serum ferritin (µg/L)	32	38	25	22	27

(Kaufer and Casanueva, 1990)

The total red cell mass and hemoglobin levels increase; however, they do not increase as quickly as the plasma volume does (72). Therefore, the red cell count and hemoglobin concentrations decline during pregnancy (72). The combination of these events leads to the 'physiological anemia of pregnancy' for most women (71). Correspondingly, the lower plasma volume expansion in SGA pregnancies as compared to AGA pregnancies could create a higher serum ferritin concentration at comparable gestational ages due to lack of hemodilution (9,70). Therefore, one possible explanation for the underlying cause of elevated maternal serum ferritin in SGA pregnancies is lack of plasma volume expansion.

*Infections, inflammation and SGA infants*

Infectious and inflammatory processes are associated with the delivery of SGA infants (61,74). In addition, serum ferritin is commonly elevated in the presence of infection or inflammation (14). Consequently, it is speculated that elevated serum ferritin levels during pregnancy may be a marker for infection or inflammation.

Infections such as syphilis, cytomegalovirus, toxoplasmosis, rubella, parvovirus B 19, hepatitis A and B, herpes simplex types 1 and 2, human immunodeficiency virus (HIV) and malaria are associated with the delivery of SGA infants (9,61,62). Infections can affect fetal growth through maternal factors (i.e. fever, medicine), adversely altering fetal cell division or damaging the placenta (3,9,61).

Infections and inflammation have been identified as possible causes of damage to the placenta (61). The placenta is essential for the materno-fetal transfer of nutrients and gases (72). In addition, the placenta allows for removal of fetal waste products (75). Therefore, damage to the placenta may impair fetal growth (9,61,62). For example, Salafia et al (1995) found that SGA infants had increased numbers of villous infarcts (37.5% vs 3.5%  $p < 0.001$ ) and chronic villitis (6.3% vs 1.5%  $p < 0.001$ ).

Inflammatory responses can be activated by chronic infection or tissue injury (74). For example, preeclampsia may be the result of an intravascular inflammatory reaction during pregnancy (74,77). In a normal pregnancy, at about 16 weeks gestation the spiral arteries in the placental bed allow an increase in blood supply by widening. In a pregnancy complicated by preeclampsia this does not occur, thus restricting the amount of blood flow in the placenta (10,61,74).

Cytokines can be an indicator of underlying infection or inflammation since inflammatory processes stimulate the release of cytokines (78). It is speculated that cytokines then induce the release of acute phase proteins (i.e. serum

ferritin). IL-1 (interleukin-1) or TNF (tumor necrosis factor) are two cytokines that may mediate changes in serum ferritin levels during inflammation (14). During inflammatory processes, TNF and IL-1 respond to inflammatory processes by producing a wide variety of effects on immune system, the central nervous system and neuroendocrine system (78). However, the exact process is unknown and it is likely to be a complex relationship between certain cytokines and the induction of serum ferritin (an acute phase protein) (18). During pregnancy, cytokines can be elevated due to conditions such as preeclampsia and chorioamnionitis (10).

In 1995, Stallmach et al performed a study to determine the quantity of cytokines found in the different compartments of the feto-maternal unit during normal pregnancy and with intrauterine disease (79). All women underwent a Cesarean section during the third trimester of pregnancy (26 to 40 weeks gestational age). Women were classified into two groups of intrauterine disease. The first group was intrauterine growth retarded (IUGR) infants with or without signs of preeclampsia and signs of hypoxia. The second group had chorioamnionitis, an ascending infection. Chorioamnionitis occurs when bacteria or mycoplasmas enter the extraplacental fetal membranes and spread to the amniotic fluid (80).

Table 8 indicates the level of cytokines found in IUGR pregnancies and pregnancies complicated by chorioamnionitis as compared to normal pregnancies. In pregnancies with IUGR, TNF- $\alpha$  is increased in amniotic fluid with and a decrease in GM-CSF and G-CSF. For cytokines associated with

inflammatory disease, in the amniotic fluid IL-1 $\beta$  decreased and there was no change in IL-6 and IL-8. However, in the group with chorioamnionitis all cytokines measured except for GM-CSF were increased in the amniotic fluid. Therefore, while altered cytokines are associated with IUGR the levels are different than what is found with chorioamnionitis.

**TABLE 8**  
**Cytokines in Pregnancies Complicated by IUGR or Chorioamnionitis**

CYTOKINE	SOURCE	WEEK GEST	IUGR	CHORIOAM
G-CSF	Amniotic fluid	26-40 weeks	Decrease	Increase
	Fetal blood	26-40 weeks	No change	Increase
	Maternal blood	26-40 weeks	No change	Increase
GM-CSF	Amniotic fluid	26-40 weeks	Decrease	No change
	Fetal blood	26-40 weeks	No change	No change
	Maternal blood	26-40 weeks	No change	No change
TNF- $\alpha$	Amniotic fluid	26-40 weeks	Increase	Increase
	Fetal blood	26-40 weeks	No change	No change
	Maternal blood	26-40 weeks	No change	No change
IL-1 $\beta$	Amniotic fluid	26-40 weeks	Decrease	Increase
	Fetal blood	26-40 weeks	No change	No change
	Maternal blood	26-40 weeks	No change	No change
IL-6	Amniotic fluid	26-40 weeks	No change	Increase
	Fetal blood	26-40 weeks	No change	Increase
	Maternal blood	26-40 weeks	No change	Increase
IL-8	Amniotic fluid	26-40 weeks	No change	Increase
	Fetal blood	26-40 weeks	Increase	Increase
	Maternal blood	26-40 weeks	Increase	No change

(Stallmach, 1995)

Stallmach et al (1995) performed another study to determine the expression of cytokines under four different conditions. The four conditions were: at the time of Cesarean section performed between 25 and 38 weeks gestational age without uterine contraction, in normal pregnancy with labor established, pregnancy complicated by amniotic infection, and under the condition of preeclampsia with fetal intrauterine dystrophy (81). The two conditions of interest to this paper were pregnancy with amniotic infection and pregnancies with preeclampsia with fetal intrauterine dystrophy. IUGR was defined by fetal growth less than the 5<sup>th</sup> percentile based on ultrasound examination. Amniotic infection was diagnosed by fever, leukocytosis and rising levels of C-reactive protein in the mother's serum and was confirmed by histological examination of the placenta. Elevation of several cytokines (GM-CSF, G-CSF, IL-1, IL-6) was found with amniotic infection. By contrast, in the group of women with preeclampsia with fetal dystrophy a reduction of some cytokines (G-CSF, IL-1) was found (Table 9).



**TABLE 9**  
**Cytokines in Pregnancies Complicated by**  
**Amniotic Infection or Preeclampsia**

CYTOKINE	SOURCE	WEEK GEST	AMNIOTIC INFECTION	PRE-ECLAMP
GM-CSF	Amniotic fld	25-38 weeks	Increase	Decrease
	Fetal atl bd	25-38 weeks	None	None
	Fetal vs bd	25-38 weeks	None	None
	Matl rpl bd	25-38 weeks	Increase	None
	Matl prl bd	25-38 weeks	Increase	None
G-CSF	Amniotic fld	25-38 weeks	Increase	Decrease
	Fetal atl bd	25-38 weeks	Increase	None
	Fetal vs bd	25-38 weeks	Increase	None
	Matl rpl bd	25-38 weeks	Increase	None
	Matl prl bd	25-38 weeks	Increase	None
IL-1	Amniotic fld	25-38 weeks	Increase	Decrease
	Fetal atl bd	25-38 weeks	No difference	Decrease
	Fetal vs bd	25-38 weeks	None	None
	Matl rpl bd	25-38 weeks	Increase	None
	Matl prl bd	25-38 weeks	None	None
IL-6	Amniotic fld	25-38 weeks	Increase	None
	Fetal atl bd	25-38 weeks	N/A*	None
	Fetal vs bd	25-38 weeks	N/A*	None
	Matl rpl bd	25-38 weeks	Increase	None
	Matl prl bd	25-38 weeks	No none	Increase
IL-8	Amniotic fld	25-38 weeks	N/A*	No none
	Fetal atl bd	25-38 weeks	N/A*	Increase
	Fetal vs bd	25-38 weeks	N/A*	Increase
	Matl rpl bd	25-38 weeks	N/A*	None
	Matl prl bd	25-38 weeks	N/A*	Increase

(Stallmach, 1995)

Amniotic fld (amniotic fluid)

Fetal atl bd (fetal arterial blood)

Fetal vs bd – (fetal venous blood)

Matl rpl bd – (maternal retroplacental blood)

Matl prl bd – (maternal peripheral blood)

N/A\* Not available

Heyborne et al (1994) performed a case-control study to determine the levels of IL-10 in SGA and AGA pregnancies. Amniotic fluid was collected on the average at 16 weeks. It was found that IL-10 was elevated in SGA pregnancies as compared to control pregnancies. Since IL-10 is an immunosuppressive cytokine, the results of this study indicate that abnormal immune activation, not inadequate immune suppression, may affect fetal growth impairment (82).

Though seldom seen in the United States, malaria is another example of an infection that should be explored to understand the relationship of infections, cytokines and serum ferritin levels during pregnancy. Moormann et al (1999) found that malaria-infected placentas had two to four times the amount of interleukin IL-1 $\beta$ , IL-8 and TNF- $\alpha$  mRNA and about half as much IL-6 and TGF- $\beta$ 1 mRNA as that in uninfected placentas (Table 10). There was an increase in the proinflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$ . In addition, there was a decrease anti-inflammatory (immunosuppressive) cytokines IL-6 and TGF- $\beta$ 1. This indicates that malaria may adversely affect the placenta. In addition, it was found that increased TNF- $\alpha$  or IL-8 expression in malaria-infected placentas was associated with IUGR (83).

**TABLE 10****Cytokines in Pregnancies Complicated by Malaria**

CYTOKINE	SOURCE	INFECTED
TNF- $\alpha$	Placenta	Increase
IL-6	Placenta	Decrease
IL-8	Placenta	Increase
IL-1 $\beta$	Placenta	Increase
TGF- $\beta$ 1	Placenta	Decrease

(Moorman, 1999)

Allen et al (1998) performed a prospective study and did not find a positive relationship between malaria infection at delivery and IUGR. However, the impact of malaria on IUGR is complicated and the placental and peripheral blood parasitaemia may have been resolved by the time of delivery (84). Sullivan et al (1999) found that parasitemia and/or clinically diagnosed malaria in the antenatal period was associated with IUGR. In fact, women were more than three times as likely to deliver an IUGR infant if they were diagnosed with parasitemia or a clinical episode of malaria during the antenatal period. However, parasitemia at delivery was not associated with IUGR, and therefore supports the results found by Allen et al (1998).

**Studies relating maternal serum ferritin to preterm labor*****Low maternal serum ferritin***

Three studies have evaluated the association of low maternal serum ferritin and preterm delivery. Goepel et al (1988) and Ulmer and Goepel (1988) found a strong association between low maternal serum ferritin and preterm

labor. However, they did not control for the variation of serum ferritin that occurs during pregnancy. Scholl et al (1992) controlled for the variation in serum ferritin by screening the women at similar weeks of gestation. Scholl et al (1992) found only a moderate association between low maternal serum ferritin and preterm labor.

Goepel et al (1988) was a prospective study on 150 women who were mostly in their last trimester of pregnancy. They found that 48% of women who had maternal serum ferritin levels below 10  $\mu\text{g/L}$  had premature labor contractions while only 11% of the women with maternal serum ferritin levels above 20  $\mu\text{g/L}$  had premature labor (<37 weeks) contractions ( $p < 0.001$ ).

In 1988, Ulmer and Goepel performed a prospective study. Three hundred women from a high-risk obstetric population in the 28<sup>th</sup> to the 41<sup>st</sup> week of gestation were included in the study. Since maternal serum ferritin was measured from 28 to 41 weeks' gestation, the study did not control for the variation of maternal serum ferritin that occurs normally during pregnancy. One hundred and three of the women went into preterm labor (<37 weeks). They found that the mean value of maternal serum ferritin in women with preterm labor was 12.35  $\mu\text{g/L}$  while the mean level of maternal serum ferritin in women without preterm labor was 28.23  $\mu\text{g/L}$  ( $p < 0.001$ ).

Scholl et al (1992) performed a prospective study on 826 women from two prenatal clinics. Maternal serum ferritin levels were measured at entry to prenatal care (average of 17 weeks' gestation). It was found that the adjusted odds ratio of preterm delivery (37 weeks' gestation) for women with iron

deficiency anemia (anemia plus ferritin  $<12\mu\text{g/L}$ ) was 2.66 (95% CI 1.15-6.17). In contrast, the adjusted odds ratio of preterm delivery for women without anemia, but with low maternal serum ferritin concentrations ( $<12\mu\text{g/L}$ ) at entry to prenatal care was not elevated (OR1.04 (95% CI 0.49-2.23)).

### *High maternal serum ferritin*

Three studies have evaluated the association of high maternal serum ferritin and preterm delivery. Tamura et al (1996), Goldenberg et al (1996) and Scholl et al (1998) found an association between high maternal serum ferritin and preterm labor. In addition, the studies controlled for the variation in serum ferritin that occurs during pregnancy by screening the women at similar weeks of gestation.

Tamura et al (1996) performed a nested case-control study on 94 women. The cases included 31 women with a spontaneous delivery of  $\leq 32$  weeks. There were two control groups, one with spontaneous delivery at 33-36 weeks ( $n=32$ ) and the other with spontaneous delivery at  $\geq 37$  weeks ( $n=31$ ). Maternal serum ferritin levels were measured at 24 weeks gestation. For women with maternal serum ferritin levels  $\geq 42\mu\text{g/L}$  as compared to women with lower maternal serum ferritin levels, they found that the odds ratio of having a delivery at  $\leq 32$  weeks versus 33-36 weeks was 2.99 (95% CI 1.13 – 7.89). For women with maternal serum ferritin levels  $\geq 42\mu\text{g/L}$  as compared to women with lower maternal serum ferritin levels, the odds ratio of having a preterm delivery ( $\leq 32$  weeks) versus at term delivery ( $\geq 37$  weeks) was 3.28 (95% CI 0.99 – 10.90).

Goldenberg et al (1996) performed a prospective study to examine the relationship between plasma ferritin and preterm delivery ( $\leq 32$  weeks). The study population consisted of 580 African-American women from a randomized, double blind study on the effects of oral zinc supplementation on pregnancy outcome. Blood samples were taken at 19, 26, and 36 weeks' gestation. It was found that high plasma ferritin levels in the highest quartile at 26 weeks' gestation were associated with preterm delivery (adjusted odds ratio 2.7, 95% CI 0.99-7.6).

Scholl et al (1998) assessed maternal serum ferritin levels for 1,162 pregnant women at entry to prenatal care (average 15 weeks' gestation) and at 28 weeks' gestation. If serum ferritin levels did not decline from entry to prenatal care, then at 28 weeks' gestation elevated levels of maternal serum ferritin ( $\geq 41.5$   $\mu\text{g/L}$ ) were associated with a nine-fold increase (AOR 8.77, 95% CI 3.90-19.72) for the risk of very preterm delivery ( $< 33$  weeks gestation). Furthermore, clinical chorioamnionitis was two times more frequent and flu symptoms (as a proxy for undiagnosed maternal infection) were six times more frequent when serum ferritin was elevated at 28 weeks gestation and did not decrease from entry to prenatal care.

Table 11 summarizes the results from studies on both low and high maternal serum ferritin in relation to preterm delivery. In addition, two methodological issues are included to demonstrate how they may affect the results of the study. In all of the studies, non-differential misclassification of serum ferritin levels by commercial kits may attenuate the association between low or high maternal serum ferritin levels and preterm delivery. However, if there

is a high-dose 'hook' effect then low or high serum ferritin levels may be overestimated or underestimated, respectively (27). This differential misclassification may bias the results of the study to the null. The gestational age estimation method in Goepel et al (1988) and Ulmer et al (1988) is not specified in the studies. However, studies that relied primarily on ultrasound to determine gestational age could attenuate the association between low or high levels of maternal serum ferritin and preterm delivery by non-differential misclassification of gestational age (Scholl et al (1992), Tamura et al (1996), Goldenberg et al (1996) and Scholl et al (1998)).

**TABLE 11**  
**Maternal Serum Ferritin and Preterm Delivery**

<b>STUDY</b>	<b>RESULT</b>	<b>GESTATIONAL AGE ESTIMATION METHOD</b>	<b>MEASURE OF FERRITIN LEVELS</b>
	<b>Low Serum Ferritin</b>		
Goepel, 1988	(+) 48% with serum ferritin <10 µg/l preterm labor contraction	Not specified	Non-differential or differential – may attenuate association
Ulmer, 1988	(+) serum ferritin value for PTD 12.35 µg/l	Not specified	Non-differential or differential – may attenuate association
Scholl, 1992	(+) AOR of PTD for serum ferritin <12 µg/l with anemia was 2.66	Non-differential – LMP and ultra – may attenuate association	Non-differential or differential – may attenuate association
	<b>High Serum Ferritin</b>		
Tamura, 1996	(+) OR of PTD was 3.28 for serum ferritin ≥42 µg/l	Non-differential – LMP and ultra – may attenuate association	Non-differential or differential – may attenuate association
Goldenberg, 1996	(+) OR of PTD (≤32 wks) was 2.7 w/ ferritin in highest quartile measured at 26 wks	Non-differential – LMP and ultra – may attenuate association	Non-differential or differential – may attenuate association
Scholl, 1998	(+) AOR 8.77 when serum ferritin did not decrease by week 28	Non-differential – LMP and ultra- may attenuate association	Non-differential or differential – may attenuate association



### **Studies relating maternal serum ferritin to small-for-gestational-age infants**

Five studies have evaluated maternal serum ferritin levels in relation to fetal growth. All have found a positive association between high levels of maternal serum ferritin and the delivery of SGA infants. However, Bhargava et al (1991), Abbas et al (1994) Rondo et al (1997) and Rondo et al (1999) did not control for the variation of serum ferritin that occurs during pregnancy. Hou et al (2000) controlled for the variation in serum ferritin by screening the women at similar weeks of gestation.

Bhargava et al (1991) performed a cross-sectional study of 308 mothers in labor. They found that severe anemia was associated with elevated maternal serum ferritin levels (101.60 versus 56.23  $p < 0.001$ ), lower birth weight (2183 g versus 2599 g  $p < 0.001$ ) and lower gestational age (36.36 weeks versus 37.72 weeks  $p < 0.001$ ) at the time of labor. The increase in maternal serum ferritin levels in anemic women could be due to infection especially since the study took place in New Delhi where iron deficiency is often complicated by infections. In fact, out of twelve women with severe anemia, six had urinary tract infections and three had tuberculosis (Bhargava 1991).

Abbas et al (1994) performed a case-control study with a population of women from King's College Hospital Medical School, London. This study measured maternal and fetal serum ferritin and cobalamin in 20 mother-infant pairs with IUGR. The control group was obtained from previous studies (Nicolaidis 1989, Abbas 1993). It was found that the mothers with growth retarded fetuses had significantly increased ( $p\text{-value} < 0.05$ ) maternal serum

ferritin levels at 26 to 36 weeks' gestation as compared to the appropriate normal mean for gestational age.

Rondo et al (1997) performed a case-control study with a population from four hospitals in Campinas City, Brazil. The study consisted of 356 mothers that delivered IUGR infants and a control group of 356 mothers that delivered AGA infants. A cord sample was taken at birth and a maternal blood sample was taken between twelve hours and three days after delivery. It was found that high maternal ferritin ( $\geq 51 \mu\text{g/L}$ ), maternal body weight  $\leq 50 \text{ kg}$ , per capita income, cigarette smoking, maternal weight gain  $\leq 7 \text{ kg}$ , prior history of low birth weight, beer intake, and coffee intake were risk factors for IUGR.

Rondo et al (1999) used the same data from the Rondo et al (1997) case-control study, but this study focused on the issue of high maternal serum ferritin. It was found that maternal levels of ferritin  $>50 \mu\text{g/L}$  were more common ( $p < 0.001$ ) in IUGR mothers than in AGA mothers. In addition, it was found that 47% of IUGR and AGA mothers were anemic (hemoglobin  $\leq 11.0 \text{ g/dL}$ ), but only about four percent of these mothers had low maternal serum ferritin levels ( $\leq 10 \mu\text{g/L}$ ) (Rondo 1999). Rondo et al (1999) speculated that the elevated maternal ferritin levels were due to subclinical infection.

Hou et al (2000) performed a prospective study of 1,545 African-American and white multiparas. In this study, asymmetric fetal growth retardation is defined as appropriate length, but with a low weight for gestational age. This type of growth retardation often occurs due to uteroplacental insufficiency usually from maternal hypertension or diabetes (3). Symmetric fetal growth retardation is

defined as small in all dimensions. This type of growth retardation is often due to constitutionally small infants, chromosomal anomalies, malnutrition, smoking or maternal infection (3). Maternal serum ferritin levels in the highest quartile at 25 weeks (adjusted odds ratio (AOR) 3.4, 95% CI 1.6-7.2) and 36 weeks (AOR 2.7, 95% 1.3-5.8) gestation were associated with asymmetric fetal growth retardation. However, maternal serum ferritin levels in the lowest quartile at 36 weeks gestation were associated with symmetric fetal growth retardation (AOR 2.2, 95% CI 1.01-4.6).

Table 12 summarizes the results of the studies and indicates the possible effect of misclassification on the results of the study. Non-differential misclassification of maternal serum ferritin levels by the commercial kits may attenuate the association between elevated serum ferritin levels and the delivery of SGA infants. However, if there is a high-dose 'hook' effect then elevated serum ferritin levels may be underestimated (27). This differential misclassification would underestimate the association between elevated serum ferritin levels and the delivery of SGA infants.

Abbas et al (1994) did not specify the method used to estimate gestational age. Bhargava et al (1991) used LMP and confirmed the gestational age with Ballard scoring system in preterm infants. LMP could result in differential misclassification of gestational age and create a false association between low serum ferritin and the delivery of SGA infants. Rondo et al (1997) and Rondo et al (1999) used the Capurro method to estimate gestational age. The Capurro method may create a false association between elevated levels of maternal

serum ferritin and the delivery of SGA infants. However, Hou et al (2000) primarily relied upon early ultrasound to estimate gestational age. Early ultrasound may create a false association between low levels of maternal serum ferritin and the delivery of SGA infants.

**TABLE 12**  
**High Maternal Serum Ferritin Levels and**  
**Small-for-Gestational-Age Infants**

<b>STUDY</b>	<b>RESULT</b>	<b>GESTATIONAL AGE ESTIMATION METHOD</b>	<b>MEASURE OF FERRITIN LEVELS</b>
Bhargava, 1991	(+) Elevated serum ferritin levels assoc. w/ decreased bw and GA	Differential - LMP & Ballard scoring – false association with low ferritin levels	Non-differential or differential – may attenuate association
Abbas, 1994	(+) Growth retarded fetuses had increased maternal serum ferritin	Not specified	Non-differential or differential – may attenuate association
Rondo, 1997	(+) High maternal serum ferritin risk factor for IUGR	Differential - Capurro method – create false positive association	Non-differential or differential – may attenuate association
Rondo, 1999	(+) High maternal serum ferritin levels more common in IUGR infants	Differential - Capurro method – create false positive association	Non-differential or differential – may attenuate association
Hou, 2000	(+) High maternal serum ferritin with asymmetric FGR at 25 weeks (OR 3.4)	Differential – LMP and ultra – false association with low ferritin levels	Non-differential or differential – may attenuate association

## **CHAPTER 2**

### **STUDY OF MATERNAL SERUM FERRITIN LEVELS IN MID-PREGNANCY AND FETAL GROWTH**

#### **Methods**

Women were sampled from a large prenatal screening program at Michigan State University from November 1, 1988 through March 1, 1992. The women were screened for fetal abnormalities using maternal serum alpha-fetoprotein (MSAFP) during the 15<sup>th</sup> to 19<sup>th</sup> week of pregnancy. Fifteen to nineteen weeks' gestation is the typical screening time for MSAFP since it is clinically useful to determine the presence of abnormalities at an early gestational age. Gestational age was based on the first day of the last menstrual period (LMP) unless the early ultrasound age differed from the LMP age by two or more weeks. In that case, the gestational age was based on the ultrasound estimated age.

Women who were neither white nor African-American as well as women who had certain medical conditions that may have resulted in high MSAFP levels were excluded from the study. The medical conditions that led to exclusion were diabetes before pregnancy, multiple fetuses or a fetus with a structural defect or chromosomal abnormality identified around the time of screening. In addition, if a woman had two pregnancies screened from 1988-1992, only the first pregnancy was included.

The MSAFP levels were converted to multiples of the median (MoM) adjusted for gestational week and maternal weight with separate medians for

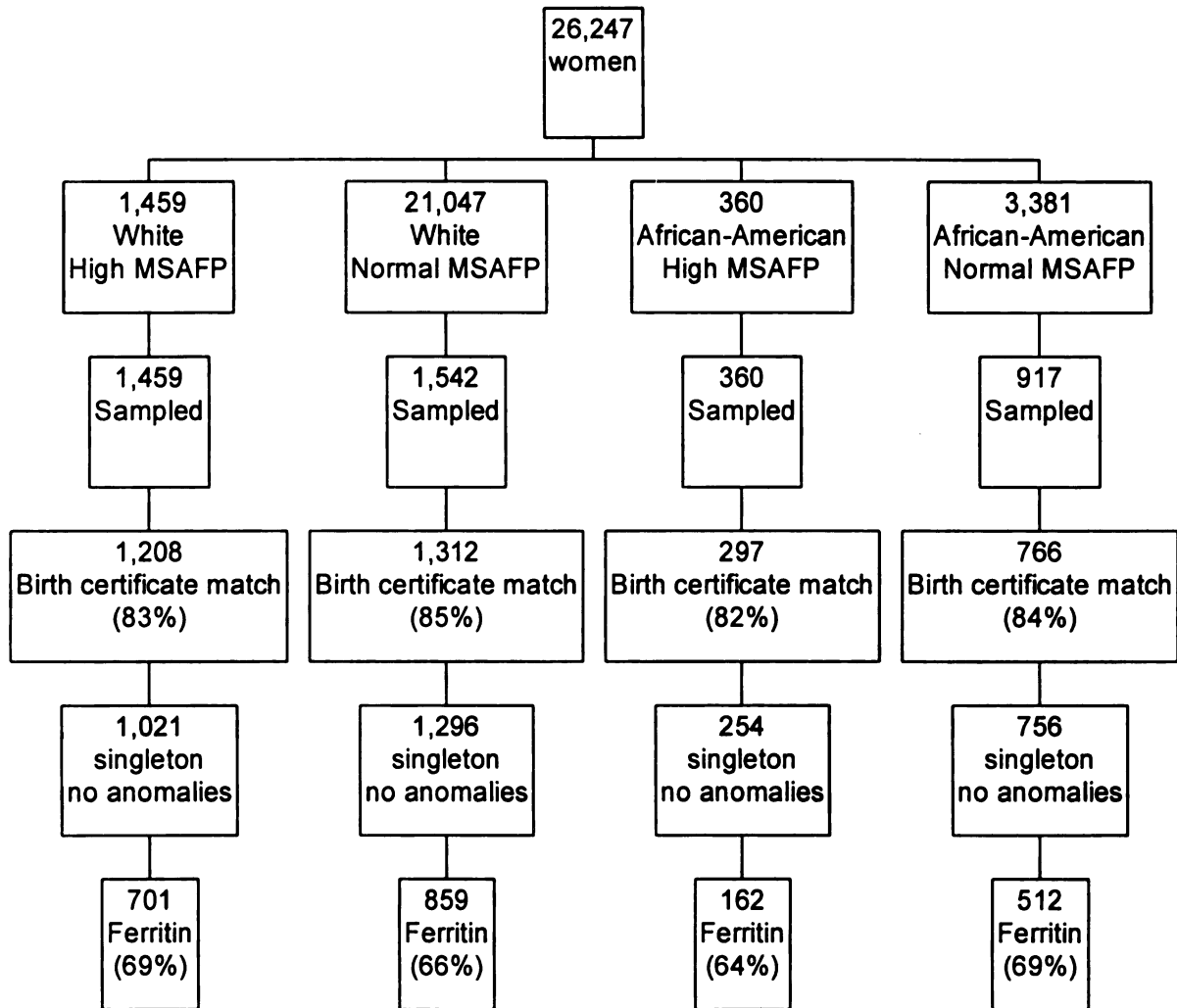
white and African-American women. The women were sampled and matched based on ethnicity (white, African-American) and MSAFP levels (0.5-1.5 MoM,  $\geq 2$  MoM). Women with normal MSAFP levels (0.5-1.5 MoM) were randomly sampled to attain an approximate 1 to 1 (normal to high MSAFP) frequency match for whites (N=1,542) and a 2.5 to 1 (normal to high MSAFP) frequency match for African-Americans (N=917). All women with unexplained high MSAFP levels ( $\geq 2$  MoM) were selected for the study (1,456 whites, 360 African-Americans). After the data on eligible women were stratified into four groups, based upon race (African-American, white) and MSAFP levels (0.5-1.5 MoM,  $\geq 2$  MoM) they were then linked to Michigan birth certificates. During the matching process to birth certificates, multiple births or infants with structural defects that were initially missed were excluded from the study. Matches for all four groups of women ranged from 82% to 85%. Information on ethnicity, gravidity, parity and maternal weight was collected from the prenatal screening request form. While information on age of mother, education of mother, smoking, and Medicaid status was collected from the birth certificates.

Gestational age at birth was calculated for the analysis from the gestational age at screening and the date on the birth certificate. Small-for-gestational-age (SGA) infants were defined as those infants below the 10<sup>th</sup> percentile in birthweight based upon the Arbuckle et al (1993) growth curves for gestational age (86). Appropriate-for-gestational-age (AGA) infants were above the cut-off. The percentiles were calculated separately by gender, but not by ethnicity. The birthweight percentiles were compared to the birthweight for each

infant plotted against their gestational age. Since underestimation of gestational age could attenuate the association between high serum ferritin levels and the delivery of SGA infants, 29 infants who were likely to have an underestimated gestational age (born before 37 weeks with birth weight above the 99<sup>th</sup> percentile) were removed from the study.

Women with available stored sera were retrospectively screened for maternal serum ferritin levels. Women without available sera were removed from the study (N=1,093). The final study sample consisted of 1,371 women with normal MSAFP levels (859 white, 512 African-American) and 863 women with high MSAFP levels (701 white, 162 African-American) (Figure 1). The sera were stored at –20 degrees centigrade and serum ferritin was measured using a radioimmunoassay kit (Incstar, double antibody).

**FIGURE 1**  
**Study Sample**





## **Analytic methods**

Maternal serum ferritin concentration was initially analyzed by quartiles within the four groups based on ethnicity (white and African-American) and MSAFP levels (normal and high). The cut point for the serum ferritin quartiles was created using the ferritin distribution for women with normal MSAFP (white and African-American). Since women with high MSAFP might have other factors that may contribute to poor fetal growth, it was speculated that the association between maternal serum ferritin and the delivery of SGA infants in the high MSAFP group might be apparent at lower levels of maternal serum ferritin. Therefore, the association was analyzed separately for women with normal and high MSAFP levels.

Variables were included in the multivariate model if they were known to be associated with the delivery of SGA infants, but were not mediators in the pathway of high maternal serum ferritin and the delivery of SGA infants. Variables were allowed to stay in the model if they had a p-value  $<0.10$  even if the variables did not significantly alter the magnitude of the ferritin to SGA relationship. Adjusted odds ratios (AOR) and 95% confidence intervals (CIs) were computed. All statistical analyses were performed using Proc Logistic within SAS (The SAS System for Windows, Release 8, SAS Institute Inc., Cary, NC).

## **Results**

A summary of background characteristics for women with serum ferritin measurement is given in Table 13. The women with measurement for maternal serum ferritin were similar to women without maternal serum ferritin measurement with respect to maternal demographic, behavioral and reproductive characteristics. Table 14 is stratified into two columns by MSAFP (normal and high) status. The number and percentage of SGA infants are listed for the four maternal serum ferritin quartiles. In addition, the total number and percentage of AGA and SGA infants are listed. The relationship between maternal serum ferritin and the risk of delivering an SGA infant exhibited an apparent threshold effect that differed by MSAFP status. The cut point for high maternal serum ferritin was at the fourth quartile (>98 ng/ml) for normal MSAFP and at the median (>52 ng/ml) for high MSAFP. The thresholds were approximately similar for whites and African-Americans so the data were combined for both ethnic groups.

**TABLE 13**

**Maternal Demographic, Behavioral and Reproductive Characteristics**

<b>VARIABLE</b>	<b>% OF TOTAL (N=2,234*)</b>
<b>Ethnicity</b>	
White	69.8% (1,560)
African-American	30.2% (674)
<b>Maternal Age</b>	
≤18	12.0% (267)
19-26	47.5% (1,062)
≥27	40.5% (905)
<b>Maternal Education</b>	
<12	21.5% (479)
12	43.1% (963)
>12	35.1% (785)
<b>Medicaid Insured</b>	
No	54.0% (1,206)
Yes	46.0% (1,028)
<b>Gravidity</b>	
1	39.8% (890)
>1	60.2% (1,344)
<b>Parity</b>	
0	53.2% (1,188)
≥1	46.8% (1,046)
<b>Maternal Weight (15-19 weeks)</b>	
≤135	35.4% (791)
136-160	32.5% (725)
≥161	32.0% (715)
<b>Smoking</b>	
0	72.7% (1,624)
1-19 cigs per day	12.8% (285)
≥ 20 cigs per day	6.5% (146)

\* Education missing 7 women; Maternal weight missing 3 women;  
Smoking missing 179 women

**TABLE 14****Number and Percentage of SGA Births within Ferritin Quartiles**

<b>FERRITIN QUARTILES BASED ON NORMAL MSAFP</b>		<b>Normal MSAFP N=1,371</b>	<b>High MSAFP N=863</b>
	<b>ng/ml</b>	<b>%SGA (N)</b>	<b>%SGA (N)</b>
Quartile # 1	≤28	7.9% (27)	5.2% (10)
Quartile # 2	29-52	9.0% (31)	9.3% (19)
Quartile # 3	53-98	8.8% (30)	12.9%(32)
Quartile # 4	≥99	17.4%(60)	14.8%(32)
All Quartiles		10.8% (148)	10.8% (93)

In the normal MSAFP group, women who were African-American, younger, less educated, Medicaid insured, primiparous, lighter weight at 15-19 weeks' gestation and smokers were more likely to deliver SGA infants (Table 15). Older women and heavier women at 15-19 weeks' gestation were more likely to have ferritin levels in the fourth quartile, but they were less likely to deliver an SGA infant. Failure to include maternal age and weight in the multivariate model could underestimate the effect of ferritin.

**TABLE 15**

**Maternal Characteristics, Serum Ferritin Levels and the Delivery of SGA Infants for Women with Normal MSAFP Levels**

VARIABLE	FERRITIN		SGA	
	QUARTILE 1-3 N=1,026	QUARTILE 4 N=345	NO N=1,223	YES N=148
<b>Ethnicity</b>				
White	63.0%	61.7%	64.0%	51.3%
African-American	37.0%	38.3%	36.0%	48.7%*
<b>Age</b>				
≤18	14.2%	9.3%	12.8%	14.9%*
19-26	48.8%	44.9%	46.9%	55.4%
≥27	37.0%	45.8%*	40.3%	29.7%
<b>Education**</b>				
<12	23.6%	19.2%	21.1%	34.0%*
12	42.7%	43.9%	43.3%	40.1%
>12	33.7%	36.9%	35.6%	25.9%
<b>Medicaid</b>				
No	50.2%	54.5%	52.9%	37.8%
Yes	49.8%	45.5%	47.1%	62.2%*
<b>Gravidity</b>				
1	38.8%	43.5%	39.1%	47.3%
>1	61.2%	56.5%	60.9%	52.7%
<b>Parity</b>				
0	52.5%	56.8%	52.2%	65.5%*
≥1	47.5%	43.2%	47.8%	34.5%
<b>Weight** (15-19 wks)</b>				
≤135	37.6%	32.7%	34.8%	49.3%*
136-160	34.1%	31.6%	33.5%	33.1%
≥161	28.3%	35.7%*	31.7%	17.6
<b>Smoking**</b>				
0	81.9%	79.4%	82.7%	69.6%
1-19 cig/day	12.2%	14.9%	11.6%	23.2%
≥20 cig/day	5.9%	5.7%	5.7%	7.2%*

\*Significant difference (p-value <0.05)

\*\*Missing information on 4 women for education, 1 woman for weight and 106 women for smoking.

In the high MSAFP group, women who were less educated, lighter weight and smokers were more likely to deliver SGA infants. Women who were older, heavier weight, primigravida, primiparous and smokers were more likely to have maternal serum ferritin levels in the third or fourth quartiles (Table 16). Smoking was associated with both maternal serum ferritin levels in the third or fourth quartiles and the delivery of SGA infants. In this case, failure to include smoking in the multivariate model would erroneously overestimate the association. Women who are heavier were more likely to have ferritin in the third or fourth quartile, but less likely to deliver an SGA infants. Failure to include maternal weight in the multivariate model would underestimate the association between elevated maternal serum ferritin and the delivery of SGA infants.

**TABLE 16**

**Maternal Characteristics, Serum Ferritin Levels and the Delivery of SGA Infants for Women with High MSAFP Levels**

VARIABLE	FERRITIN		SGA	
	QUART 1-2 N=398	QUART 3-4 N=465	NO N=770	YES N=93
<b>Ethnicity</b>				
White	83.4%	79.3%	81.4%	79.6%
African-American	16.6%	20.7%	18.6%	20.4%
<b>Age</b>				
≤18	12.1%	8.8%	10.3%	10.8%
19-26	49.7%	44.7%	47.1%	46.2%
≥27	38.2%	46.5%*	42.6%	43.0%
<b>Education**</b>				
<12	21.6%	18.6%	18.8%	30.1%*
12	42.6%	44.5%	44.3%	37.6%
>12	35.8%	36.9%	36.9%	32.3%
<b>Medicaid</b>				
No	57.5%	58.9%	58.6%	55.9%
Yes	42.5%	41.1%	41.4%	44.1%
<b>Gravidity</b>				
1	33.9%	44.6%*	39.5%	40.9%
>1	66.1%	55.4%	60.5%	59.1%
<b>Parity</b>				
0	48.0%	56.3%*	52.2%	54.8%
≥1	52.0%	43.7%	47.8%	45.2%
<b>Weight** (15-19 wks)</b>				
≤135	39.5%	29.3%	31.9%	52.2%*
136-160	32.0%	30.0%	31.9%	22.8%
≥161	28.5%	40.7%*	36.2%	25.0%
<b>Smoking**</b>				
0	80.3%	71.1%	77.6%	56.6%
1-19cig/day	11.3%	19.1%	13.9%	28.9%
≥20 cig/day	8.4%	9.8%*	8.5%	14.5%*

\*Significant difference (p-value <0.05)

\*\* Missing information on 3 women for education, 2 women for weight and 73 women for smoking

For women with normal MSAFP, the unadjusted odds ratio for the relationship between high serum ferritin levels and the delivery of SGA infants was 2.2 (95% CI 1.5-3.2). For women with high MSAFP, the unadjusted odds ratio was 2.3 (95% CI 1.4-3.8) (Table 17). Next, this relationship was next evaluated while adjusting for potential confounding variables (Tables 15 and 16). For women with normal MSAFP, the adjusted odds ratio was 2.5 (95% CI 1.7-3.7). For women with high MSAFP, the adjusted odds ratio was 2.5 (95% CI 1.5-4.1). The adjusted odds ratio when controlling for additional variables associated with the delivery of small-for-gestational-age infants was 2.4 (95% CI 1.7-3.6) for normal MSAFP and 2.5 (95% CI 1.5-4.1) for high MSAFP. Since accuracy and precision were not greatly compromised when controlling for these additional variables, the variables were allowed to remain in the model if they were statistically significant.

**TABLE 17**

**The Relationship between Elevated Serum Ferritin,  
MSAFP and the Delivery of SGA Infants**

<b>Variables</b>	<b>NORMAL MSAFP N=1,262 OR (95% CI)</b>	<b>HIGH MSAFP N=785 OR (95% CI)</b>
<b>Unadjusted</b>	2.2 (1.5-3.2)	2.3 (1.4-3.8)
<b>Adjusted</b>	*2.4 (1.7-3.6)	**2.5 (1.5-4.1)

\*parity, maternal weight, ethnicity, smoking, Medicaid use

\*\*maternal weight, smoking

Criteria for remaining in model: Likelihood ratio test with p-value <0.10



Time in storage may degrade the sera and alter ferritin levels (Chard). Therefore, statistical analysis was performed in order to determine if the storage time of serum ferritin might have biased the results of the study. It was found that storage time was unlikely to bias study results since the mean of log ferritin for each year of sampling did not vary significantly. It is still possible that the absolute ferritin levels measured are affected by storage, but the ferritin levels are not affected differentially by duration of storage in this study.

Statistical analysis was performed in order to determine if inaccuracy of gestational age estimation might have biased the results of the study. Since early ultrasound is considered to be the most practical and accurate method of determining gestational age in clinical use, the analyses were repeated after dividing the women into two subgroups: Those with early ultrasound and those without early ultrasound. In the subgroup with early ultrasound, the estimated magnitude of the association was slightly stronger in the normal MSAFP group (AOR 2.7, 95% CI 1.6-4.7) group and diminished below significance in the high MSAFP group (AOR 1.6, 95% CI 0.9-3.0) when compared to the original analysis. In the subgroup without ultrasound, the magnitude of the association for high serum ferritin and the delivery of SGA infants for women with normal was similar to the original analysis MSAFP (AOR 2.4, 95% CI 1.4-4.2) and increased for women with high MSAFP (5.9, 95% CI 2.2-16.0).

It is not possible with the data available to determine the reason for the increased risk for the delivery of an SGA infant for women with high MSAFP levels in the group without ultrasound. It is important to recognize that the

number of SGA infants for women with high MSAFP in the ultrasound group (N=33) and in the LMP group (N=31) is rather small. In addition, another consideration is that the increased risk could be due to an overestimation of gestational age. Since maternal serum ferritin levels decrease during pregnancy, an overestimation of gestational age could create a spurious association between the delivery of SGA infants and high levels of maternal serum ferritin.

## **CHAPTER 3**

### **DISCUSSION**

High maternal serum ferritin levels measured at 15-19 weeks gestation were associated with more than a two-fold increased risk of the delivery of small-for-gestational-age infants. Although there was a threshold effect for elevated serum ferritin that differed by MSAFP status, the strength of the association was similar for both women with normal MSAFP levels and women with high MSAFP levels (adjusted OR 2.43 and 2.48, respectively). The results of this study agree with past studies that have found a relationship between high maternal serum ferritin and the delivery of SGA infants (4,5,6,7,8).

One possible explanation for the association between elevated maternal serum ferritin levels and the delivery of SGA infants is the lack of plasma volume expansion during SGA pregnancies. Plasma volume during a normal pregnancy can increase by 50% (70). However, in SGA pregnancies the increase in plasma volume may only be 25% (9). The lower plasma volume expansion in SGA pregnancies as compared to AGA pregnancies could create a higher serum ferritin concentration due to lack of hemodilution. There are few accurate ways to measure hemodilution during pregnancy and these methods were not available for this study.

Since there is some evidence that the amount of plasma volume expansion is related to maternal weight in early pregnancy, maternal weight was controlled for in the analysis (87,88). In addition, high levels of maternal serum

ferritin in this study were associated with high maternal weight. It would be expected that high maternal serum ferritin levels would be associated with low maternal weight if lack of plasma volume expansion were the reason for elevated ferritin levels. Yet, maternal weight should not be viewed as an exact measure of plasma volume expansion. As a result, it is not possible to eliminate lack of plasma volume expansion as an explanation for the relationship between elevated maternal serum ferritin and the delivery of SGA infants. In addition, maternal weight may be a mediator in the causal pathway to the delivery of SGA infants. Controlling for maternal weight as a confounder may thus introduce iatrogenic bias and attenuate the results of the study to the null.

The placenta is vital to normal fetal growth since nutrients such as glucose, nitrogen, and lipids are transported across the placenta to the fetus. Infections and inflammation can cause damage to the placenta and are associated with poor fetal growth (10,61). In addition, there is biological evidence to indicate that maternal infection or noninfectious inflammatory conditions may lead to high maternal serum ferritin levels (4,16,20). In fact, serum ferritin has been shown to act as an acute phase reactant in inflammatory diseases. During infection, the body withholds iron from pathogenic microorganisms in part by increased ferritin synthesis (13,14,19). During inflammation, it is speculated that the body probably withholds iron to avoid tissue damage from free radical generation (14).

Anemia is generally caused by iron deficiency. Therefore, it is speculated that anemia in the presence of increased ferritin levels (usually reflective of total

body iron stores) may indicate an infectious or inflammatory process (4). Two studies found that even in the presence of anemia, maternal serum ferritin levels were elevated among the mothers of SGA infants (4,7). This indicates that infection or inflammation, not iron overload, may have increased the maternal serum ferritin levels. However, the current study did not have a measure of maternal infection so was unable to support or refute this hypothesis.

Previous studies have indicated that maternal hypertension and especially preeclampsia are associated with inflammatory cell response (74,89,90,91). The delivery of small-for-gestational-age infants is associated with maternal hypertension (chronic hypertension, preeclampsia, eclampsia) (10,59,77). In addition, elevated levels of maternal serum ferritin have been associated with preeclampsia (92).

The current study and Hou et al (2000) failed to find elevated maternal serum ferritin levels associated with maternal hypertension. However, in the current study, diagnosis of PIH in this study was taken from the birth certificate. Studies have shown that birth certificates underreport the occurrence of pregnancy complications (93,94). This underreporting could attenuate the association between elevated serum ferritin and maternal hypertension. Hou et al (2000) speculated that the lack of association in their study could be due to the inability to distinguish between the different forms a maternal hypertension.

In future studies it may be useful to classify SGA infants as asymmetric or symmetric in order to fully understand the role of serum ferritin as a marker for poor fetal growth. Asymmetric fetal growth retardation often occurs due to

uteroplacental insufficiency usually from maternal hypertension or diabetes (3). This type of growth retardation has been associated with elevated levels of maternal serum ferritin (8). Symmetric fetal growth retardation is often due to constitutionally small infants, chromosomal anomalies, malnutrition, smoking or maternal infection (3). This type of growth retardation has been associated with low levels of maternal serum ferritin (8).

Studies should actively screen women for infections and maternal hypertension. Bhargava et al (1991) found that 18 women had an apparent clinical infection with severe anemia and elevated serum ferritin levels (mean 33 ng/ml). In addition, 55 women with anemia had higher than expected ferritin levels (>10 ng/ml) without apparent clinical infection. This suggests that some women have subclinical infections that may remain undiagnosed. Therefore, future studies could actively screen for maternal infections and hypertension. One way to actively screen for maternal infections and hypertension is by examination of placentas. In addition, accurate reporting of maternal hypertension may further help to distinguish between placental damage due to infection or maternal hypertension.

Additional markers may be useful for disentangling the association between serum ferritin and the delivery of SGA infants. In the current study, it was found that women with high MSAFP had a lower cut point for elevated maternal serum ferritin levels. In addition, other potential markers for the delivery of SGA infants should be measured. Cytokines, especially interleukin-1 and tumor necrosis factor, should be measured since they may mediate changes in

serum ferritin levels during inflammation (14). Accurate and complete measurement of these potential markers could allow for the development of a predictive model for physicians. This could indicate to physicians a subset of women that need to be followed closely throughout pregnancy and allow for timely interventions.

More research should be performed in order to determine if the association is a biomarker for infection or non-infectious inflammation. If elevated maternal serum ferritin levels are a marker for inflammatory processes, then medical treatment of the infection or inflammation in mid-pregnancy may improve the prognosis of SGA infants. The relationship deserves further investigation as maternal serum ferritin may prove to be an early marker for slow fetal growth and allow for interventions to prevent infant morbidity and mortality.

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