CRISTIANE BARBIERI

MEDIDAS ULTRA-SONOGRÁFICAS DA SECÇÃO TRANSVERSAL DO CORDÃO UMBILICAL E DE SEUS VASOS, SEGUNDO IDADE GESTACIONAL, EM GESTAÇÕES DE BAIXO RISCO

Dissertação de Mestrado

ORIENTADOR: Prof. Dr. JOSÉ GUILHERME CECATTI

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Dissertação de Mestrado apresentada à Pós-Graduação da Faculdade de Ciências Médicas da Universidade Estadual de Campinas para obtenção do Título de Mestre em Tocoginecologia, área de Tocoginecologia.

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Aluno: CRISTIANE BARBIERI

Orientador: Prof. Dr. JOSÉ GUILHERME CECATTI

Membros:

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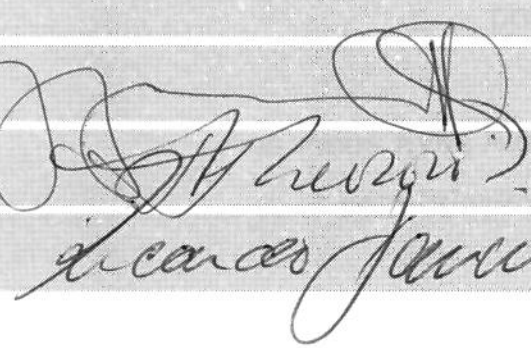

Curso de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas

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Dedico este trabalho...

À minha família

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por terem me dado a vida.

Aos meus avós Aldo (*in memorian)* e Cida,
pela criação, formação e educação.

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SUMÁRIO

Símbolos, Siglas e Abreviaturas

A	*Umbilical Artery*
AC	*Abdominal circumference*
AGA	*Adequate for gestational age*
AIG	Adequado para a idade gestacional
BPD	*Biparietal diameter*
C	*Umbilical cord*
CA	Circunferência abdominal
CAISM	Centro de Atenção Integral à Saúde da Mulher
CC	Circunferência cefálica
CEP	Comitê de Ética em Pesquisa
CF	Comprimento do fêmur
95% CI	*95% Confidence Interval*
DBP	Diâmetro bi-parietal
DP	Desvio padrão
DTG	Departamento de Tocoginecologia
E	especificidade
EFG	*Estimated fetal weight*
FL	*Femur length*
GA	*Gestational age*
GIG	Grande para a idade gestacional
GW	Geléia de Wharton
IBGE	Instituto Brasileiro de Geografia e Estatística
IC 95%	Intervalo de confiança de 95%

ICC *Intraclass correlation coefficient*

IG Idade gestacional

IRB *Institutional Review Board*

IUGR *Intrauterine growth restriction*

LGA *Large for gestational age*

LMP *Last menstrual period*

Log Logarítmo

NPV *Negative predictive value*

p Nível descritivo do teste estatístico (significância estatística)

PFE Peso fetal estimado

PIG Pequeno para a idade gestacional

PPV *Positive predictive value*

R Coeficiente de correlação linear de Spearman

R^2 Coeficiente de determinação

RCIU Restrição do crescimento intra-uterino

S Sensibilidade

_SD_ *Standard deviation*

Sen *Sensitivity*

SGA *Small for gestational age*

Spec *Specificity*

TCLE Termo de consentimento livre e esclarecido

UNICAMP Universidade Estadual de Campinas

_US_ *Ultrasound*

_V_ *Umbilical Vein*

VVN Valor preditivo negativo

VPP Valor preditivo positivo

_WJ_ *Wharton Jelly*

_Ø_ *Diameter*

RESUMO

Introdução: mais recentemente, demonstrou-se que o diâmetro do cordão umbilical pode se modificar nos casos de diabetes mellitus, pré-eclâmpsia, restrição de crescimento intra-uterino e baixo peso ao nascimento, podendo talvez ser utilizado como um marcador para detecção precoce destas condições.

Objetivo: obter intervalos de referência das medidas ultra-sonográficas da área do cordão umbilical, dos diâmetros de seus vasos e da área da superfície da Geléia de Wharton da secção transversa do cordão umbilical em função da idade gestacional em gestações de baixo risco, entre 12 e 40 semanas, avaliar a variabilidade inter- e intra-observador destas medidas e investigar sua correlação com o peso fetal estimado.

Método: foram avaliadas 2310 gestantes no período entre junho de 2005 e dezembro de 2006, seguindo critérios de inclusão e exclusão pré-estabelecidos. Em uma sub-amostra destas gestantes foi avaliada a variabilidade inter- e intra-observador, estimando-se o coeficiente de correlação de Spearman, o coeficiente de correlação intra-classe e o alfa de Crombach. Para cada idade gestacional, foi avaliado um número mínimo de 59 casos, calculando-se a

média e seu respectivo desvio-padrão e os percentis 10, 50 e 90 de cada uma das medidas. Para a análise estatística foram utilizados os testes t de Student, Anova e Wilcoxon para amostras independentes. Os intervalos de referência foram estimados por regressão polinomial de terceiro grau. Foi avaliado também o desempenho da área da secção transversa do cordão umbilical, do diâmetro do cordão umbilical e da área de geléia de Wharton do cordão umbilical em predizer alterações do peso fetal estimado (PFE) nestas gestações, estimando-se sua sensibilidade, especificidade, valor preditivo positivo e negativo.

Resultados: Foram obtidas elevadas correlação, reprodutibilidade e confiabilidade na avaliação da variabilidade inter e intra-observador das medidas do cordão umbilical e de seus vasos. Os intervalos de referência apresentaram valores crescentes até cerca de 32 semanas e depois estabilizaram-se. As medidas avaliadas apresentaram baixa sensibilidade para predição de alterações do PFE. Portanto, elas não devem ser utilizadas para rastreamento com esta finalidade.

Conclusões: os valores normais padronizados para essas medidas em todas as idades gestacionais, para gestações de baixo risco na população de referência, foram determinados, havendo a necessidade de que sejam posteriormente validados como preditores de situações perinatais adversas.

Palavras-chave: ultra-sonografia, curva de normalidade, cordão umbilical, veia umbilical, artéria umbilical, geléia de Wharton, peso fetal estimado.

ABSTRACT

Introduction: recently it has been demonstrated that the diameter of the umbilical cord may be modifyed in the case of diabetes, preeclampsia, intrauterine growth restriction and low birthweight. In this way it could perhaps be used as a marker for early detection of these conditions.

Objective: to obtain reference ranges for the ultrasonographic measurements of the umbilical cord area, the diameters of its vessels and the area of the Wharton Jelly surface from a cross sectional plan of the umbilical cord according to gestational age in low risk pregnancies between 12 and 40 weeks; to evaluate the inter- and intra-observer variability of these measurements; and to investigate their correlation with the estimated fetal weight.

Method: a total of 2310 pregnant women were evaluated in the period from June 2005 and December 2006, following previously stablished inclusion and exclusion criteria. Inter and intra-observer variability were evaluated in a sub sample of these pregnant women, with the estimation of Spearman correlation coefficient, the intraclass correlation coefficient and alfa of Crombach. For each gestational age a minimum number of 59 cases were evaluated. For statistical analysis mean and standard deviation and the percentiles 10, 50 and 90 for

each one of the measurements were estimated. Student t, Anova and Wilcoxon tests for independent samples were used. The reference ranges were estimated by third degree polynomial regression. The performance of the area of the transverse section of umbilical cord, its diameter and the area of the Wharton Jelly in predicting deviations of the estimated fetal weight (EFW) was also estimated among these pregnancies, with their sensitivity, specificity, positive and negative predictive values.

Results: High correlation, reproductibility and feasibility were obtained when evaluating the inter- and intra-observer variability of the measurements of umbilical cord and its vessels. The reference intervals presented increasing values up to around 32 weeks, and afterwards they estabilized. The measurements evaluated showed very low sensitivity for predicting deviations of the EFW. Therefore they should not be used for screening with this purpose.

Conclusions: the normal standardized values for these measurements in all gestational ages, for low risk pregnancies in the reference population, were determined. There is still the need of them being validated as predictors of adverse perinatal conditions.

Key words: ultra-sound, normal curve, umbilical cord, umbilical vein, umbilical artery, Wharton Jelly, estimated fetal weight.

1. INTRODUÇÃO

O exame ultra-sonográfico tem sido usado em obstetrícia há mais de cinco décadas. O exame é útil na detecção de malformações congênitas, no diagnóstico de gestações múltiplas, localização da placenta, crescimento fetal, assim como na identificação de pacientes com risco de pós maturidade e restrição de crescimento intra-uterino (Eik-Nes et al., 1984; Persson et al., 1983).

A ultra-sonografia obstétrica tem diferentes finalidades em cada fase da gestação. O exame realizado no primeiro trimestre busca identificar a gravidez intra-uterina, identificar o número de fetos, estimar a idade gestacional por meio do comprimento cabeça-nádega, com uma precisão de 3 a 5 dias, e avaliar a medida da translucência nucal como forma de rastreamento genético (Callen, 2002). O exame realizado no segundo trimestre, preferencialmente entre 19 e 20 semanas, pode ser útil para excluir a maioria das anormalidades fetais, por meio de um exame morfológico minucioso das estruturas fetais, assim como da placenta, do cordão umbilical e da quantidade de líquido amniótico. No terceiro trimestre, a avaliação morfológica fetal se torna mais difícil, mas é útil na

avaliação da situação e apresentação fetais, determinação do peso fetal, volume de líquido amniótico, avaliação da maturidade placentária e das trocas materno-fetais, por intermédio do estudo Doppler na artéria umbilical.

Entre as estruturas anatômicas identificadas durante o exame, encontra-se o cordão umbilical, tradicionalmente limitado à detecção da presença de três vasos, sendo duas artérias e uma veia (Wu et al., 1997) e para a avaliação do fluxo placentário, com a análise da onda Doppler (Goffinet et al., 1997).

O cordão umbilical começa a ser visibilizado na oitava semana de gravidez, como uma estrutura reta e relativamente espessa. Nesta fase, seu comprimento é aproximadamente igual ao comprimento cabeça-nádegas do embrião (Lyndon et al., 1994). O diâmetro do cordão normalmente é inferior a 2 cm (Weissman et al.,1994). O cordão contém duas artérias e uma veia. A veia umbilical leva sangue oxigenado da placenta para o feto e conecta-se com a veia porta esquerda no fígado fetal. O sangue desoxigenado da aorta fetal passa para as artérias hipogástricas que caminham superior e medialmente para depois entrar no cordão com as artérias umbilicais, as quais são contínuas com as artérias ilíacas internas. Os vasos no interior do cordão são circundados pela geléia de Wharton, um tecido conjuntivo gelatinoso que protege os vasos umbilicais contra compressão (Benirschke et al., 1990). O cordão umbilical tem sido estudado sob os aspectos anatômicos, patológicos e fisiológicos (Hill et al.,1987; Clausen I, 1989). Alterações da sua estrutura, tais como dilatação do cordão, dos vasos, presença de cistos e edema podem ser detectados durante

os exames ultra-sonográficos em qualquer época da gestação. Vizza et al. relataram em 1996 que o colágeno da geléia, estudado por microscopia eletrônica apresenta um sistema de cavidades interconectadas que promove a difusão de água e outros metabólitos dos vasos do cordão e do líquido amniótico. Os diferentes aspectos do cordão ao nascimento podem estar relacionados ao conteúdo de água e à diferente quantidade de geléia (Scott et al., 1978). Estudos de patologia mostram que as células da geléia de Wharton podem sofrer contrações, tais como as células musculares e participar da regulação do fluxo sanguíneo umbilical (Gebrane –Younes et al., 1986)

A morfologia do cordão umbilical, incluindo seu diâmetro e a quantidade de geléia de Wharton, tem sido associada a efeitos perinatais adversos, tais como doença hipertensiva (Raio et al., 2002), diabetes mellitus gestacional (Weissman et al., 1997), restrição de crescimento intra-uterino (Di Naro et al., 2002), peso fetal abaixo do esperado ao nascimento (Degani et al., 2001), sofrimento fetal durante o trabalho de parto e indicação de parto cesárea (Ezimokhai et al., 2000). Em 2004, Raio et al avaliaram 160 fetos com mais de 20 semanas, cujas áreas de secção transversa do cordão umbilical estavam abaixo do p10 para a respectiva idade gestacional. Foram avaliadas também as áreas de secção transversa das artérias e da veia, além da área de superfície da quantidade de geléia de Wharton. O estudo mostrou que entre fetos portadores de cordões finos, o risco de resultados perinatais adversos é inversamente proporcional à área do cordão. Eles observaram que as áreas do cordão umbilical e da veia foram significativamente menores nos fetos

admitidos em unidades de terapia intensiva após o nascimento. Esse achado é consistente com outros estudos onde a redução do calibre do cordão umbilical está associada a desordens de crescimento e desenvolvimento fetais ou sofrimento fetal durante o trabalho de parto. Bruch et al. também demonstraram que as áreas do cordão e da veia são menores em fetos com diagnóstico de restrição de crescimento intra-uterino. Não só o calibre da veia, mas também a espessura da parede das artérias e da veia são diferentes nestes fetos quando comparados aos fetos sem anormalidades, com ou sem parâmetros Doppler anormais. O mesmo ocorre nas pacientes com hipertensão crônica e pré-eclâmpsia (Inan et al., 2002). Com a redução da luz da veia, há um aumento na resistência do fluxo sanguíneo e uma remodelação da hemodinâmica feto-placentária.

Por outro lado, há casos em que são encontrados cordões umbilicais com aumento da sua espessura. Esse aumento de espessura do cordão é atribuído às alterações moleculares dos componentes da matriz extracelular, por uma expressão genética anormal em algumas proteínas estruturais, além de uma alteração na distribuição das fibras conjuntivas, com grandes espaços vazios entre elas, resultando em um aumento de superfície, já diagnosticada em gestações de 24 semanas, sugerindo que o envolvimento do cordão nos fetos de mães diabéticas ocorre precocemente (Weissman et al., 1997). Resulta em acúmulo anormal de fluido e edema na geléia de Wharton (Ghezzi et al., 2002). Outros autores, por meio de estudos retrospectivos, avaliaram os diâmetros externos do cordão umbilical e de seus vasos internos, além da quantidade de

geléia de Wharton, na inserção do cordão na parede abdominal em fetos com diagnóstico de alterações cromossômicas, entre 14 e 23 semanas de gestação. Essas medidas foram comparadas com uma tabela de normalidade pré-existente, descrita por Predanic et al., entre 14 e 22 semanas de gestação. Eles concluíram que a maioria dos fetos com aneuploidia apresentou a medida do diâmetro do cordão umbilical acima do percentil 95 (Predanic et al., 2004).

Em 1995, Jackson et al. encontraram paredes arteriais mais finas nas placentas de fetos com diagnóstico de restrição de crescimento intra-uterino, quando comparadas às placentas de fetos normais (Jackson et al., 1995). Recentemente, Di Naro et al. avaliaram a área do cordão umbilical, a quantidade de geléia de Wharton e o fluxo sangüíneo na veia umbilical em 116 pacientes com gestações únicas, 24 horas antes do parto a termo, observando que há uma correlação significativa entre a presença de um cordão umbilical fino e inadequada vitalidade fetal ao nascimento (Di Naro et al., 2001).

Atualmente é consenso que o estudo ultra-sonográfico do cordão umbilical não se deva restringir à simples verificação do seu número de vasos e Dopplervelocimetria da artéria umbilical, ante as importantes informações passíveis de se obter, pois as alterações da espessura do vaso ocorrem precocemente, antes mesmo da detecção da diminuição do volume do líquido amniótico.

Cada vez mais, especialistas e estudiosos dedicados à avaliação fetal intra-útero vêm buscando novos parâmetros de normalidade, que podem antecipar as suspeitas de algumas doenças gestacionais, como pré-eclâmpsia, restrição de crescimento intra-uterino e outros.

Muitas anormalidades do cordão umbilical podem ser detectadas pela ultra-sonografia e podem estar associadas a anomalias fetais, cromossômicas e complicações potenciais durante a gravidez. O conhecimento de sua anatomia e de seu desenvolvimento normal são importantes para o diagnóstico pré-natal.

O conhecimento adequado da morfologia do cordão umbilical e de seu diâmetro pode preceder as mudanças hemodinâmicas que podem levar a um maior risco de restrição de crescimento intra-útero, desenvolvimento de pré-eclâmpsia e sofrimento fetal. Desta forma, a construção de intervalos de referência de medidas do cordão umbilical ao longo da gestação permitiria a identificação de gestações com medidas fora do normal para a possível predição de situações perinatais adversas.

2. OBJETIVOS

2.1. Objetivo geral

Avaliar ultra-sonograficamente a espessura do cordão umbilical, dos seus vasos, da sua área e da superfície da Geléia de Wharton, em um corte transversal do cordão umbilical, em gestações de baixo risco entre 12 e 40 semanas, e correlacionar essas medidas com parâmetros de crescimento fetal.

2.2. Objetivos específicos:

- Avaliar a variabilidade inter- e intra-observador das medidas ultra-sonográficas da area de secção transversal do cordão umbilical e dos diâmetros de seus vasos internos em gestações de baixo risco entre 12 a 40 semanas de idade gestacional.

- Construir intervalos de referência da área e do diâmetro da secção transversa do cordão umbilical e dos diâmetros de seus vasos internos, em fetos de 12 a 40 semanas de gestação.

- Construir intervalos de referência da área da superfície da Geléia de Wharton na secção transversa do cordão umbilical em função da idade gestacional e investigar sua correlação com o peso fetal estimado.

- Avaliar o desempenho da área da secção transversa do cordão umbilical, do diâmetro do cordão umbilical e da área de geléia de Wharton do cordão umbilical em predizer alterações do peso fetal estimado (PFE) em gestações de baixo risco, entre 20 e 40 semanas.

3. SUJEITOS E MÉTODO

3.1. Desenho do estudo

Este foi um estudo prospectivo para a determinação dos intervalos de referência das medidas da espessura do cordão umbilical, do diâmetro de seus vasos internos, da superfície de sua área e da superfície da área da Geléia de Wharton, em gestações de baixo risco. Para cada idade gestacional, foram calculados os percentis 10, 50 e 90. Foi também realizada uma avaliação da variabilidade inter- e intra-observador em cerca de 10% do total da amostra.

3.2. Técnica

Para a medida do diâmetro do cordão umbilical em qualquer idade gestacional, a partir de 12 semanas, foi realizado um corte ultra-sonográfico transversal da circunferência abdominal fetal, na qual se observa a inserção do cordão na parede abdominal, no nível da bexiga. Em gestações de 12 a 14 semanas, a

medida dos diâmetros do cordão umbilical e de seus vasos internos foi realizado em um corte longitudinal do cordão umbilical, no máximo a uma distância de até 2,0 cm da inserção abdominal. Em gestações de mais de 14 semanas, inicialmente, com o transdutor perpendicularmente ao eixo longitudinal do cordão, rodando-se o transdutor 90 graus, sem incliná-lo, obteve-se a secção transversal do cordão umbilical. A medida do diâmetro do cordão umbilical foi realizada com o calibrador de medida na parede externa do cordão, de uma extremidade à outra, bem próximo da inserção do cordão na parede abdominal, ou no máximo até 2,0 cm da mesma. Para a avaliação do diâmetro dos vasos internos, o calibrador de medidas foi colocado na parede interna do vaso. Com o recurso do calibrador de medidas na forma elíptica, obteve-se tanto o valor dos diâmetros dos vasos, quanto o valor de suas áreas. No caso da medida das artérias umbilicais, apenas uma teve seu diâmetro avaliado, cada examinador escolhendo aquela com melhor visibilização de seus contornos. Para a obtenção da área de superfície de geléia de Wharton, subtrairam-se do valor da área total do cordão umbilical, as áreas dos seus vasos internos.

Sabe-se que é comum encontrar, no primeiro trimestre, a chamada herniação fisiológica do intestino à base do cordão umbilical, criando-se uma massa focal proeminente, entre 9 e 10 semanas, podendo persistir até 11 semanas de gestação, devido à rotação de 90º em sentido anti-horário do intestino em torno da base da artéria mesentérica superior. A massa resolve-se por volta da 11ª semana, quando o intestino retorna à sua localização intra-abdominal. Neste

caso, pode-se repetir a medida uma semana depois, quando já não há mais a presença de herniação. Em gestantes normais o diâmetro da base do cordão contendo o intestino herniado deve ser inferior a 7mm e não deve haver nenhuma herniação visível depois de que o comprimento cabeça-nádegas do feto atingir 45mm ou mais. Nestes casos, pode estar havendo um diagnóstico precoce de gastrosquise ou onfalocele. O estudo nesta idade gestacional deve ser realizado por via transvaginal, não por via transabdominal, pois facilita a visualização das estruturas fetais, ainda muito pequenas. A partir do segundo trimestre, a avaliação ocorre por via transabdominal, devendo-se realizar a medida do diâmetro do cordão umbilical seguindo-se o mesmo método acima descrito.

Além disso, foi realizada a avaliação da estimativa do peso fetal, calculada a partir das medidas da circunferência abdominal, craniana, diâmetro biparietal e comprimento do fêmur, utilizando-se a tabela de Hadlock et al. (1991) existente nos aparelhos de ultra-som e as técnicas previamente padronizadas para essas medidas.

3.3. Tamanho amostral

Foram avaliadas no mínimo 59 gestantes para cada idade gestacional, entre 12 e 40 semanas de gestação. Utilizando os dados aplicados de Raio et al., de 1999, que mostram um valor médio para o diâmetro do cordão umbilical às 35 semanas de 16,27mm com desvio padrão de 2,67mm (o maior entre os valores

de todas idades gestacionais) e, admitindo-se um erro do tipo I de 0,05 para detectar uma diferença mínima entre a média populacional e amostral de 0,68mm correspondente a 25% do valor do desvio padrão, o número mínimo necessário é de uma amostra independente de 59 casos em cada idade gestacional, com intervalos de uma semana.

3.4. Seleção dos sujeitos

Foram selecionadas gestantes encaminhadas ao Serviço de Ultra-sonografia do CAISM (32% da amostra) ou à Clínica CDE (68% da amostra) para exames de rotina do pré-natal, encaminhadas dos Ambulatórios de pré-natal ou de serviços de saúde pública e privada da cidade de Campinas e região. As mulheres foram convidadas a participar do estudo, após explicação e assinatura do termo de consentimento livre e esclarecido.

Critérios de Inclusão

- Gestantes entre 12 e 40 semanas, com gestações únicas
- Gestantes de baixo risco

Critérios de exclusão

- Gestantes com doença hipertensiva gestacional ou hipertensão crônica
- Gestantes com diabetes gestacional ou diabetes mellitus
- Gestações gemelares
- Fetos com diagnóstico ecográfico de malformações

- Fetos com suspeita clínica de restrição de crescimento intra-uterino
- Gestantes com diagnóstico de pré-eclâmpsia
- Diagnóstico de amniorrexe
- Presença de oligohidrâmnio ou polidrâmnio.
- Anomalias do cordão umbilical, tais como a presença de cistos no cordão, presença de artéria umbilical única e cordões lisos.

3.5. Variáveis

3.5.1. Variável dependente

Diâmetro do cordão umbilical, de seus vasos internos, da área de superfície do cordão e da área de superfície da geléia de Wharton, medidos ultra-sonograficamente entre 12 e 40 semanas de gestação, próximo à inserção do cordão na parede abdominal fetal (em mm).

3.5.2. Variável Independente

Idade gestacional, calculada a partir do primeiro dia da última menstruação, quando confiável, confirmada pelo exame ultra-sonográfico a partir do comprimento cabeça-nádegas do feto (em semanas e dias) realizado no primeiro trimestre da gestação. Quando incerta a data da última menstruação, a idade gestacional foi calculada pela medida do comprimento crânio-caudal fetal por ultra-sonografia.

3.5.3. Variáveis de controle

- Idade materna, em anos completos no momento do exame
- Antecedentes obstétricos: número de gestações, abortos, partos e de filhos vivos referidos pela gestante
- Raça materna, autoclassificada pelo sujeito segundo as categorias utilizadas no censo demográfico de 2000, pelo Instituto Brasileiro de Geografia e Estatística (IBGE), como branca, preta, parda, amarela, indígena ou outra.
- Medida do peso fetal estimado, realizado durante o exame de ultra-som, em gramas, calculado a partir das medidas do diâmetro biparietal, circunferência intracraniana, comprimento do fêmur e circunferência abdominal fetais, segundo a fórmula de Hadlock et al. (1991).

3.6. Coleta, processamento e análise de dados

Durante a realização dos exames na Seção Técnica de Ultra-sonografia ou na clínica privada, os dados do diâmetro do cordão umbilical e demais medidas, além da idade gestacional, foram colocados em uma ficha pré-codificada (Anexo 3) pelo pesquisador. Os dados foram posteriormente digitados em um banco de dados em programa Epi-Info para microcomputador.

Para cada intervalo de idade gestacional de uma semana, foi avaliado um número mínimo de 59 casos, calculando-se a média e seu respectivo desvio-

padrão, e também os percentis 10, 50 e 90, utilizando-se posteriormente um processo de alisamento da curva obtida por regressão polinomial de segundo e terceiro grau.

As medidas foram também realizadas independentemente por outro médico e depois novamente pelo pesquisador, para a comparação da variabilidade inter- e intra-observador, em cerca de 10% do total de casos da amostra. Ambos examinadores fizeram treinamento específico para a realização destas medidas e para sua obtenção de forma independente e cega, utilizando-se posteriormente das imagens fotografadas para a obtenção dos valores.

3.7. Aspectos éticos

As gestantes incluídas no estudo foram aquelas que rotineiramente são examinadas pela Seção Técnica de Ultra-Sonografia por solicitação da Área de Obstetrícia do CAISM ou na Clínica privada. Não foram submetidas a nenhum procedimento fora das rotinas dos serviços. O projeto do estudo foi previamente avaliado e aprovado pela Comissão de Pesquisa do Departamento de Tocoginecologia da FCM/UNICAMP, bem como pelo Comitê de Ética em Pesquisa (CEP) da FCM/UNICAMP (Anexo 1). As mulheres elegíveis para o estudo foram convidadas a participar, sendo explicados todos os procedimentos para o mesmo. Tais medidas não acrescentaram mais do que 5 minutos no tempo estimado do exame. As que concordaram, assinaram o Termo de

Consentimento Livre e Esclarecido (Anexo 2). Foi assegurada a confidencialidade sobre a fonte das informações. As mulheres não receberam nenhum tipo de compensação financeira por sua participação. Foram obedecidos os princípios enunciados na Declaração de Helsinki e na Resolução 196/96 do conselho Nacional de Saúde (Brasil, 1996). As gestantes assim incluídas no estudo foram imediatamente submetidas a um exame ultra-sonográfico obstétrico em que se avaliaram os parâmetros que fazem parte da rotina deste exame, incluindo nesse caso específico as medidas do cordão umbilical e seus vasos. Além do laudo ultra-sonográfico rotineiramente emitido pelo serviço, as informações de interesse para o estudo foram transcritas para a Ficha de coleta de dados (Anexo 3) específica para o estudo.

4. PUBLICAÇÕES

Artigo 1

Artigo submetido a publicação no *Journal of Clinical Ultrasound* 2007 (Anexo 4):

- Barbieri C, Cecatti JG, Souza CEO, Marussi EF, Costa JV. Inter- and intraobserver variability in sonographic measurements of the cross-sectional area of the umbilical cord and its vessels during pregnancy.

Artigo 2

Artigo submetido a publicação no *Ultrasound in Obstetrics and Gynecology* 2007 (Anexo 5)

- Barbieri C, Cecatti JG, Krupa FG, Marussi EF, Costa JV. Sonographic measurement of the area of the umbilical cord and the diameters of its vessels during pregnancy.

Artigo 3

Artigo submetido a publicação no *Acta Obstetricia et Gynecologica Scandinavica* 2007 (Anexo 6):

- Barbieri C, Cecatti JG, Surita FG, Marussi EF, Costa JV. Wharton Jelly area as an estimate of thickness of umbilical cord and its relationship with estimated fetal weight.

Artigo 4

Artigo submetido a publicação na *Acta Obstetricia et Gynecologica Scandinavica* 2007 (Anexo 7)

- Barbieri C, Cecatti JG, Krupa FG, Marussi EF, Costa JV. Validity of the reference curves of ultrasound measurements of umbilical cord for identifying deviations of the estimated fetal weight.

4.1. Artigo 1

Inter- and intraobserver variability in sonographic measurements of the cross-sectional area of the umbilical cord and its vessels during pregnancy

Running title: Umbilical cord measurement variability

C. BARBIERI

J.G. CECATTI

C.E.O. SOUZA

E.F. MARUSSI

J.V. COSTA

Department of Obstetrics and Gynecology

School of Medical Sciences

Universidade Estadual de Campinas - UNICAMP

Correspondence:

Jose Guilherme Cecatti

P O Box 6030

13083-881 Campinas-SP

Brazil

E-mail: cecatti@unicamp.br

ABSTRACT

Purpose: To evaluate inter- and intraobserver variability in sonographic measurements of the cross-sectional area of the umbilical cord and the diameters of its vessels in low-risk pregnancies of 12 to 40 weeks of gestation.

Methods: A prospective cross sectional study was performed to measure the diameters of the arteries and umbilical vein, as well as the diameter and area of the umbilical cord by ultrasonography in 221 pregnant women at different gestational ages. Measurements were carried out also by a second observer to evaluate interobserver variability and repeated once again by the first observer to assess intraobserver variability. The linear correlation between the measurements (Spearman's coefficient of correlation) and their reliability through the intraclass correlation coefficient (ICC), the Cronbach's alpha coefficient and the limits of agreement proposed by Bland and Altman were evaluated.

Results: Interobserver and intraobserver variability did not show any significant difference between examiners. A good linear correlation between the measurements and reliability, with values of R, ICC and Cronbach´s alpha above 9, excepting the values for umbilical artery, were obtained.

Conclusion: Inter- and intraobserver variability in the measurements of the umbilical cord and its vessels was small; their reliability and agreement were good.

KEY WORDS*:* ultrasonography; pregnancy; umbilical cord; umbilical vessels; variability; reliability.

INTRODUCTION

Ultrasonography has been known for quite some time to be a useful tool for the detection of congenital abnormalities, in the diagnosis of multiple pregnancies, in locating the placenta, evaluating fetal growth, and in identifying pregnant women at risk of postmaturity or intrauterine growth restriction[1,2]. More recently, morphology of the umbilical cord, including its diameter and the amount of Wharton's jelly, have been associated with adverse perinatal events, such as preeclampsia[3], gestational *diabetes mellitus*[4], intrauterine growth restriction[5], small-for-gestational-age fetuses[6], fetal distress during labor and indication for Cesarean delivery[7]. Bruch et al.[8] also showed that the areas of the umbilical cord and vessels are smaller in fetuses with a diagnosis of intrauterine growth restriction. The same occurs in pregnant women with chronic hypertension and preeclampsia[9]. Under these conditions, the reduction in the vein lumen area would lead to an increase in resistance to blood flow and a consequent remodeling of fetal-placental hemodynamics.

Using an ultrasound scanner with high image resolution and amplification that permits adequate vision of the vessels and their contours, the umbilical cord is easily identified even in the initial stages of pregnancy, permitting early detection of any changes in its thickness[10]. Therefore, this could theoretically, become another useful tool for the prognostic selection of cases in which associated adverse effects are more likely to develop, particularly in the case of high risk pregnancies[11].

Nevertheless, sonographic measurement of the diameter of the umbilical cord and its vessels is yet to become routine practice in obstetrics[12]. Although there is no clear explanation for this, some of the difficulties that are presumed to be the principal determining factors in the technique not having yet been incorporated into routine

healthcare during pregnancy include the absence of a universally accepted reference curve for these measurements, the lack of effective validation of these measurements in different populations, and possible technical difficulties in performing the measurements[13]. This present study is part of a larger study designed to construct a reference curve of measurements of the cross-sectional area of the umbilical cord in low-risk pregnancies. The objective of this study was to evaluate inter- and intraobserver variability in sonographic measurements of the diameter of the umbilical artery, the umbilical vein, the umbilical cord and the cross-sectional area of the umbilical cord.

METHODS

This was a prospective cross sectional study to compare the variability in sonographic measurements of the umbilical cord and its vessels when carried out by the same evaluator or by different evaluators. It was estimated that 214 exams would be necessary to assess inter and intraobserver variability of these measurements, considering a type I error of 0.05 and a power of 80%, without considering gestational age. A total of 221 patients with low risk pregnancies of gestational ages ranging between 12 and 40 weeks, who had been referred for routine ultrasonography, were evaluated once between June 2005 and December 2006.

Inclusion criteria comprised: single gestation, live fetus, gestational age previously established by the date of last menstrual period (LMP) if reliable or ultrasonography carried out in the first trimester, unruptured membranes, and normal amniotic fluid index[14]. Patients with *diabetes mellitus*, gestational diabetes, hypertension of any etiology, fetal malformations, oligoamnios or polyhydramnios, fetuses with signs of intrauterine growth restriction (estimated fetal weight below the

10th percentile) or signs of fetal macrosomia (estimated fetal weight above the 90th percentile) and abnormalities in the morphology of the umbilical cord up to the moment of the ultrasound exam, were excluded from the study.

A Toshiba Power Vision 6000 ultrasound scanner (model SSA-370)® or a Voluson 730 PRO® scanner with a 3.5 mHz transabdominal convex transducer, adopted as the standard equipment for obstetrical examinations, were used for all the ultrasonography scans carried out in this study.

Patients were submitted to routine ultrasonography in the semi-seated position. Parameters for the estimation of fetal weight were measured (biparietal diameter, head and abdominal circumferences and femur length), and the amniotic fluid index, location and grade of the placenta, and fetal position were evaluated. Patients who fulfilled the inclusion criteria were then informed about the study and any queries were answered, after which they were invited to participate in the study. All patients who agreed to participate signed an informed consent form. The research protocol was previously approved by the Institutional Review Board of the institution (approval #268/2005).

Next, the diameter and the cross-sectional area of the umbilical cord and the diameters of its vessels (arteries and vein) were measured in all women after 14 weeks of gestation. Measurements were carried out in a cross-sectional plane to the cord, adjacent to its insertion into the fetal abdominal wall, within a maximum distance of 2.0cm, using the elliptical calipers of the ultrasound scanners, at the outer borders of the cord and at the borders of the vessels (umbilical vein and arteries), as shown in Figure 1A (method used by Ghezzi et al.)[15]. In the case of pregnancies of 12-14 weeks of gestational age, cranial-caudal length was measured during a period of fetal rest in a longitudinal section. In some cases, a 7.5 mHz endovaginal probe was also used. The

diameters of the cord and its internal vessels were measured in a free loop of cord adjacent to its insertion into the fetal abdominal wall, placing the markers at its outer borders and, with maximum image magnification, along its longitudinal axis, according to the technique described by Ghezzi et al.[16] (Figure 1B).

To evaluate inter- and intraobserver variability, measurements of the umbilical cord (area of the cord, diameters of the cord, vein and arteries) were initially made always by the same first examiner. Next, another second examiner, previously informed about the nature of the study, took the same measurements with no knowledge of the previous results. Finally, the same first examiner repeated the measurements according to the criteria established above. All measurements were independently recorded and photographed.

To evaluate inter- and intraobserver variability of the measurements of the cross-sectional area of the umbilical cord and the diameters of its vessels, the mean difference in the measurements of the two observers was first calculated, as well as their respective standard deviations (inter: measurement 2 – measurement 1; intra: measurement 3 – measurement 1), and 95% confidence intervals (95%CI) with the statistical significance of these mean differences evaluated using the Mann-Whitney non-parametric test. P-values < 0.05 were considered statistically significant. Next, the following analyses were performed: the linear correlation between measurements (Spearman's coefficient of correlation), with values >0.7 being considered indicative of good agreement[17]; the reliability of the measurements evaluated by their reproducibility (intraclass coefficient of correlation – ICC)[18], with values >0.8 being considered as excellent[19]; and internal consistency (Cronbach's alpha), with values >0.8 being considered indicative of good reliability[20,21]. Finally the 95% agreement limits were graphically evaluated according to

the method proposed by Bland and Altman[22], using proportions of the difference between both measurements in relation to the mean value.

RESULTS

The principal characteristics of the 221 pregnant women evaluated are shown in Table 1. Most of the women were white, and 46.6% were nulliparous. Forty-four percent were between 20 and 29 years of age. The ultrasonographic evaluations were carried out at different gestational ages.

Comparison between the measurements of the first and second evaluators (interobserver variability) indicated a trend to slightly overestimate the diameter of the umbilical vein, and the umbilical cord and its area, and to underestimate the diameter of the umbilical artery (Table 2). However, these differences were not statistically significant. The difference between the measurements obtained by the two different examiners was found to be dispersed around the mean, with no clear trend towards over- or underestimation by either one of these examiners, as graphically seen through the 95% agreement limits of Blend and Altman (Figure 2).

The linear correlation between the measurements (Spearman's coefficient of correlation), their reliability (intraclass correlation coefficient – ICC) and internal consistency (Cronbach's alpha) were significantly high for all the measurements, being < 0.9 only in the case of the diameter of the umbilical artery (Table 2). Figure 3 illustrates the linear correlation between the interobserver measurements of the diameter and area of the cord.

In the comparison between the two sets of measurements carried out by the first evaluator (intraobserver variability), there was a trend towards underestimation of the

diameters of the cord, the artery and the area of the cord, with a small overestimation in the diameter of the umbilical vein. Nevertheless, again none of these differences was statistically significant. The linear correlation between the measurements, their reliability and internal consistency were significantly high for all the measurements, being < 0.9 only in the case of the diameter of the artery (Table 3). The linear correlations between the intraobserver measurements for the parameters of the diameter and area of the cord are illustrated in Figure 4.

DISCUSSION

The objective of using two different investigators to measure the cross-sectional area of the umbilical cord and the diameter of its internal vessels was to determine the precision of the method for use as an early screening tool for the detection of abnormalities that could be harmful to the fetus or the pregnancy. Reliability, reproducibility and precision are terms used to describe the extent to which the measurements of a stable phenomenon, repeated by different persons or instruments at different times or in different places, achieve similar results[21]. This evaluation is fundamental in assuring the predictive value of a measurement.

In the present study, in pregnancies of 12-14 weeks of gestational age, the measurements were carried out on a longitudinal section due to the greater difficulty in obtaining images of the cross-sectional area of the cord, while in the remaining women measurements were performed on a transversal section. This may represent a limitation to the study and consequently in its results due to the different techniques used in evaluating different gestational ages. Historically, this procedure was first reported in 1994 in a study carried out by Weissman et al.[12] in which the diameters of the umbilical

artery, vein and cord, and the surface area of Wharton's jelly were measured between 8 and 42 weeks of pregnancy. For this evaluation, a longitudinal section of the umbilical cord close to its point of insertion into the fetal abdominal wall was used, since there was no difference between the measurements carried out using this section compared to the diameters measured in a transversal section of the cord; in addition, it provided better visualization in early pregnancies. Moreover, the diameters of the artery and the umbilical vein were measured only after 14 weeks of pregnancy.

Some years later, another study used the cross-sectional area of the umbilical cord to construct a normality curve of the diameters of the umbilical cord and its vessels in relation to fetal size[22]. According to these investigators, the cross-sectional area of the cord is a more reliable parameter, since the measurement of the diameter of the cord is influenced by the amount of Wharton's jelly. Moreover, the cross-section of the umbilical cord is not precisely circular, and this may lead to a slight underestimation in measurement. Recently, Togni et al.[23] also established normality curves using the cross-sectional areas of the umbilical cord and its vessels and the quantity of Wharton's jelly, and correlated them with fetal anthropometric parameters in low risk pregnancies of 24-39 weeks.

In the comparison of the umbilical cord measurements carried out by the different examiners, the present study shows that these differences were not statistically significant at all. The choice of each individual examiner with respect to the part of the cord in which to carry out the measurements (while respecting the standard distance of a maximum of 2.0cm from the insertion of the umbilical cord into the fetal abdomen), and the presence of coiling along the cord may partially explain these differences. It should also be remembered that up to 40 coils may be present in the umbilical cord as its length

increases with gestational age[24]. If the examiners randomly select the best transversal section in which to carry out their measurements within the standard distance from the umbilical insertion but in different locations within the coils, small variations in measurements would be expected.

In the case of the umbilical arteries, the diameter of only one artery was measured, each examiner selecting the one in which the contours were more visible. Generally, the umbilical arteries have similar lumen diameters; however, it is known that in around 0.7 to 1.4% of cases one of the umbilical arteries is smaller than the other[25]. Differences of around 1-3mm have also been reported in their diameters[26], leading to differences in blood flow parameters and greater resistance in the vessel with lower caliber[27]. This may also contribute towards the differences found in this measurement.

On the other hand, in the evaluation of the differences obtained in the measurements of the vessels of the umbilical cord carried out by the same examiner, the small variations detected were not statistically significant. Spearman's correlation coefficient indicated good agreement between the measurements carried out by the different examiners for all the parameters studied, both with respect to inter- and intraobserver variability, thereby allowing us to assume that these measurements may be safely carried out by different examiners at different times in different locations.

Ultrasonographic findings of abnormalities in the umbilical cord may be associated with fetal or chromosomic abnormalities, intrauterine fetal growth restriction and other pathological conditions related to an increase in fetal and neonatal morbidity and mortality[3,5,6]. Early detection of these changes may be important for maternal and fetal prognosis, but this was not one of the objectives of the present study. First, standards that would be valid for the reference population have to be established for

these measurements. The ability of these standards to accurately predict conditions apparently associated with abnormalities of the umbilical cord would have to be validated. These are tasks to be undertaken in the near future.

Many of these findings are not isolated. For this reason, careful evaluation of the umbilical cord by measuring its vessels and the umbilical cord itself throughout the different phases of pregnancy may become a routine part of obstetrical care[28], and should not be restricted to detecting the number of umbilical vessels, the presence of cysts and Doppler evaluation of blood flow, as is current practice[29]. This will permit a qualitative evolution in perinatal care even during pregnancy by identifying those cases with a greater probability of developing maternal and fetal neonatal complications so that surveillance may be improved and prophylactic or therapeutic measures may be instituted at an earlier stage. If future studies confirm the predictive capability of abnormalities in these measurements for the various associated pathological conditions, the present study will have contributed towards demonstrating that these measurements are technically reproducible.

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Legends for Illustrations:

Figure 1. Ultrasonographic measurement A. of the cross-sectional area of the umbilical cord (C), the diameter of the umbilical vein (V) and the umbilical artery (A); and B. of the longitudinal section of the umbilical cord (between 12 and 14 weeks).

Figure 2. Interobserver agreement by plotting the proportional differences (difference between both values, divided by their mean, multiplied by 100) against the mean values (method of Blend & Altman proportional) for the following measurements: A. Diameter of the umbilical cord (cord $_1$ and cord $_2$ are respectively the measurements of observer 1 and 2); and B. diameter of the area of the umbilical cord (area $_1$ and area $_2$ are respectively the measurements of observer 1 and 2).

Figure 3. Linear correlation (r = correlation coefficient of Spearman) of the interobserver ultra-sonographic measurements: A. of the diameter of the umbilical cord (cord $_1$ and cord $_2$ are respectively the measurements of observer 1 and 2); and B. of the cross-sectional area of the umbilical cord (area $_1$ and area $_2$ are respectively the measurements of observer 1 and 2).

Figure 4. Linear correlation (r = correlation coefficient of Spearman) of the intraobserver ultrasonographic measurements: A. of the diameter of the umbilical cord (cord $_1$ and cord $_3$ are respectively the first and second measurements of observer 1); and B. of the cross-sectional area of the umbilical cord (area $_1$ and area $_3$ are respectively the first and second measurements of observer 1).

Table 1. Characteristics of the patients

Characteristics	*n*	*%*
Nulliparous	103	46.6
Previous abortion	27	12.2
At least one living child	118	53.4
White	168	76.0
Age (years)		
14 – 19	21	9.5
20 - 29	98	44.3
30 - 39	85	38.5
40 - 45	17	7.7
Gestational age (weeks)		
14 – 24	53	24.0
25 – 28	43	19.4
29 – 32	50	22.7
33 – 36	30	13.5
37 - 40	45	20.4
Total	**221**	

Table 2. Interobserver variability in the ultrasonographic measurements of the umbilical cord (n=221)

Characteristics	*Mean Difference (SD)*	*95%CI*	*p**	*R*	*ICC*	*Crombach's alpha*
Diameter of the cord (mm)	0.024 (1.208)	(-0.136; 0.185)	0.896	0.90	0.94	0.97
Diameter of the artery (mm)	-0.057 (0.677)	(-0.147; 0.032)	0.728	0.79	0.83	0.91
Diameter of the vein (mm)	0.045 (0.907)	(-0.075; 0.166)	0.800	0.90	0.91	0.95
Area of the cord (mm^2)	1.088 (30.120)	(-2.905; 5.081)	0.903	0.90	0.92	0.96

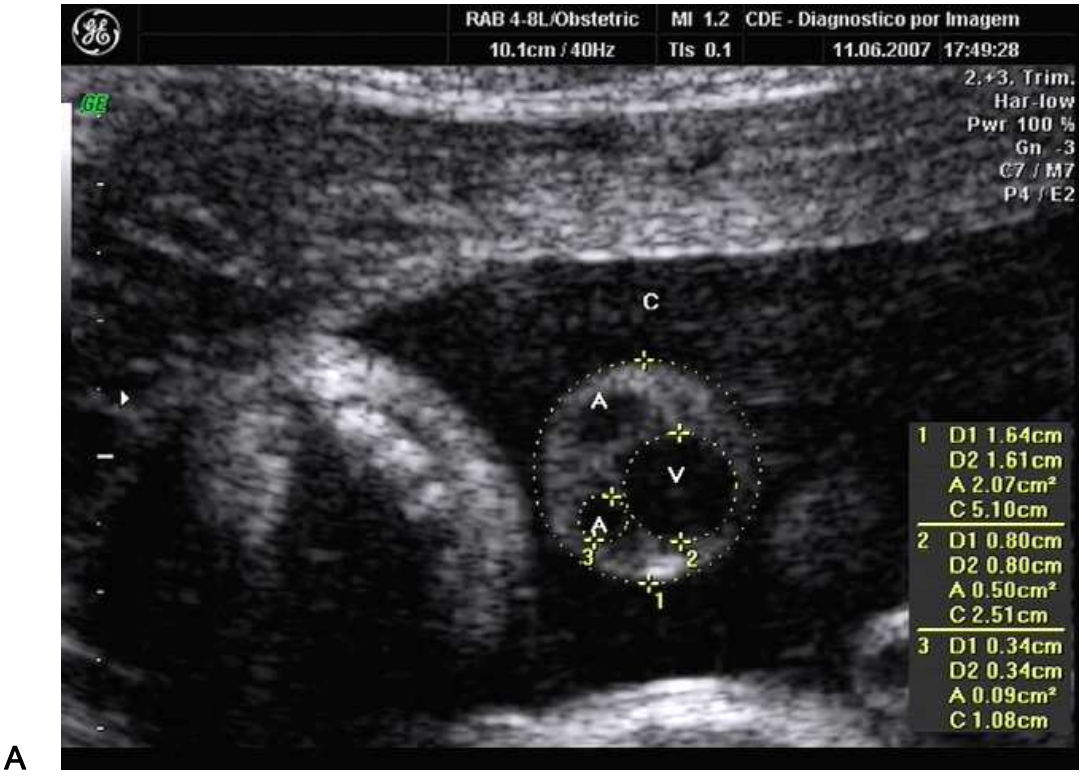
* Mann-Whitney's non parametric test.
R: Spearman's correlation coefficient ($p<0.0001$)
ICC: intraclass correlation coefficient (internal consistency)

Table 3. Intraobserver variability in the ultrasonographic measurements of the umbilical cord (n=221)

Characteristics	*Mean Difference (SD)*	*(95%CI)*	*p**	*R*	*ICC*	*Crombach's alpha*
Diameter of the cord (mm)	-0.120 (0.960)	(-0.248; 0.007)	0.734	0.95	0.96	0.98
Diameter of the artery (mm)	-0.137 (0.576)	(-0.213; -0.06)	0.332	0.86	0.88	0.94
Diameter of the vein (mm)	0.011 (0.763)	(-0.09; 0.113)	0.917	0.92	0.94	0.97
Area of the cord (mm^2)	-2.500 (24.390)	(-5.74; 0.73)	0.732	0.95	0.95	0.97

* Mann-Whitney's non parametric test.
R: Spearman's correlation coefficient ($p<0.0001$)
ICC: intraclass correlation coefficient (internal consistency)

Figure 1: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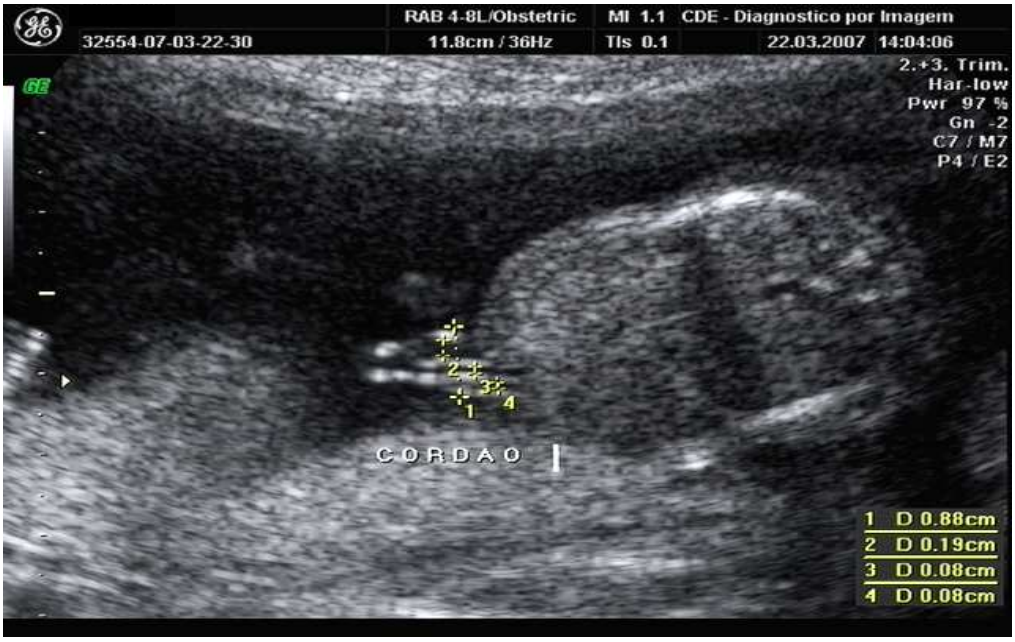
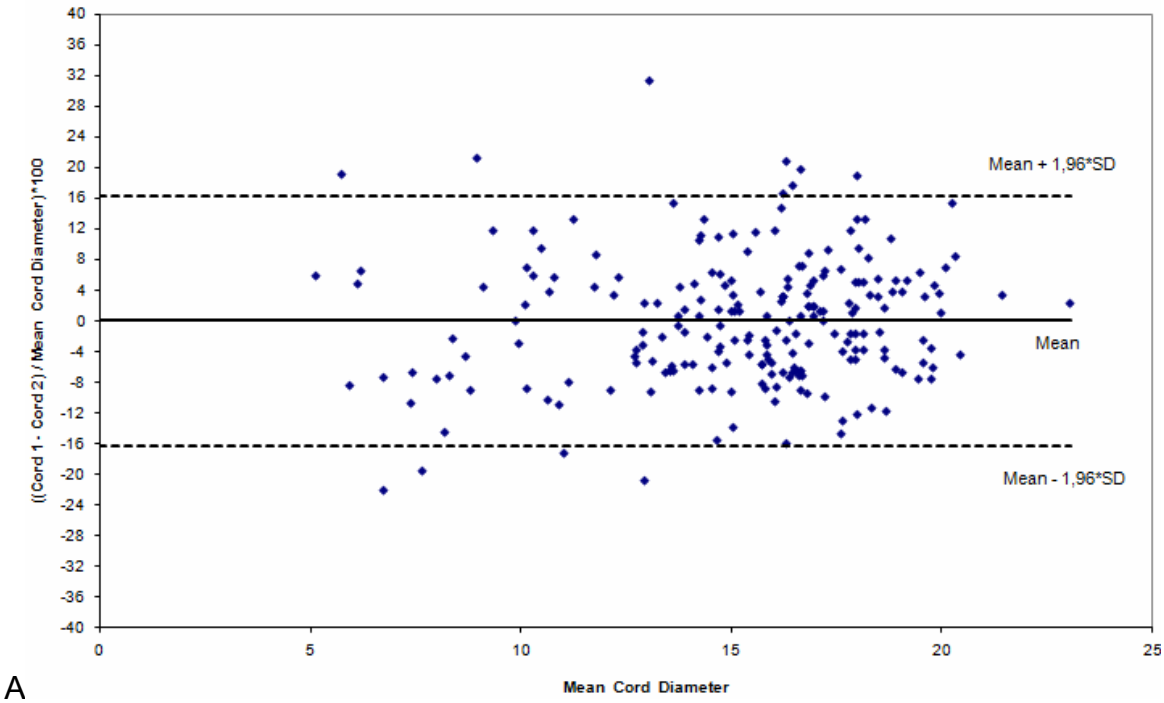
A 2.07cm²
C 5.10cm
2 D1 0.80cm
D2 0.80cm
A 0.50cm²
C 2.51cm
3 D1 0.34cm
D2 0.34cm
A 0.09cm²
C 1.08cm

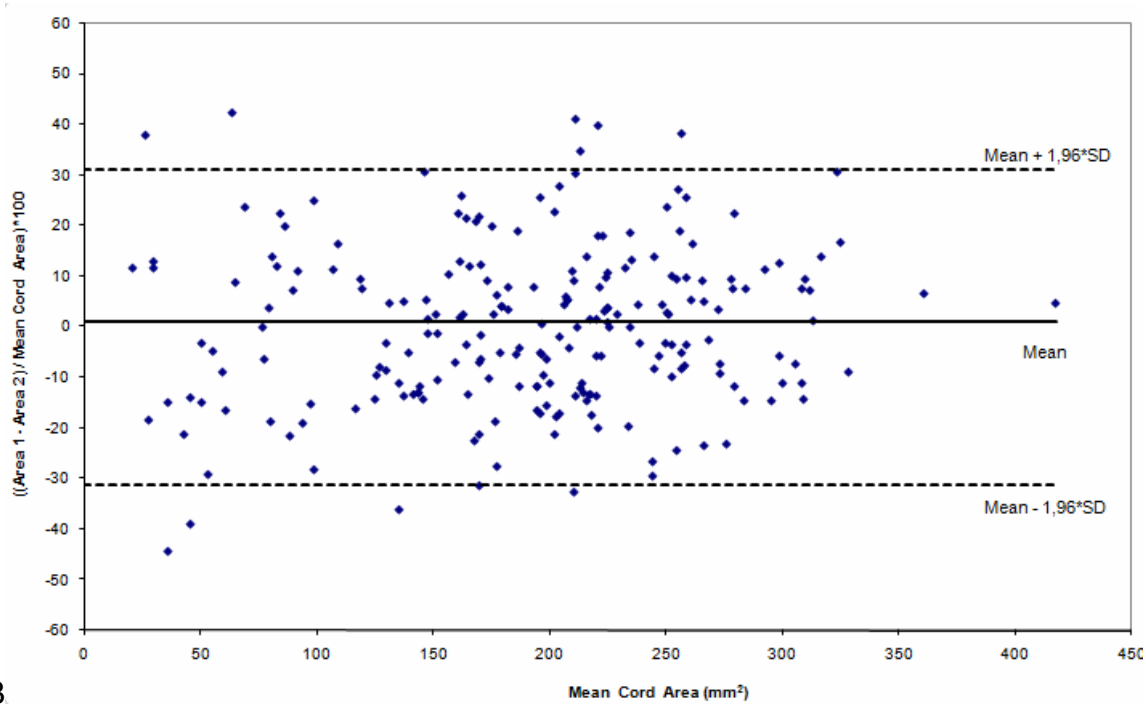
A


RAB 4-8L/Obstetric
MI 1.1
CDE - Diagnostico por Imagem
32554-07-03-22-30
11.8cm / 36Hz
TIs 0.1
22.03.2007 14:04:06
2.+3. Trim.
Har-low
Pwr 97 %
Gn -2
C7 / M7
P4 / E2
CORDAO
1 D 0.88cm
2 D 0.19cm
3 D 0.08cm
4 D 0.08cm

B

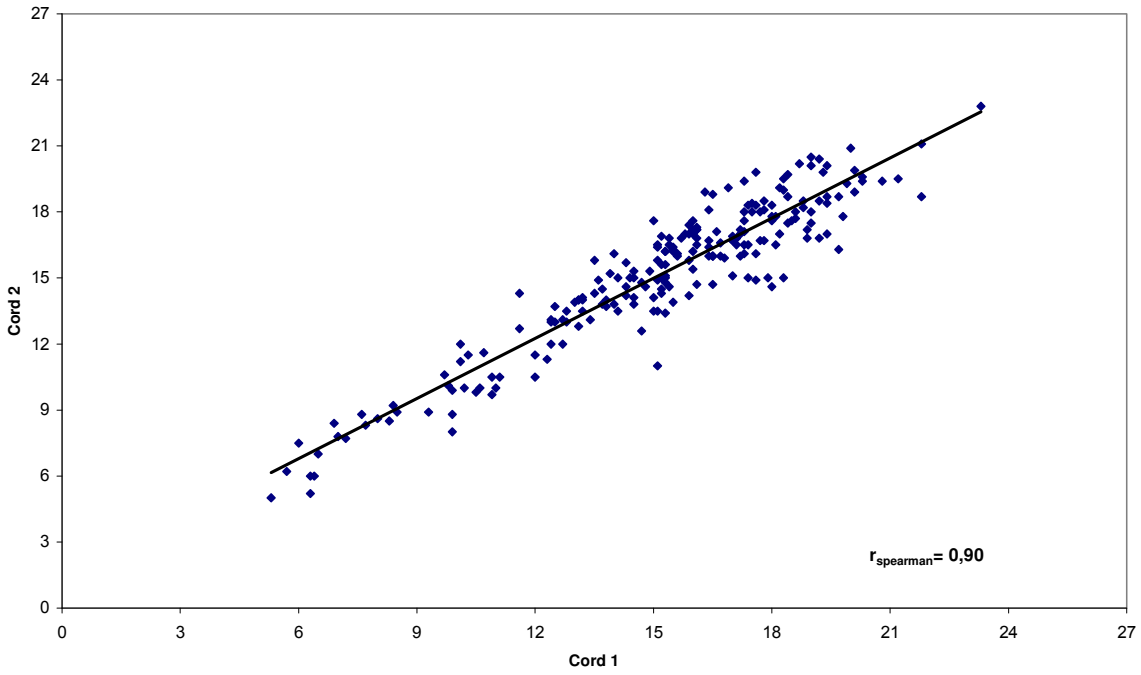

((Cord 1 - Cord 2) / Mean Cord Diameter)*100
Mean + 1,96*SD
Mean
Mean - 1,96*SD
Mean Cord Diameter

A

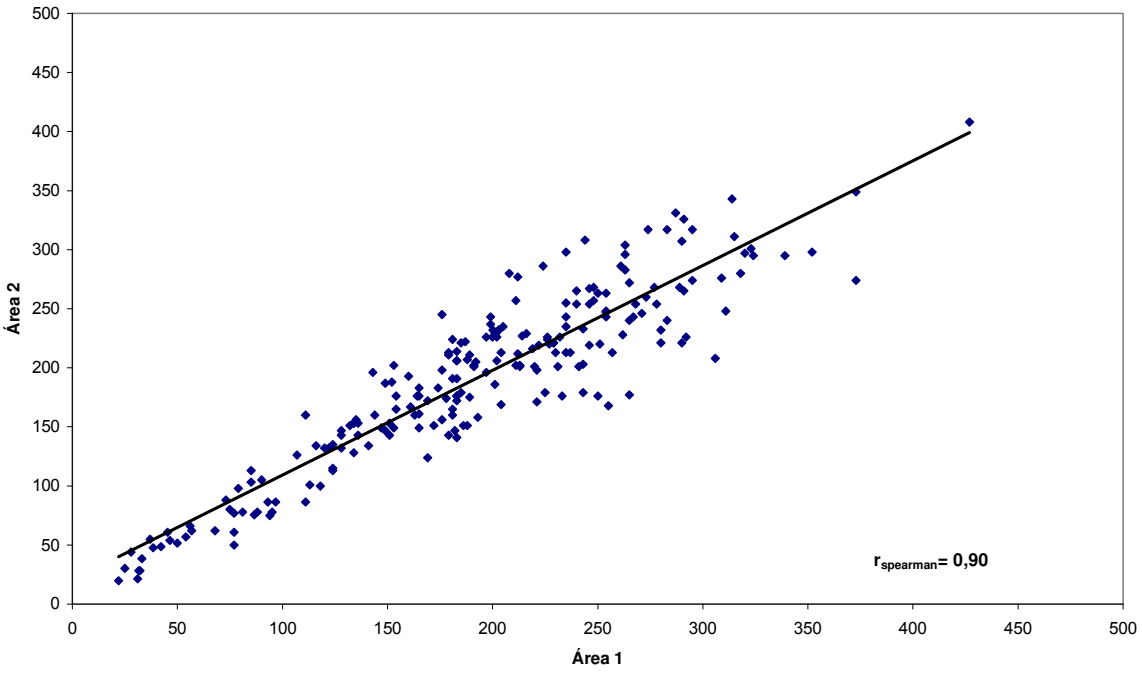

((Area 1 - Area 2) / Mean Cord Area)*100
Mean + 1,96*SD
Mean
Mean - 1,96*SD
Mean Cord Area (mm²)

B

Figure 2.

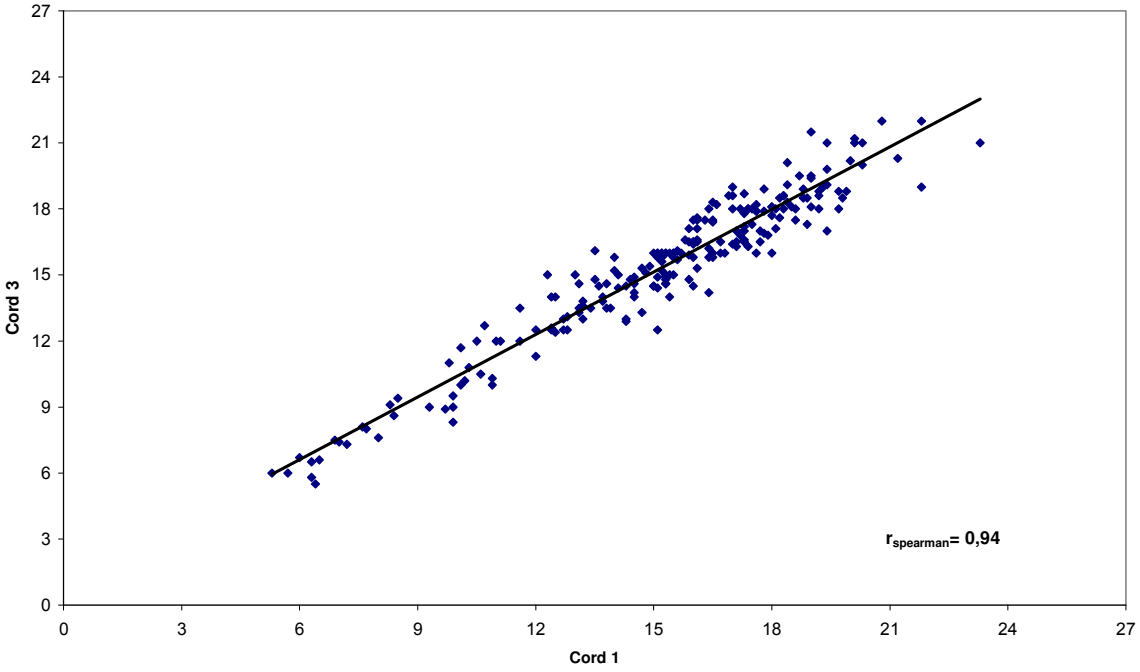


A



B

Figure 3.



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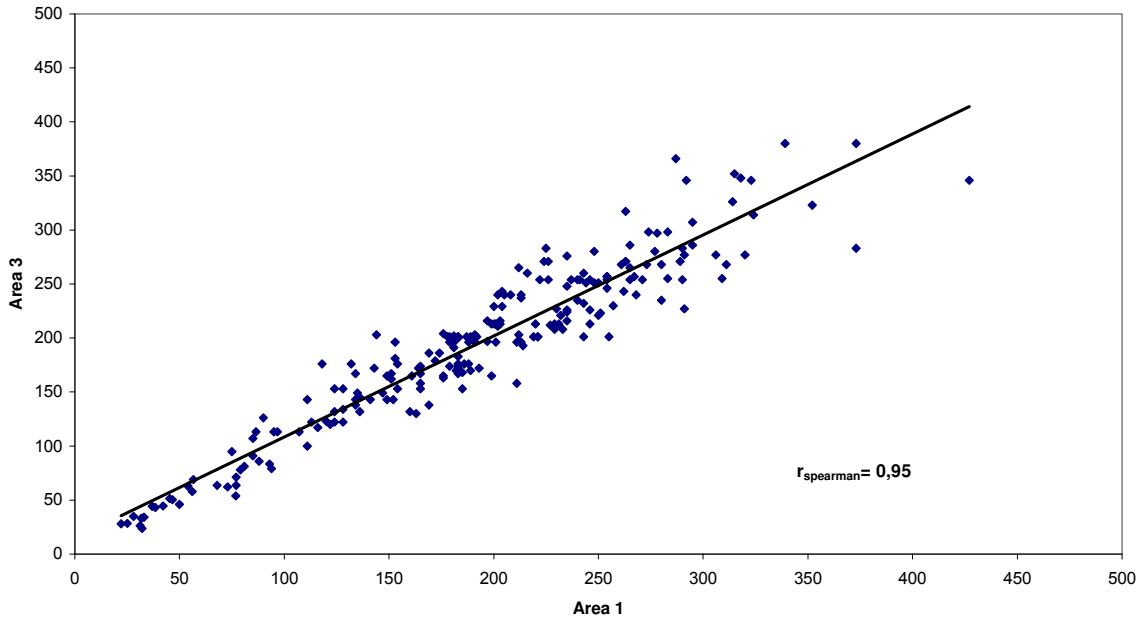

B

Figure 4.

4.2. Artigo 2

Sonographic measurement of the area of the umbilical cord and the diameters of its vessels during pregnancy

Short title: umbilical cord measure

C. BARBIERI

J.G. CECATTI

F. KRUPA

E.F. MARUSSI

J.V. COSTA

Department of Obstetrics and Gynecology

School of Medical Sciences

Universidade Estadual de Campinas - UNICAMP

Correspondence:

Jose Guilherme Cecatti

P O Box 6030

13083-980 Campinas-SP

Brazil

E-mail: cecatti@unicamp.br

Abstract

Objective: To evaluate the cross-sectional area of the umbilical cord, its diameter and the diameter of its internal vessels in low-risk pregnancies of 12-40 weeks to establish a reference curve for these parameters.

Methods: A prospective study was carried out between June 2005 and December 2006 in 2310 low-risk pregnancies to determine the diameter of the umbilical arteries and vein, and the cross-sectional area of the umbilical cord. Toshiba-Power Vision-6000® (model SSA-370) and Voluson 730 PRO® scanners were used for all evaluations. A minimum of 59 cases was evaluated for each gestational age. Means and their respective standard deviations were calculated, as well as the 10^{th}, 50^{th} and 90^{th} percentiles for each measurement. Mann-Whitney, Kruskal-Wallis and Wilcoxon tests for independent variables were used in the statistical analysis. Polynomial regression analysis was used to calculate percentiles.

Results: Diameters of the umbilical cord, artery and vein, and the area of the cord increased significantly with gestational age. The area of the cord also varied significantly with parity. The reference curve of the cross-sectional area of the umbilical cord and its vessels was calculated using polynomial regression, and an almost linear increase in values was found up to 32 weeks of pregnancy, tending to stabilize from then onwards. The regression equation of the area of the umbilical cord according to gestational age (GA) was: $-1.417+0.3026*GA-0.008*GA^2+0.000007*GA^3$.

Conclusion: Sonographic measurements of the umbilical cord and its vessels increase with gestational age and this progressive increase was observed up to 32 weeks of pregnancy.

Key words: ultrasonography; pregnancy; umbilical cord; umbilical vessels.

INTRODUCTION

In the last century, there was a belief that the presence of thin umbilical cords with a small amount of Wharton's jelly affected fetal blood supply. In 1961, Hall described the thin cord syndrome in a report on two cases, emphasizing the danger involved, which could only be confirmed following delivery[1]. Later, other studies on the physiology and microscopic anatomy of the umbilical cord were carried out[2,3] in an attempt to define its role during fetal development and its influence on the development of complications during pregnancy[4]. The morphology of the umbilical cord may be affected by fetal-placental blood flow and by the pressure in the arterial vessels[5], the quantity of amniotic fluid[6,7] and by genetic variations[8].

Among the various structures identified during a routine, prenatal sonographic scan, the umbilical cord is probably the one that receives least attention, its examination usually being limited to identification of its three blood vessels, two arteries and the single vein[9]. Under special circumstances, Doppler velocimetric evaluation of its blood flow is carried out[10]. More recently, the morphology of the umbilical cord, including its diameter and the quantity of Wharton's jelly, has been associated with adverse perinatal effects such as hypertensive disease[11], gestational *diabetes mellitus*[12], intrauterine growth restriction[13], lower than expected fetal birthweight[14], fetal distress during labor and indication for Cesarean section[15]. More frequently, a reduction in the area of the umbilical cord is associated with abnormalities in fetal growth and development, or fetal distress during labor[16].

On the other hand, an abnormally large amount of Wharton's jelly may lead to an increase in the thickness of the umbilical cord in diabetic mothers[12]. In fetuses with some form of chromosomal abnormality, an increase in the diameter of the umbilical

cord may also be found, and this is more evident in the first trimester of pregnancy[17]. Identification of abnormalities in the dimensions of the umbilical cord and its vessels, in addition to the detection of cords with single arteries[18], the presence of cysts[19], absence of coiling[20], and abnormal or circular insertion, may serve as an early warning sign of possible abnormalities[21].

Since the quantity of Wharton's jelly is known to undergo modifications in its composition during pregnancy that may lead to variations in the thickness of the umbilical cord, some studies have directly compared the curves of the quantity of Wharton's jelly to biometric fetal parameters in low-risk pregnancies. The areas of the curve of the umbilical cord and the diameter of its vessels have also been determined[22-25].

The first curve was published in 1994 and showed that values increased according to gestational age until a plateau in the diameter of the umbilical cord and its vessels was reached at around 36 weeks of gestation[22], at which time a progressive reduction also occurs in the quantity of water in the Wharton's jelly[26]. In 1999, Raio et al[23] established reference curves for the area and diameter of the umbilical cord and reported a peak at around 33-34 weeks of gestation followed by a decrease. In 2001, Ghezzi et al[24] reported a statistically significant correlation between the area of Wharton's jelly and the area of the umbilical cord compared to biometric fetal measurements and estimated fetal weight in low-risk pregnancies of 15-41 weeks; however, in this study the peak in growth of the area of the cord occurred at 32 weeks and remained stable until 41 weeks. Recently, Togni et al[25] published curves of the area of Wharton's jelly and the umbilical cord and its vessels between 24 and 39 weeks of

gestation, and reported results similar to those described by Ghezzi et al[24], although values reached maximum levels at around 35 weeks of gestation.

In view of the results reported in the international scientific literature and of the potential of the umbilical cord as a predictor of adverse maternal-fetal or perinatal situations, it is essential to establish standardized normal values for these measurements at all gestational ages for low risk-pregnancies in the reference population so that these measurements may later be validated as predictors of adverse perinatal situations. Therefore, the objective of this study was to construct a reference curve for the area of the umbilical cord and the diameters of its vessels in low-risk pregnancies of 12 to 40 weeks and to evaluate their variation according to certain sociodemographic characteristics.

METHODS

A prospective, cross-sectional study was carried out in the Ultrasonography Unit of the Women's Integrated Healthcare Center of the *Universidade Estadual de Campinas* and in the CDE *Diagnóstico por Imagem* Clinic in Campinas, Brazil, between June 2005 and December 2006 in a total of 2310 pregnant women, who had been referred to one of these clinics for a routine sonographic scan at different gestational ages of a low-risk pregnancy.

For the calculation of sample size, the reference taken into consideration was a study carried out by Raio et al[23] in which a mean measurement of the diameter of the umbilical cord of 16.27±2.67mm at 35 weeks of gestational age was considered (since this measurement had the greatest standard deviation), with a minimum admitted difference between the sample mean and the population mean of 0.68 mm (around 25%

of the standard deviation). Admitting a type I error of 0.05, the minimum number of cases required for each gestational age was calculated at 59, with an independent sample of pregnant women for each gestational age.

Inclusion criteria comprised: a single pregnancy, living fetus, gestational age previously established according to the date of the last menstrual period when reliable or according to an ultrasonographic scan performed in the first trimester, intact membranes and a normal amniotic fluid index[27]. Exclusion criteria comprised *diabetes mellitus*, arterial hypertension of any etiology, fetal malformations, oligoamnios or polyhydramnios, fetuses with signs of intrauterine growth retardation (estimated fetal weight below the 10th percentile) or signs of fetal macrosomia (estimated weight above the 90th percentile) and morphological abnormalities of the umbilical cord.

The following equipment was used to perform the ultrasonographic scans: Toshiba-Power Vision-6000 (model SSA-370®) and Voluson 730 PRO® scanners with a 3.5mHz transabdominal convex transducer, adopted as the standard equipment for obstetrical exams. Specific inter- and intra-observer variability was considered minimal in this study. The linear correlation coefficients were 0.9 and 0.95, respectively, for the measurements of the diameter of the cord; 0.79 and 0.86 for the diameter of the umbilical artery, 0.9 and 0.92 for the diameter of the umbilical vein, and 0.9 and 0.95 for the area of the umbilical cord.[28]

The women were submitted to a routine sonographic scan in a semi-seated position, at which time the parameters for estimating fetal weight were measured (according to biparietal diameter, cranial and abdominal circumferences and the length of the femur) in addition to the other parameters routinely evaluated during pregnancy. If the patients fulfilled the inclusion criteria, they were then provided with information

regarding the nature of the study, and invited to participate. If they voluntarily agreed to participate, they then signed an informed consent form in compliance with the regulations of the local Institutional Review Board, which approved the protocol prior to initiation (approval 268/2005).

Next, the diameter and the cross-sectional area of the umbilical cord and the diameters of its vessels (arteries and vein) were measured in all women of more than 14 weeks of gestation. Measurements were carried out in a cross-sectional plane to the cord, adjacent to its insertion into the fetal abdominal wall within a maximum distance of 2.0cm from insertion, using the elliptical calipers of the ultrasonography scanners at the outer borders of the cord and at the borders of the vessels, according to the method used by Raio et al[23] and Weissman et al[22]. In the case of pregnancies of 12-14 weeks of gestational age, cranial-caudal length was measured during a period of fetal rest in a longitudinal section. In some cases, a 7.5mHz endovaginal probe was also used. The diameters of the cord and its internal vessels were measured in a free loop of cord adjacent to its insertion into the fetal abdominal wall, placing the markers at its outer borders and, with maximum image magnification, along its longitudinal axis, according to the technique described by Ghezzi et al [24].

For the statistical analysis, first the mean and standard deviation of the area of the umbilical cord were calculated in accordance with certain sociodemographic characteristics. The differences were evaluated using Wilcoxon-Mann-Whitney and Kruskal-Wallis tests for non parametric data. Based on the measurements obtained, the mean and standard deviation of the cross-sectional area of the umbilical cord and the diameters of the umbilical cord, the umbilical artery and the umbilical vein were calculated for the different gestational ages. Differences between them were evaluated

using Wilcoxon's test for independent samples. Next, the smoothed values of the 10^{th}, 50^{th} and 90^{th} percentiles of these measurements were estimated for each gestational age using polynomial regression analysis. The respective regression equations and the coefficients of determination with respect to the adjusted regression model (R^2) were calculated. Values <0.05 were considered statistically significant.

RESULTS

The principal characteristics of the 2310 women evaluated are shown in Table 1. The majority of them were white (80.3%), nulliparous (53.9%) and under 29 years of age (57.3%). Parity was shown to be significantly associated with the area of the umbilical cord, values being higher in women of parity 5 and lower in women of parity 0 and 7.

Table 2 shows the number of cases for each gestational age and the principal mean values (± SD) of the measurements of the diameters of the umbilical artery, the umbilical vein and the umbilical cord, as well as the cross-sectional area of the umbilical cord. These mean values increased throughout the entire duration of pregnancy, thereby confirming that the increase was significant.

Tables 3 and 4 show, respectively, the 10^{th}, 50^{th} and 90^{th} percentiles of the diameters of the umbilical artery and vein, and the diameter of the umbilical cord and its cross-sectional area for each gestational age studied.

Calculation of the regression equation that defines the diameter of the umbilical artery in accordance with gestational age (GA) resulted in: $Log_{10}(artery)= -1.902 + 0.21*GA - 0.00585*GA^2 + 0.00006*GA^3$, and the degree of adjustment (R^2) was 0.82 (Table 3). For the diameter of the umbilical vein according to gestational age, the result was: $Log_{10}(vein)$: $-1.717 + 0.21*GA - 0.0056*GA^2 + 0.00005*GA^3$, and the degree of

adjustment (R^2) was 0.87 (Table 3). The regression equation found for the cross-sectional area of the umbilical cord according to gestational age was: $Log_{10}(cord)= -1.417+0.3026*GA – 0.008*GA^2 + 0.00007*GA^3$, and the degree of adjustment (R^2) was 0.89 (Table 4). For the diameter of the umbilical cord according to gestational age, the equation was: $Log_{10}(area)= -0.677+0.154*GA – 0.0042*GA^2 + 0.00004*GA^3$, and the degree of adjustment (R^2) was 0.89 (Table 4).

The figures show the curves of the values of the diameter of the umbilical artery, the umbilical vein, umbilical cord and the cross-sectional area of the umbilical cord according to gestational age (Figures 1, 2, 3 and 4, respectively) with the 10^{th}, 50^{th} and 90^{th} percentiles. In general, it may be said that the values increase consistently and uniformly until around 32 weeks, after which their speed of growth diminishes, practically reaching a plateau, particularly in the case of the area of the cord.

DISCUSSION

The present study shows that there is a significant, consistent and practically regular increase in the measurements of the diameters of the umbilical artery and vein, the umbilical cord and the cross-sectional area of the cord until around 32 weeks of gestational age after which these measurements remain practically constant until the end of pregnancy.

For many years, little was known with respect to the morphology and functions of the umbilical cord during pregnancy. The observation that the presence of thin cords or ones with a small amount of Wharton's jelly was related to adverse perinatal effects or even to the presence of oligoamnios and fetuses with low birthweight has been described in the literature in a case report[4]. With progress and the consequent

improvement in the resolution of ultrasonographic scanners, the umbilical cord began to be studied in greater detail and at earlier stages of pregnancy[29].

From 1994 onwards, the hypothesis that the umbilical cord may serve as another parameter for the early identification of fetuses in situations of risk, such as intrauterine growth restriction, preeclampsia or *diabetes mellitus*, stimulated various investigators to construct reference curves for the diameters of the umbilical cord and its vessels and surface area in low-risk pregnancies at different gestational ages. The first of these studies[22] reported maximum umbilical cord diameter at around 38 weeks of gestation and a maximum area of Wharton's jelly at around 34 weeks, later than the dates found in the present study. The second study[23] identified these maximum measurements as occurring at 33-34 weeks, also later than those found in the present study, and showed a correlation between the cross-sectional area of the umbilical cord and its diameter and fetal anthropometric parameters.

In 2005, Predanic et al. published a retrospective study carried out in 650 pregnant women in whom these investigators correlated the measurement of the diameter of the umbilical cord at 18-23 weeks with estimated fetal weight and gestational age[30]. The curve obtained was compared with that described by Raio et al[23] and no difference was found between the two.

Recently, Togni et al published another reference curve of the cross-sectional areas of the umbilical cord and its components in low-risk pregnancies of 24-39 weeks, and showed a statistically significant correlation between the area of Wharton's jelly, calculated from the areas of the umbilical cord and its vessels, and gestational age. In this case, the increase in area occurred up to 31 weeks of gestation, followed by a plateau until around 35 weeks, with values decreasing from 36 weeks onwards[25].

With respect to the growth curve of the areas of the umbilical arteries and vein, an increase in both was also found up to 35-36 weeks of gestation, with values stabilizing at 38 weeks [22,25]. The present study did not evaluate the areas of the umbilical arteries and vein, but their diameters and the area of the umbilical cord showed signs of stabilizing earlier, at around 32 weeks of gestational age.

As shown, various reference curves have been published in the international literature with respect to the diameters and areas of the umbilical cord and its components at different intervals of analysis of gestational age and using different techniques for carrying out these measurements. Therefore, the objective of this study was to construct a reference curve for these parameters in low-risk pregnancies of 12 to 40 weeks using the most reliable techniques available. This cross-sectional study of an independent sample of 2310 women includes the largest sample size reported for a study of this type up to the present time, thereby resulting in greater statistical power for these measurements. Moreover, the careful methodology and the techniques applied in this study are reflected in the small variability in measurements, as previously described[28].

Some possible limitations of this study include, for example, the technique used for measuring the umbilical cord in a longitudinal plane at 12-14 weeks of gestation. Initially, this technique was standardized according to the recommendations of Ghezzi et al[24] precisely because of the greater technical difficulty in obtaining measurements using a transversal plane at these gestational ages. Nevertheless, no great difference was found in the serial measurements and their respective percentiles taken between 12 and 14 weeks of gestation and those taken after 15 weeks with another technique, thereby suggesting that there is no significant discrepancy between the methods adopted for obtaining these measurements.

A second potentially controversial point concerns the statistically significant variation found in the values of the area of the umbilical cord as a consequence of parity. We failed to find any plausible justification for this finding, which has not been reported by the other authors who have published reference curves of measurements of the diameters and areas of the umbilical cord and its vessels[22-25]. Initially, we believed that this finding would merit further investigation and we proposed the construction of two curves, one curve specifically for nulliparous women and another for parous women. However, a more in-depth observation led us to conclude that no positive linear relationship exists between the measurement of the area of the umbilical cord and parity, the existence of which would complicate the use and interpretation of these measurements.

Early identification of umbilical cord parameters that may be capable of detecting abnormalities in low- or high-risk pregnancies may be useful in the prevention of associated complications or in the more rigorous follow-up of these cases so that timely intervention may be made. However, once a curve with values that serve as parameters for the evaluation of the umbilical cord and its vessels has been established in a reference population, diagnostic validation studies would be required to determine the actual performance of the curves for this purpose before any differences found could be truly considered abnormal and possibly predictive of adverse perinatal situations. This is our proposal and it represents a challenge for the continuation of the work carried out in the present study.

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Table 1. Mean cross-sectional area of the umbilical cord according to race, age and parity.

Characteristics	n	%	Mean ± SD	P value
Race/ethnicity				
White	1855	80.3	169.5±90.6	0.944*
Others	455	19.7	169.9±89.9	
Age				
≤ 29 years	1324	57.3	167.1±90.7	0.125*
≥ 30 years	986	42.7	172.9±90.0	
Parity				
Nullipara	1244	53.9	163.8±90.6	0.002*
≥ 1	1066	46.1	176.4±89.8	
Parity				0.003**
0	1244		163.8±90.6	
1	592		171.4±91.4	
2	296		178.9±87.0	
3	87		192.5±91.1	
4	66		187.0±82.9	
5	16		206.8±81.3	
6	5		156.9±126.4	
7	4		99.4±65.2	
Total	**2310**			

* Wilcoxon-Mann-Whitney test
** Kruskal-Wallis test

Table 2. Mean values of the measurements of the diameters of the umbilical artery, umbilical vein and umbilical cord and the cross-sectional area of the umbilical cord according to gestational age.

GA	n	Cord Area in mm^2	Ø Artery in mm	Ø Cord in mm	Ø Vein in mm
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
12	60	16.5±5.2	0.7 ±0.2	4.5 ±0.7	1.2 ±0.3
13	60	20.7 ±5.3	0.9 ±0.3	5.1 ±0.6	1.6 ±0.4
14	59	26.9 ±7.5	1.1 ±0.3	5.8 ±0.8	1.8 ±0.5
15	60	35.5 ±7.5	1.4 ±0.4	6.7 ±0.7	2.4 ±0.6
16	60	49.6 ±13.3	1.6 ±0.4	8.0 ±1.4	2.7 ±0.5
17	61	58.7 ±18.4	1.9 ±0.5	8.5 ±1.3	3.2 ±0.8
18	60	63.3 ±17.3	2.1 ±0.5	9.0 ±1.2	3.6 ±0.9
19	62	92.7 ±23.7	2.2 ±0.5	10.8 ±1.4	4.2 ±1.1
20	110	100.1 ±23.2	2.7 ±0.8	11.3 ±1.3	4.9 ±1.1
21	102	115.5 ±30.3	2.8 ±0.6	12.1 ±1.5	5.1 ±1.1
22	91	124.4 ±35.3	3.0 ±0.7	12.5 ±1.8	5.3 ±1.0
23	82	140.3 ±35.5	3.2 ±0.7	13.4 ±1.7	5.8 ±1.2
24	60	168.4 ±34.7	3.5 ±0.7	14.7 ±1.6	6.3 ±1.4
25	59	171.9 ±37.8	3.7 ±0.8	15.0 ±1.8	6.6 ±1.4
26	63	190.2 ±38.6	4.0 ±0.8	15.4 ±1.6	7.3 ±1.6
27	62	193.1 ±44.9	3.9 ±0.8	15.6 ±1.8	7.1 ±1.4
28	91	210.4 ±55.1	3.8 ±0.8	16.2 ±2.2	7.8 ±1.3
29	80	218.1 ±54.4	4.3 ±1.0	16.6 ±2.0	7.9 ±1.4
30	91	226.0 ±53.7	4.4 ±0.9	16.8 ±2.0	8.1 ±1.4
31	103	239.2 ±59.1	4.5 ±0.9	17.4 ±2.1	8.9 ±1.9
32	101	235.2 ±56.1	4.5 ±0.8	17.4 ±2.0	8.7 ±1.6
33	96	231.7 ±74.1	4.3 ±1.1	17.0 ±2.5	9.0 ±1.8
34	102	237.7 ±54.4	4.5 ±0.9	17.3 ±2.0	9.0 ±1.9
35	121	241.9 ±60.3	4.6 ±0.9	17.6 ±2.3	8.9 ±1.9
36	102	230.8 ±55.7	4.5 ±0.8	17.1 ±2.1	8.8 ±1.4
37	99	235.7 ±63.5	4.6 ±0.9	17.4 ±2.2	9.0 ±1.8
38	95	238.1 ±65.6	4.7 ±1.0	17.5 ±2.3	9.2 ±1.7
39	59	241.1 ±57.2	4.8 ±1.0	17.5 ±2.2	8.9 ±2.0
40	59	252.0 ±63.6	4.6 ±0.7	17.8 ±2.2	9.1 ±1.3
P value		<0.00001	<0.00001	<0.00001	<0.00001

Ø: diameter GA: gestational age
Wilcoxon Two-Sample Test

Table 3. Estimated values of the diameter of the umbilical artery and umbilical vein by ultrasonography according to the 10th, 50th and 90th percentiles, using polynomial regression.

		Umbilical artery			Umbilical Vein		
GA-US	**n**	**P10**	**P50**	**P90**	**P10**	**P50**	**P90**
12	60	0.51	0.75	1.09	0.86	1.22	1.73
13	60	0.63	0.92	1.34	1.07	1.51	2.14
14	59	0.76	1.11	1.62	1.30	1.84	2.61
15	60	0.90	1.32	1.92	1.56	2.22	3.14
16	60	1.06	1.54	2.25	1.85	2.62	3.71
17	61	1.22	1.78	2.60	2.16	3.05	4.33
18	60	1.39	2.03	2.96	2.48	3.51	4.97
19	62	1.56	2.27	3.32	2.81	3.98	5.64
20	110	1.73	2.52	3.68	3.15	4.46	6.32
21	102	1.89	2.76	4.03	3.49	4.94	7.00
22	91	2.05	3.00	4.37	3.82	5.41	7.66
23	82	2.20	3.21	4.69	4.14	5.86	8.30
24	60	2.34	3.41	4.98	4.44	6.29	8.91
25	59	2.47	3.60	5.25	4.72	6.69	9.47
26	63	2.58	3.76	5.49	4.98	7.05	9.99
27	62	2.67	3.90	5.69	5.21	7.38	10.45
28	91	2.76	4.02	5.87	5.42	7.67	10.86
29	80	2.83	4.12	6.02	5.59	7.92	11.22
30	91	2.88	4.21	6.14	5.74	8.13	11.52
31	103	2.93	4.27	6.24	5.87	8.31	11.77
32	101	2.97	4.33	6.32	5.97	8.46	11.98
33	96	3.00	4.37	6.38	6.06	8.58	12.15
34	102	3.02	4.41	6.43	6.13	8.68	12.29
35	121	3.04	4.44	6.48	6.18	8.76	12.40
36	102	3.06	4.47	6.52	6.23	8.82	12.50
37	99	3.08	4.50	6.56	6.27	8.89	12.59
38	95	3.11	4.54	6.62	6.32	8.95	12.68
39	59	3.14	4.58	6.69	6.37	9.02	12.78
40	59	3.18	4.64	6.78	6.42	9.10	12.91
Equation	2310	$Log_{10}(artery) = -1.902+0.21*GA-0.00585*GA^2+0.00006*GA^3$			$Log_{10}(vein) = -1.717+0.21*GA-0.0056*GA^2+0.00005*GA^3$		
R^2		0.82			0.87		

GA-US: gestational age as assessed by ultrasonography
R^2: coefficient of determination

Table 4. Estimated values of the diameter of the umbilical cord and of a cross-sectional area of the cord by ultrasonography according to the 10^{th}, 50^{th} and 90^{th} percentiles, using polynomial regression.

		Umbilical cord			Cord Area		
GA-US	**n**	**P10**	**P50**	**P90**	**P10**	**P50**	**P90**
12	60	3.54	4.38	5.42	9.89	15.03	22.84
13	60	4.14	5.12	6.34	13.50	20.50	31.13
14	59	4.78	5.92	7.32	17.96	27.27	41.41
15	60	5.46	6.75	8.35	23.36	35.46	53.82
16	60	6.16	7.62	9.43	29.70	45.08	68.43
17	61	6.88	8.51	10.52	36.97	56.11	85.17
18	60	7.60	9.40	11.63	45.09	68.43	103.87
19	62	8.31	10.28	12.72	53.93	81.86	124.25
20	110	9.01	11.15	13.78	63.34	96.14	145.92
21	102	9.68	11.97	14.81	73.11	110.97	168.43
22	91	10.31	12.76	15.78	83.02	126.01	191.25
23	82	10.90	13.49	16.68	92.83	140.90	213.85
24	60	11.44	14.15	17.50	102.32	155.29	235.69
25	59	11.93	14.75	18.25	111.27	168.88	256.31
26	63	12.36	15.28	18.90	119.52	181.39	275.29
27	62	12.73	15.74	19.47	126.91	192.61	292.33
28	91	13.04	16.13	19.95	133.36	202.40	307.19
29	80	13.30	16.45	20.35	138.82	210.69	319.77
30	91	13.51	16.71	20.67	143.28	217.47	330.05
31	103	13.67	16.91	20.92	146.79	222.79	338.13
32	101	13.79	17.06	21.10	149.40	226.76	344.17
33	96	13.88	17.16	21.23	151.24	229.54	348.38
34	102	13.93	17.23	21.31	152.40	231.30	351.05
35	121	13.97	17.28	21.37	153.03	232.26	352.50
36	102	13.99	17.30	21.39	153.27	232.63	353.06
37	99	14.00	17.31	21.41	153.27	232.63	353.10
38	95	14.01	17.33	21.43	153.16	232.51	352.96
39	59	14.02	17.35	21.46	153.10	232.48	353.03
40	59	14.05	17.39	21.51	153.21	232.78	353.68
Equation	2310	$Log_{10}(cord) = -0.677+0.154*GA-0.0042*GA^2+0.00004*GA^3$			$Log_{10}(area) = -1.417+0.3026*GA-0.008*GA^2+0.00007*GA^3$		
R^2		0.89			0.89		

GA-US: gestational age as assessed by ultrasonography
R^2: coefficient of determination

Legends:

Figure 1: Estimated values of the diameter of the umbilical artery as assessed by ultrasonography according to the 10^{th}, 50^{th} and 90^{th} percentiles, using polynomial regression.

Figure 2: Estimated values of the diameter of the umbilical vein as assessed by ultrasonography according to the 10^{th}, 50^{th} and 90^{th} percentiles, using polynomial regression.

Figure 3: Estimated values of the diameter of the umbilical cord as assessed by ultrasonography according to the 10^{th}, 50^{th} and 90^{th} percentiles, using polynomial regression.

Figure 4: Estimated values of a cross-sectional area of the umbilical cord as assessed by ultrasonography according to the 10^{th}, 50^{th} and 90^{th} percentiles, using polynomial regression**.**

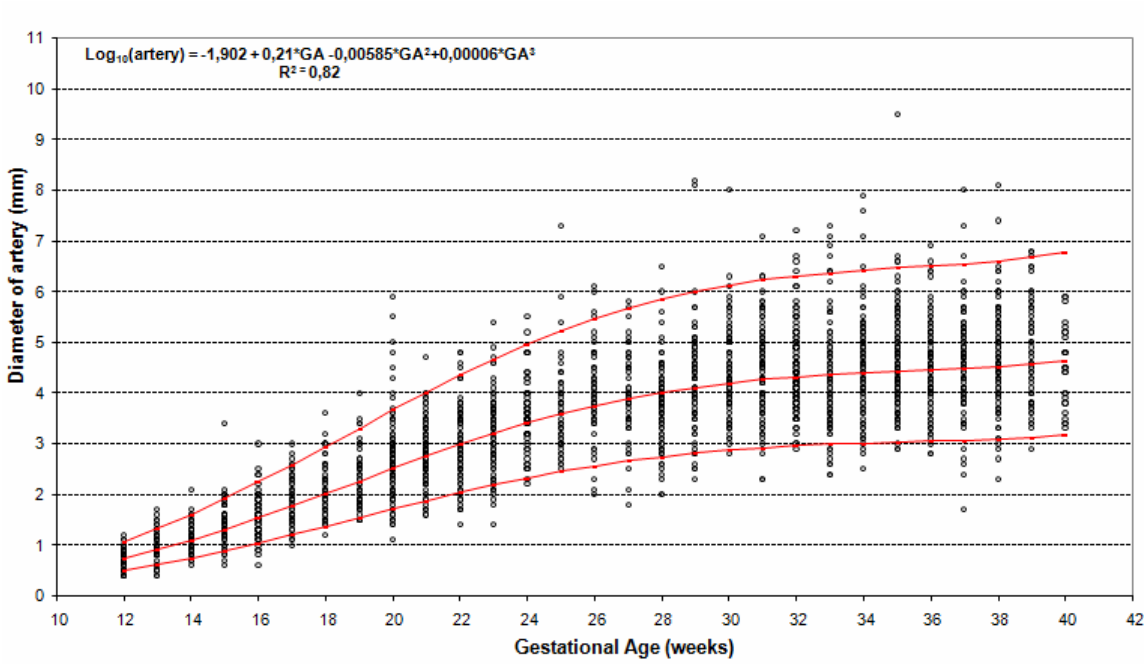
Log10(artery) = -1,902 + 0,21*GA -0,00585*GA²+0,00006*GA³
R² = 0,82
Diameter of artery (mm)
Gestational Age (weeks)
0
1
2
3
4
5
6
7
8
9
10
11
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42

Figure 1.

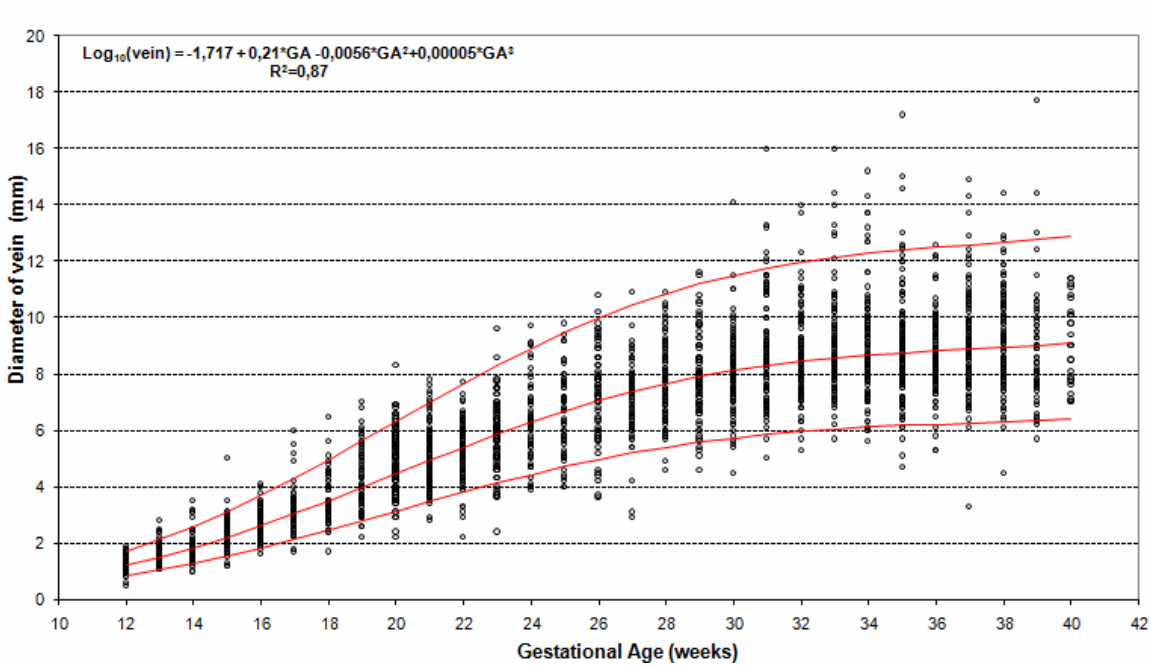
Log10(vein) = -1,717 + 0,21*GA -0,0056*GA²+0,00005*GA³
R²=0,87
Diameter of vein (mm)
Gestational Age (weeks)
0
2
4
6
8
10
12
14
16
18
20
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42

Figure 2.

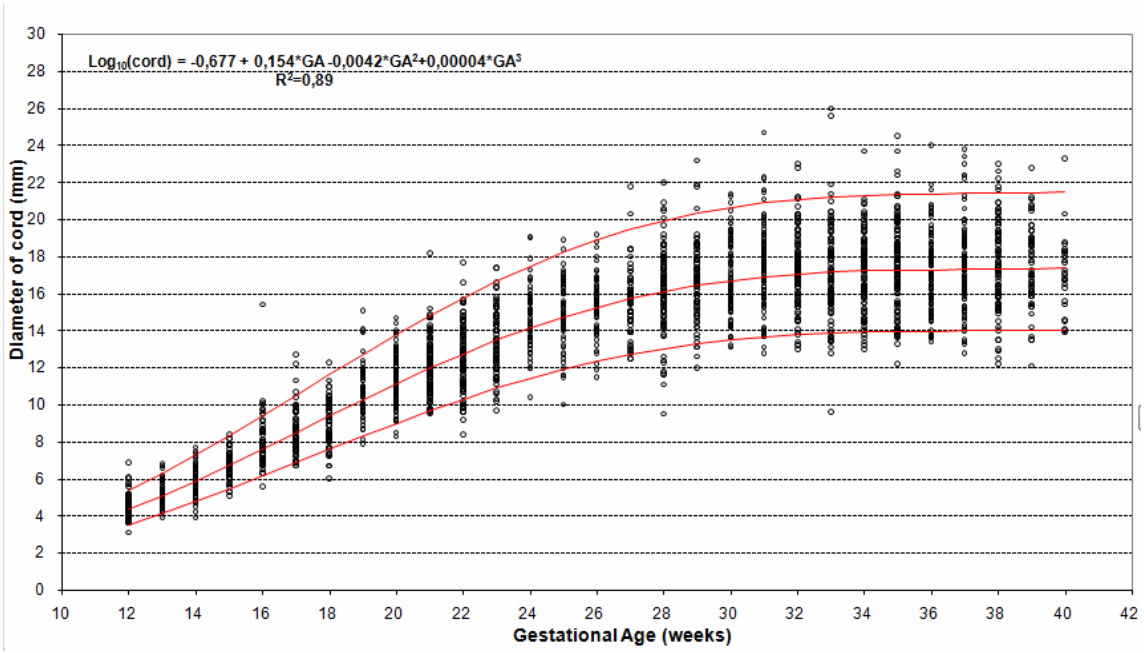
Log10(cord) = -0,677 + 0,154*GA -0,0042*GA2+0,00004*GA3
R2=0,89
Diameter of cord (mm)
Gestational Age (weeks)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42

Figure 3.

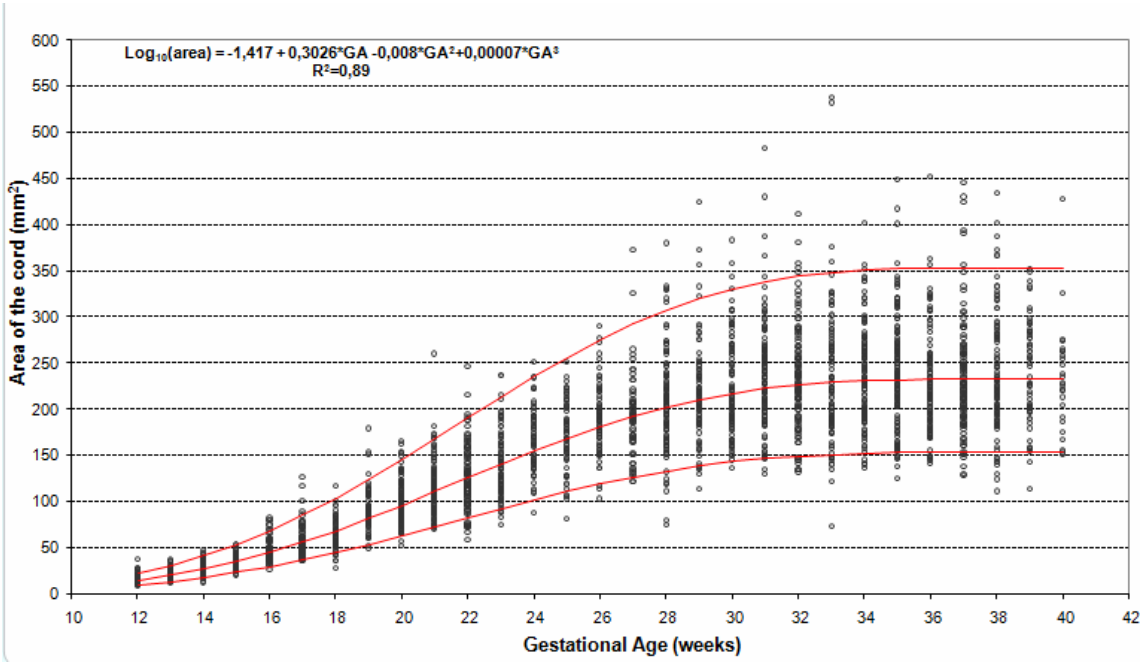
Log10(area) = -1,417 + 0,3026*GA -0,008*GA²+0,00007*GA³
R²=0,89
Area of the cord (mm²)
Gestational Age (weeks)
600
550
500
450
400
350
300
250
200
150
100
50
0
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42

Figure 4.

4.3. Artigo 3

Area of Wharton's jelly as an estimate of the thickness of the umbilical cord and its relationship with estimated fetal weight

CRISTIANE BARBIERI, MD,[a] JOSE GUILHERME CECATTI, MD, PhD,[a]* FERNANDA GARANHANI SURITA, MD, PhD,[a] EMILIO FRANCISCO MARUSSI, MD, PhD,[a] & JOSE VILTON COSTA, Stat [a]

Department of Obstetrics and Gynecology, School of Medical Sciences, Universidade Estadual de Campinas – UNICAMP [a], Campinas, São Paulo, Brazil.

*** Correspondence:**

Jose Guilherme Cecatti

P O Box 6030

13083-881 Campinas-SP

Brazil

E-mail: cecatti@unicamp.br

Running head: *Normal values of Wharton´s jelly area*

Word count (Text part of manuscript) = 1984 words

Abstract

*Background***:** The objective of this study was to construct a reference curve for the ultrasonographic measurement of the area of Wharton's jelly (WJ) in low-risk pregnancies of 13-40 weeks and to investigate the relationship between the area of Wharton's jelly and estimated fetal weight. *Methods:* A prospective study was carried out between June 2005 and December 2006 in 2,189 low-risk pregnancies to determine the area of Wharton's jelly in a cross-section of the umbilical cord. The area of WJ was calculated by subtracting the areas of the umbilical vessels from the total area of the umbilical cord and calculating the 10^{th}, 50^{th} and 90^{th} percentiles using a third-degree polynomial regression procedure. Fetal weight estimated by ultrasonography was correlated with the measurement of the area of WJ. *Results:* The estimated area of WJ increased according to gestational age (R^2=0.64, p< 0.0001), stabilizing, however, from the 32^{nd} week onwards. This relationship may be expressed by its regression equation of $Log_{10}(WJ) = -1.4307 + 0.2986*GA - 0.008*GA^2 + 0.00008*GA^3$. There was a significant linear correlation between the area of WJ and estimated fetal weight up to 26 weeks (R=0.782), values of WJ then remaining practically constant from that time until delivery (R=0.047). *Conclusion:* The reference curve constructed for the area of WJ indicates that it increases according to gestational age, showing, however, a tendency to stabilize at around 32 weeks of gestation. It is also linearly correlated with estimated fetal weight only up to 26 weeks of gestation.

Key words: *ultrasonography; pregnancy; umbilical cord; umbilical vessels; Wharton's jelly.*

Abbreviations: *A: umbilical artery, C: umbilical cord, EFW: estimated fetal weight, GA: gestational age, R: linear correlation coefficient, US: ultrasound, V: umbilical vein, WJ: Wharton's jelly*

The umbilical cord is responsible for maternal-fetal blood flow. Normally, it is composed of two arteries permeated with venous blood and a vein that transports arterial blood, cushioned by a special type of mucous connective tissue known as Wharton's jelly and by remnants of the allantois (1).

This tissue consists of a fundamental amorphous substance containing glycosaminoglycans, proteoglycans and, principally, hyaluronic acid. It also contains cells with characteristics of smooth muscle cells that permit its contractile function. These cells constitute an interconnected network of collagen that forms canaliculi and perivascular spaces (2,3), permitting adequate blood flow to the fetus in cases of compression of the umbilical cord during pregnancy or delivery (4).

Alterations in the area of WJ have been described in various different conditions such as hypertensive disease (5) and prematurity and fetal distress during labor (6). The absence of WJ around the vessels of the umbilical cord has been found in cases of perinatal mortality (7), whereas the presence of a large area of WJ has been described in cases of diabetes mellitus (8). Until recently, these data consisted in findings resulting from pathological examinations or case reports (9). With the recent progress made in ultrasonographic techniques during pregnancy, some investigators have concentrated their efforts on studying the umbilical cord.

The presence of a thin cord identified during pregnancy places the fetus at risk of restricted growth and birthweight classified as small for gestational age. This appears to be a consequence of a reduction in the area of WJ. Therefore, in 2001, Ghezzi et al.

published a reference curve for the area of WJ in accordance with fetal biometric parameters, reporting a strong statistical correlation up to 32 weeks of pregnancy and demonstrating that WJ is one of the principal components of the umbilical cord in the second and third trimesters of pregnancy (10).

Other studies have also shown a strong correlation between the anthropometric parameters used to estimate gestational age and fetal weight with the area of WJ at ultrasonography (8,9,11,12). Therefore, the objective of this study was to calculate a reference curve of the area of WJ in a cross-section of the umbilical cord as a function of gestational age in a population of low-risk pregnant women, and to correlate these values with fetal weight as calculated by routine ultrasonography.

Material and Methods

This prospective, cross-sectional study was carried out between June 2005 and December 2006 in a total of 2,189 low-risk pregnant women of gestational ages ranging from 13 to 40 weeks, who had been referred for routine ultrasonography at the Ultrasonography Department of the Center for Women's Integrated Healthcare (CAISM), *Universidade Estadual de Campinas* and at the *CDE Diagnóstico por Imagem* Clinic in Campinas, Brazil.

Inclusion criteria comprised: a low-risk pregnancy with a single living fetus, gestational age previously established by the date of the last menstrual period when reliable or by ultrasonography carried out in the first trimester, intact membranes, and normal amniotic

fluid index (13). Exclusion criteria comprised: cases of diabetes, arterial hypertension of any etiology, fetal malformation, oligoamnios or polyhydramnios, cases with clinical signs of intrauterine growth restriction or fetal macrosomia (uterine height below or above, respectively, the lower or upper limits for gestational age), and cases with morphological abnormalities in the umbilical cord or its blood flow (abnormal Doppler velocimetry).

A Toshiba-Power Vision 6000 ultrasonographic scanner, model SSA-370 ® and a Voluson 730 PRO® scanner with 3.5 mHz transabdominal convex transducers, adopted as standard for obstetric scans, were used for the ultrasonographic examinations carried out in this study. The pregnant women were submitted to routine ultrasonography in a semi-seated position during which biparietal diameter, head and abdominal circumferences and femur length were measured and estimated fetal weight (EFW) calculated according to Hadlock's formula (14); in addition, the other parameters routinely evaluated during pregnancy were also measured. Women who fulfilled all the inclusion criteria were informed of the nature of the study and invited to participate. Those who agreed to participate signed an informed consent form drawn up in accordance with the regulations of the institution's Internal Review Board, which approved the study protocol prior to commencement (Approval #268/2005).

Next, the area of the umbilical cord was measured in all patients, together with the diameters of its vessels (arteries and vein) in a cross-sectional plane of the cord adjacent to its insertion in the fetal abdominal wall, at a maximum distance of 2.0cm from the insertion point, using the elliptical calibrators of the ultrasound scanners at the outer

edges of the cord and at the edges of the vessels in accordance with the method used by Raio et al. (9) and Weissman et al. (11) (Figure 1). The surface area of WJ was calculated according to the cross-sectional area of the umbilical cord from which the areas of the two arteries and the umbilical vein were subtracted (WJ = C-V-2A). The inter- and intra-observer variability of the measurements used to calculate the area of Wharton's jelly were evaluated in a sub-sample of this population of women and were considered good (15).

For the statistical analysis, first, the mean, standard deviation and median of the area of WJ in the umbilical cord were calculated in accordance with some demographic and obstetrical characteristics, and the statistical differences between them were evaluated using the Kruskal-Wallis or Mann-Whitney non-parametrical tests. Next, the smoothed values of the 10th, 50th and 90th percentiles of these measurements were calculated for each gestational age, using third degree polynomial regression analysis, and resulting in the respective regression equations and coefficients of determination of the regression adjustment model (R^2). Finally, the values of the area of Wharton's jelly were correlated with the estimated fetal weight, and the linear coefficient of correlation (R) between them was calculated for two groups of cases: those up to 26 weeks of gestational age and those at more than 26 weeks. P values < 0.05 were considered significant.

Results

The principal characteristics of the 2,189 pregnant women are shown in Table 1. The majority were white, under 29 years of age and nulliparas. There was no statistically significant difference in the area of WJ as a function of these characteristics; the only difference being with respect to gestational age.

Table 2 shows the estimated 10^{th}, 50^{th} and 90^{th} percentiles of the area of Wharton's jelly for each gestational age between 13 and 40 weeks. To calculate the regression equation that defines the area of WJ according to gestational age (GA), the following was obtained: $Log_{10}(WJ) = -1.4307 + 0.2986*GA - 0.008*GA^2 + 0.00008*GA^3$, for which the degree of adjustment (R^2) was 0.64. Figure 2 shows the curve of these percentile values of the area of WJ according to gestational age. Note that values increase uniformly until around 32 weeks when they reach a plateau, tending to stabilize from then onwards.

Figure 3 shows the correlation between the measurement of the area of WJ and fetal weight as estimated by ultrasonography. This correlation increases linearly until 26 weeks of gestational age (R=0.782), remaining practically constant from this gestational age onwards (R=0.047).

Discussion

This study shows a direct relationship between gestational age and the area of Wharton's jelly in the umbilical cord. There is an increase in the area of WJ as a function of gestational age until around 32 weeks, after which these measurements remain

practically stable until the end of pregnancy. Our findings also show a positive and linear relationship between estimated fetal weight and the area of WJ, but only until the 26th week of gestational age, since from then onwards there is almost no change in the area of WJ compared to estimated fetal weight.

Previous case reports have shown a correlation between the presence of thin cords or a reduced area of WJ and fetal loss, premature births and inadequate fetal growth (6). In 1967, studies were initiated on the macro and microscopic structure of the umbilical cord. Later, other investigators became interested in studying the tissue components of the umbilical cord. In 1983, Klein & Meyer (2) showed the macromolecular diffusion in WJ in relation to hyaluronic acid, one of its principal components. In 1994, Weissman et al. (11) presented a reference curve for the diameter of the umbilical cord and its vessels, which had not existed in the literature up to that time. Using the values of the diameters of the umbilical cord and its vessels, these investigators calculated the area of WJ at the different gestational ages and reported a peak at around 34 weeks.

Raio et al. (9) described a reference curve for the cross-sectional area of the umbilical cord and its vessels using a slightly different technique in which they viewed the cord through a cross-section, the same technique used in the present study. These investigators found a correlation between the cross-sectional area of the umbilical cord and fetal anthropometric parameters.

Using this same technique, Ghezzi et al. in 2001 (10) established a curve of the area of WJ in a total of 659 low-risk pregnancies of 15-41 weeks. In fact, in 1994, Weissman et

al. had already defined normal values of the estimated area of Wharton's jelly in 368 uncomplicated pregnancies, and reported differences in the values obtained, which varied between 13 and 27% depending on gestational age. One possible explanation for these results may the different techniques used for measuring the umbilical cord. This same study reported that the ratio of the area of WJ in relation to the total area of the umbilical cord decreased significantly as gestational age increased, probably because of a reduction in the amount of water, one of its principal components.

In fact, WJ is the major component of the umbilical cord in the second and third trimesters (4); therefore, if the area of the umbilical cord reaches its peak at around 32 weeks (16) or 31 weeks (17), the area of WJ would be expected to follow the same pattern.

However, the results of the present study are closer to those reported by Ghezzi et al. (10) and by Togni et al. (12), although values are slightly higher. These investigators studied 312 pregnant women of 24-39 weeks of pregnancy and described reference curves of the cross-sectional areas of the umbilical cord (18) and its components, as well as the area of WJ (12), also reporting an increase at around 32 weeks followed by a plateau at around 35 weeks with values decreasing from 36 weeks onwards.

The correlation between the area of Wharton's jelly and anthropometric parameters (which are used to calculate fetal weight) is generally weak, as in the study carried out by Togni et al. (12), who, for example, reported a correlation of only 0.240 between the area of Wharton's jelly and estimated fetal weight. However, in 2001 Ghezzi et al. (10)

already suspected this weak correlation to be a result of the overlap of two different situations as a function of gestational age, i.e. a strong correlation for earlier gestational ages and a weak correlation for later gestational ages. These were exactly the results also found in the present study.

Investigators have been proposing reference curves for the area of the umbilical cord and its components since 1994, and have carried out evaluations on the area of WJ in the umbilical cord. A possible strength of the present study is that it has the largest sample size described up to the present time and the results obtained are in agreement with previously obtained values. These parameters should serve as a reference, principally in cases in which diseases such as diabetes, arterial hypertension, and intrauterine growth restriction are suspected that may interfere with fetal development, and in which there may be changes in the morphology and in the function of the umbilical cord and in the area of WJ. Nevertheless, appropriate validation of these curves is necessary to confirm the usefulness of these parameters, and this represents a challenge for future research studies.

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Legends to figures:

Figure 1: Ultrasonographic measurement of the cross-sectional area of the umbilical cord (C), of the diameter of the umbilical vein (V) and of the umbilical artery (A). The area of Wharton Jelly (WJ) is WJ = C-V-2A.

Figure 2: Relationship between the area of Wharton's Jelly of the umbilical cord and gestational age. The lines correspond to the 10^{th}, 50^{th} and 90^{th} percentiles.

Figure 3: Correlation between the area of Wharton's Jelly and estimated fetal weight (EFW) for low-risk pregnancies up to 26 weeks (r=0.782) and above 26 weeks (r=0.047).

Table 1. Means and medians of the area of Wharton's Jelly of the umbilical cord (mm^2) in low-risk pregnancies, according to some demographic and obstetric characteristics

Characteristics	n[£]	Mean	Standard deviation	Median	p-value
Race/ethnic					0.7011*
White	1756	110.7	56	106.9	
Non-white	433	109.3	56	107.3	
Age					0.5204*
≤29	1254	109.9	56.9	106.4	
>29	935	111.1	54.8	107.4	
Parity					0.0342*
Nullipara	1168	107.7	55.5	106.1	
≥ 1 previous pregnancies	1021	113.4	56.4	107.5	
Gestational age (US)					<0.0001**
13	18	18.3	6.9	18.7	
14	43	23.3	7.5	23.4	
15	59	27.2	8.1	26.5	
16	60	39.5	13.5	35.8	
17	61	44.1	17.0	41.7	
18	60	45.3	18.1	41.4	
19	62	69.3	21.3	66.2	

20	110	67.9	22.1	64.4
21	102	81.6	32.1	76.6
22	91	86.7	33.2	80.7
23	82	95.5	35.1	87.1
24	60	115.2	35.0	115.6
25	59	113.5	37.6	114.3
26	62	120.2	37.2	120.9
27	62	125.6	36.8	128.9
28	92	137.0	51.3	136.9
29	80	137.3	48.4	133.6
30	91	141.1	49.8	125.3
31	103	142.0	50.9	137.4
32	101	142.0	51.3	132.7
33	95	135.2	56.5	126.8
34	102	137.9	44.0	136.0
35	121	141.5	49.4	136.1
36	102	135.6	45.7	130.4
37	99	134.2	48.4	129.0
38	94	134.7	50.7	121.7
39	59	137.6	44.6	144.8
40	59	151.9	52.0	143.3

£ **N=2189** * Mann-Whitney test ** Kruskal-Wallis test US: ultrasound

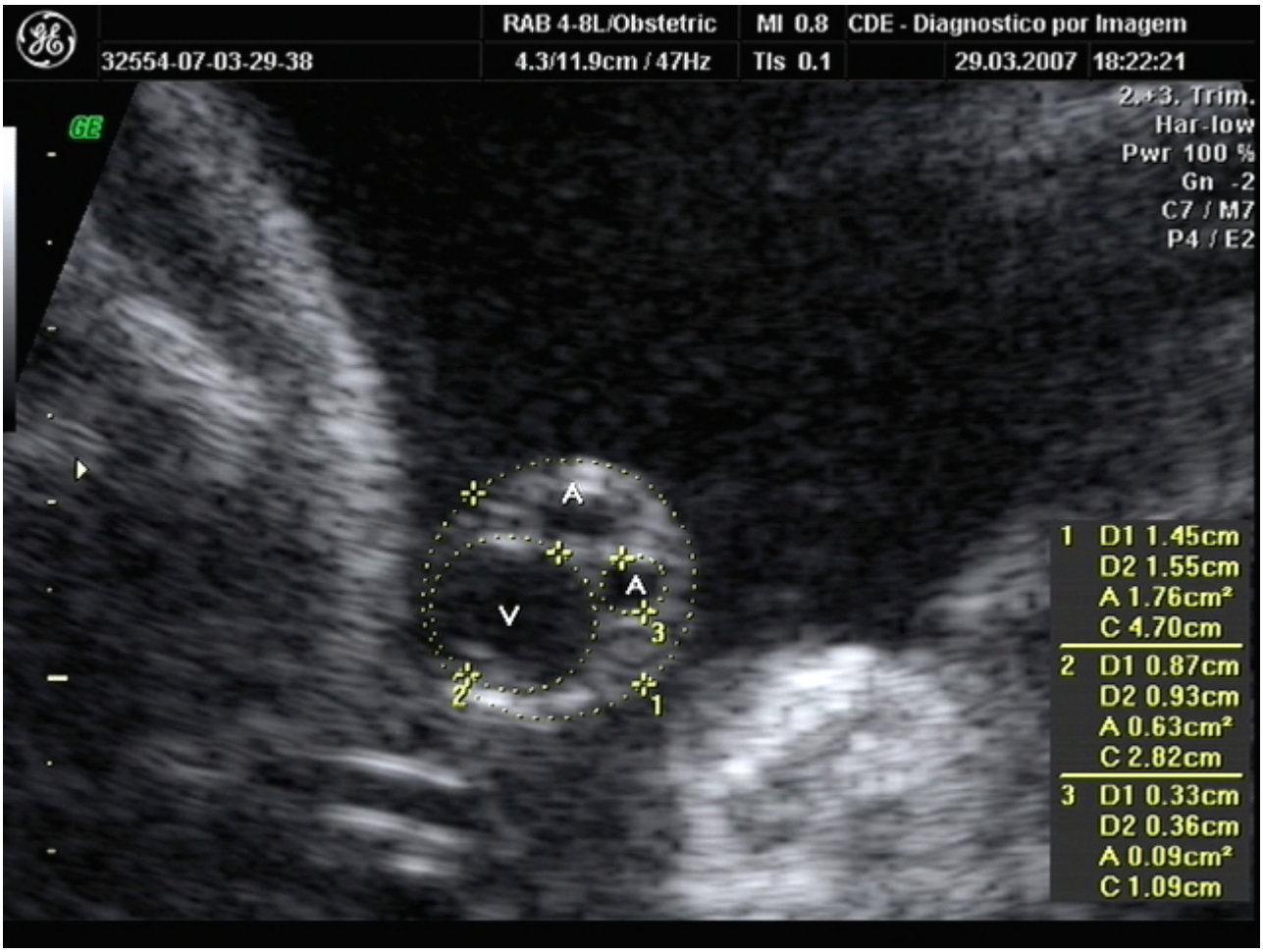
Table 2. Estimated values of percentiles of the area of Wharton's Jelly (mm^2), according to gestational age

GA-US	p10	p50	p90	n
13	8.81	16.30	30.15	18
14	11.52	21.27	39.30	43
15	14.70	27.13	50.08	59
16	18.34	33.84	62.43	60
17	22.39	41.31	76.19	61
18	26.79	49.41	91.14	60
19	31.44	57.99	106.95	62
20	36.23	66.83	123.25	110
21	41.05	75.71	139.63	102
22	45.77	84.41	155.68	91
23	50.27	92.72	171.00	82
24	54.46	100.44	185.23	60
25	58.24	107.41	198.09	59
26	61.56	113.53	209.37	62
27	64.38	118.73	218.95	62
28	66.68	122.97	226.77	92
29	68.48	126.28	232.88	80
30	69.79	128.71	237.36	91
31	70.68	130.35	240.39	103

32	71.20	131.30	242.15	101
33	71.41	131.69	242.87	95
34	71.39	131.66	242.80	102
35	71.22	131.34	242.21	121
36	70.97	130.88	241.36	102
37	70.71	130.41	240.52	99
38	70.51	130.08	239.97	94
39	70.45	130.02	239.97	59
40	70.58	130.37	240.83	59

Equation: $Log_{10}(WJ) = -1.4307 + 0.2986*GA – 0.008*GA^2 + 0.00008*GA^3$

GA-US: gestational age according to ultrasonography.


RAB 4-8L/Obstetric
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4.3/11.9cm / 47Hz
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2.+3. Trim.
Har-low
Pwr 100 %
Gn -2
C7 / M7
P4 / E2
A
V
A
3
2
1
1 D1 1.45cm
D2 1.55cm
A 1.76cm²
C 4.70cm
2 D1 0.87cm
D2 0.93cm
A 0.63cm²
C 2.82cm
3 D1 0.33cm
D2 0.36cm
A 0.09cm²
C 1.09cm

Figure 1.

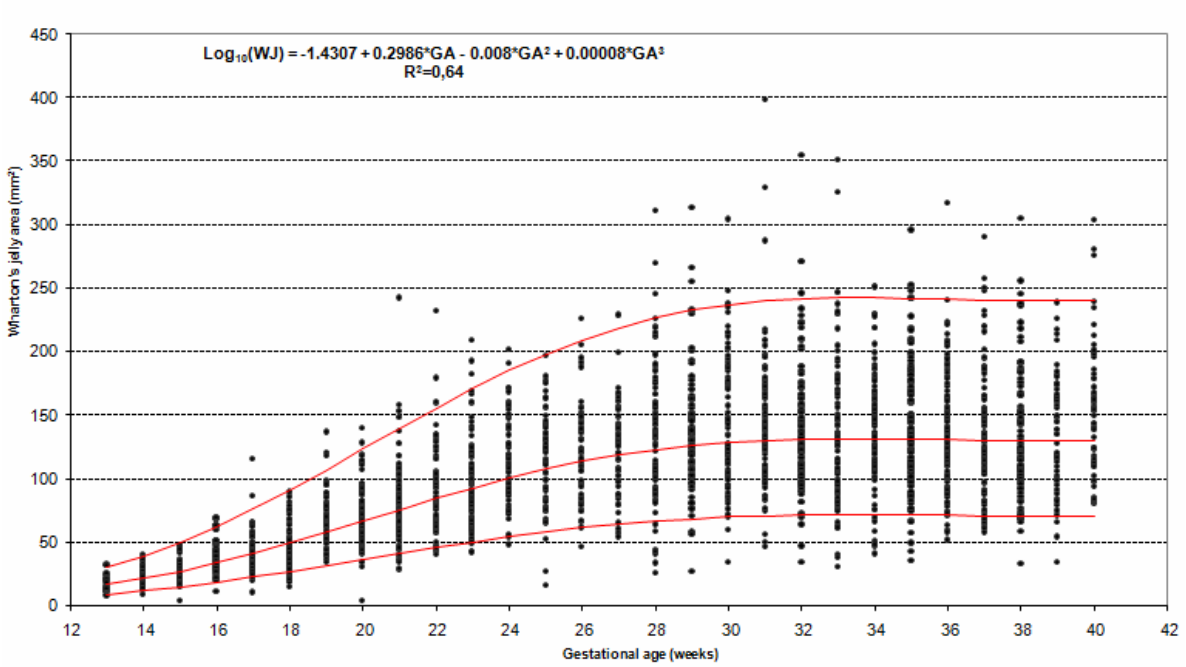
Log10(WJ) = -1.4307 + 0.2986*GA - 0.008*GA² + 0.00008*GA³
R²=0,64
Wharton's jelly area (mm²)
Gestational age (weeks)
0
50
100
150
200
250
300
350
400
450
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42

Figure 2.

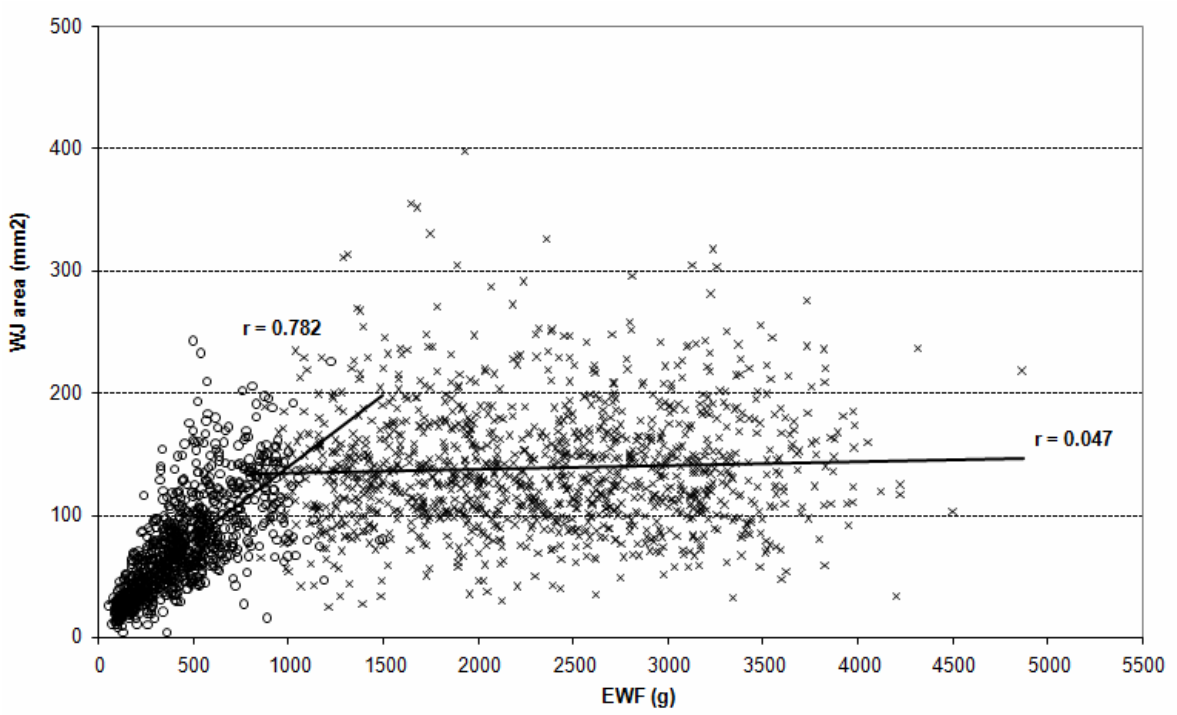
WJ area (mm2)
EWF (g)
r = 0.782
r = 0.047
0
100
200
300
400
500
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500

Figure 3.

4.4. Artigo 4

ORIGINAL ARTICLE

Validation study of the capacity of the reference curves of ultrasonographic measurements of the umbilical cord to identify deviations in estimated fetal weight

CRISTIANE BARBIERI, JOSE GUILHERME CECATTI, FABIANA KRUPA, EMILIO FRANCISCO MARUSSI & JOSE VILTON COSTA

Department of Obstetrics and Gynecology, School of Medical Sciences, University of Campinas – UNICAMP, Campinas, SP, Brazil.

Correspondence:

José Guilherme Cecatti

P.O. Box 6081

13083-881 Campinas, SP, Brazil

E-mail: cecatti@unicamp.br

Running headline: Umbilical cord thickness and EFW

Word count (Text part of manuscript) = 2014 words

Abstract

Background: The objective of this study was to evaluate the capacity of the cross-sectional area and diameter of the umbilical cord, and the area of Wharton's jelly to predict abnormalities in estimated fetal weight (EFW) in 20-40 week, low-risk pregnancies. *Methods:* A validation study was performed in 1,828 pregnant women. Fetal weight was estimated by ultrasonography and classified as: small for gestational age (SGA), appropriate for gestational age (AGA) or large for gestational age (LGA) according to the 10^{th} and 90^{th} percentiles of the reference curve. Measurements of the parameters of the cord were used to classify it as thin, normal or thick using the 10^{th} and 90^{th} percentiles of the reference curves as limits. The capacity of the diameter and total area of the cord and the area of Wharton's jelly to predict abnormal EFW were calculated for different gestational ages. *Results:* The capacity of the diameter of thin cords to predict SGA fetuses (S=8.3%, PPV=16.5%) or thick cords to predict LGA fetuses (S=5.5%, PPV=30.1) was weak, similar to the capacity of the area of the umbilical cord to predict SGA (S=8.3%; PPV=16.3%) or LGA fetuses (S=5.5%; PPV=27.8%). The capacity of the area of Wharton's jelly to predict SGA fetuses (S=5.7%, PPV=11.7%) was similar to its capacity to predict LGA fetuses (S=4%, PPV=27.1%). *Conclusion:* Despite the correlation between the diameter and cross-sectional area of the cord and EFW, these measurements were not found to be useful for predicting alterations in EFW and should not be used for this purpose.

Key words: estimated fetal weight; ultrasound; intrauterine growth restriction; umbilical cord measurements.

Abbreviations: *AC: abdominal circumference, AGA: appropriate for gestational age, BPD: biparietal diameter, EFW: estimated fetal weight, FL: femur length, HC: head circumference, IUGR: intrauterine growth restriction, LGA: large for gestational age, LMP: last menstrual period, NPV: negative predictive value, PPV: positive predictive value, S: sensitivity, SGA: small for gestational age, Spec: specificity, US: ultrasound, WJ: Wharton's Jelly.*

For some time now, various studies have been carried out to detect abnormalities in fetal growth at an early stage in pregnancy by using ultrasonography in obstetrical practice (1,2). Birthweight is a reflection of intrauterine fetal growth, which is determined by the nutrients received from the mother, and of the capacity of the placenta to provide these nutrients in sufficient quantity (3).

Intrauterine growth restriction (IUGR) of fetal origin may be caused by chromosomal abnormalities, infections, malformations or innate errors in metabolism. Maternal causes are principally low weight increase during pregnancy, low pre-gestational weight, smoking, hypertension and causes related to inadequate uteroplacental circulation. IUGR may lead to consequences in the fetus that are not restricted to the immediate effects such as neonatal hypoxia, hypoglycemia and infections, but also include mid- and long-term consequences such as impaired neurological development, cerebral palsy, diabetes type II and hypertension in adulthood (2,4,5).

In addition, changes in blood flow may be detected early by Doppler study of umbilical artery blood flow velocity waveform in fetuses with signs of IUGR as fetal response to compensate flow deficit in certain regions (6). Early detection of growth restriction may lead to a better prognosis. Cnattingius et al. (7) found that when fetuses with IUGR were identified and monitored, the risk of intrauterine death diminished and the neonatal period was less complicated.

Nevertheless, other diagnostic parameters for the evaluation of fetal growth continue to be sought. Among them, morphology of the umbilical cord, including its diameter and

the amount of Wharton's jelly, have been associated with intrauterine growth restriction (8) and lower than expected fetal birthweight (9,10). Histological studies, carried out using computer-assisted microscopic image analysis, found that the area of the umbilical vein was smaller in these fetuses compared to healthy fetuses (11).

The presence of thin or thick cords is already known to be linked to adverse maternal-fetal outcomes and diabetes, respectively (12-14). In 1999, Raio et al. reported a progressive increase in the diameter of the umbilical cord in low-risk pregnancies of up to 32 weeks, followed by a reduction in the size of the cord and also in the area of Wharton's jelly (WJ) as gestational age increased. These results show a direct participation of the umbilical cord in fetal metabolism and suggest that its thickness is related to the nutritional status of the fetus (12,15).

Therefore, it should be theoretically possible to use reference curves of the parameters of the umbilical cord to identify fetuses with inadequate growth by comparing the expected measurements of the umbilical cord for gestational age with estimated fetal weight. Hence, the objective of this study was to evaluate the capacity of the measurements of the umbilical cord, i.e. the cross-sectional area of the umbilical cord, its diameter and the area of Wharton's jelly, to diagnose deviations in estimated fetal weight in low-risk pregnancies of 20-40 weeks using as the gold standard the normal curve of EFW for gestational age developed in a similar population (16). Other factors that may also be associated with any variation in this performance were also evaluated.

Material and methods

The objective of the present study was to validate the curves of the measurements of the umbilical cord (diameter, cross-sectional area and area of Wharton's jelly) for the diagnosis of deviations in estimated fetal weight as a function of gestational age according to their classification. The gold standard was the curve established by Cecatti et al. (16) which was constructed using data of women from the same region.

A total of 1,828 women at 20 to 40 weeks of a low-risk pregnancy were evaluated by ultrasonography between June 2005 and December 2006. Inclusion criteria comprised: a single live fetus, gestational age previously established by the date of the last menstrual period (LMP) if reliable or an ultrasonographic examination carried out in the first trimester, intact membranes and normal amniotic fluid index (17). Patients with diabetes, hypertension of any etiology, fetal malformations, oligoamnios or polyhydramnios or with abnormalities in the morphology of the umbilical cord (presence of cysts on the umbilical cord or a single umbilical artery) were excluded from the study. For the ultrasonographic examinations proposed in this study, a Toshiba-Power Vision 6000, model SSA-370 ® scanner and a Voluson 730 PRO® scanner were used with a 3.5 mHz transabdominal convex transducer adopted as standard for obstetric scans.

The pregnant women were submitted to routine ultrasonography in a semi-seated position, for the estimation of fetal weight, which was automatically calculated by software in the ultrasound scanner in accordance with Hadlock's formula (18), which is based on four parameters of fetal biometry: biparietal diameter (BPD), head

circumference (HC), abdominal circumference (AC) and femur length (FL). Later, measurements of the diameter and cross-sectional area of the umbilical cord, its vessels (19) and the area of Wharton's jelly were measured to obtain reference curves for gestational age (20). All the women were duly informed about the nature of the study and any questions were answered, after which they voluntarily signed an informed consent form designed in accordance with the regulations of the Institutional Review Board that approved the study protocol (approval #268/2005).

For the classification of EFW as a function of gestational age, a modified curve of the normal values of fetal weight estimated by ultrasonography in accordance with gestational age, which was elaborated by Cecatti et al. (16), was used, including a polynomial adjustment of second and third degree to obtain regression curves of the percentiles. The variable EFW was classified as: small for gestational age (SGA), appropriate for gestational age (AGA) or large for gestational age (LGA) according to the position of the EFW in the aforementioned curve, below the 10^{th} percentile, between the 10^{th} and the 90^{th} percentiles or above the 90^{th} percentile for gestational age, respectively.

For each one of the measurements of the umbilical cord used, sensitivity, specificity, positive predictive and negative predictive values and their respective 95% confidence intervals were calculated, both in the case of small and large EFW.

Results

The demographic and obstetrical characteristics of the 1,828 pregnant women evaluated in this study are shown in Table I according to their percentage distribution. The majority of these women (80.3%) were white, 43.1% were over 30 years of age, and 51% had had two or more pregnancies. The majority (53.6%) had no living children yet and 88.9% had no history of abortion. With respect to gestational age, the cases were evenly distributed throughout the range from 20 to 40 weeks of gestational age.

Table II shows the performance of the ultrasonographic measurement of the diameter of the umbilical cord for the diagnosis of small for gestational age (SGA) and large for gestational age (LGA) fetuses, using the curve of estimated fetal weight as the gold standard. The capacity of the diameter of thin umbilical cords to predict SGA fetuses (S=8.3%, PPV=16.5%) or of thick umbilical cords to predict LGA fetuses (S=5.5%, PPV=30.1%) was very weak.

The results shown in Table III reflect a very similar trend. This table shows the performance of the ultrasonographic measurement of the area of the umbilical cord for the diagnosis of small for gestational age (SGA) or large for gestational age (LGA) fetuses, taking the same curve of estimated fetal weight as the gold standard. The capacity of the area of thin umbilical cords to predict SGA fetuses (S=8.3%; PPV=16.3%) or of thick umbilical cords to predict LGA fetuses (S=5.5%; PPV=27.8%) was also very weak.

Finally, in Table IV, the performance of the ultrasonographic measurement of the area of Wharton's jelly of the umbilical cord for the diagnosis of small for gestational age

(SGA) and large for gestational age (LGA) fetuses, taking the curve of estimated fetal weight as the gold standard, was also weak, both for SGA fetuses (S=5.7%; PPV=11.7%) and for LGA fetuses (S=4.0%; VPP=27.1%).

Discussion

This study was carried out in low-risk pregnant women with the objective of acquiring knowledge on the correlation between the anthropometric parameters regularly used for the evaluation of fetal growth and development and the area of the umbilical cord and of its components. In general, these results showed that the capacity of the different measurements of the umbilical cord to predict deviations in fetal growth using estimated fetal weight as the gold standard was very low.

Umbilical cords considered thick are strongly associated with the presence of metabolic diseases such as diabetes, and macrosomic fetuses. On the other hand, thin cords or those with a sparse amount of Wharton's jelly may be related to the presence of oligoamnios and fetal distress during labor, leading to a greater incidence of Cesarean sections and to low weight fetuses (11,21). Following more detailed studies that were carried out on the umbilical cord and its components between the 1990's and the present date, further associations were identified. In 1999, Raio et al. followed up 860 fetuses over 20 weeks to investigate to what extent a prenatal diagnosis of thin cords in supposedly normal fetuses was related to the risk of low birthweight or fetal distress during labor. Fetuses in which the area of the umbilical cord was below the 10^{th} percentile were found to be at greater risk of low birthweight, thereby confirming the

existence of a correlation between the area of the umbilical cord and fetal birthweight (22).

Similar results have recently been described by Togni et al. following evaluation of women at 24 to 39 weeks of a low-risk pregnancy. These investigators reported a statistically significant relationship between the areas of the Wharton´s jelly and of the umbilical cord and its components and fetal anthropometric components (23,24).

In the present study, our findings show that the capacity of the diameter of a thin umbilical cord to predict SGA fetuses or of a thick umbilical cord to predict LGA fetuses was very low, and the same occurred with respect to the other parameters studied such as the area of the umbilical cord and the area of Wharton's jelly. Many studies have been published in the scientific literature on the capacity of the thickness of the umbilical cord to predict a series of adverse perinatal conditions, including those related to low birthweight or to large or small weight for gestational age at birth. To the best of our knowledge, this is the first time that a formal attempt has been made to validate the capacity of the thickness of the umbilical cord to predict actual alterations in fetal weight as estimated by ultrasonography. Since the results of this study indicate such a poor performance, the question that would seem natural is why the thickness of the umbilical cord would appear to be a good predictor of alterations in neonatal weight when it is able to identify so few deviations in fetal weight?

Although this appears a contradiction, it is true that when the associations previously found between the various parameters related to the thickness of the umbilical cord and

the anthropometric parameters used to estimate fetal weight by ultrasonography are evaluated, it may be concluded that, although there is an association, the correlation between the measurements is small (23-25). This association becomes slightly more evident when the analysis is stratified according to gestational age: the association is greater for lower gestational ages up to 28-32 weeks, after which the parameters of the cord remain practically constant until the end of pregnancy (26). Therefore, if this is the association between the parameters of the umbilical cord and the parameters used to estimate fetal weight, the outcome of this validation would be expected to be very low, as was confirmed in the present study. On the basis of these findings, it is not possible, at least at the present moment, to recommend use of the parameters of the umbilical cord for this purpose.

These data do not contradict the previous findings that the parameters of the umbilical cord may serve as good markers of adverse perinatal situations, even of low or inadequate birthweight. Therefore, the challenge remains to validate the normal reference values identified in this population by following-up a series of high and low-risk pregnancies until delivery.

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Table I. Percentage distribution of women according to their demographic and obstetrical characteristics.

Characteristics	*N*	*%*
Age (years)		
14 – 19	126	6.9
20 – 24	408	22.3
25 – 29	506	27.7
≥ 30	788	43.1
Race		
White	1467	80.3
Black	221	12.1
Others	140	7.6
Number of pregnancies		
1	895	49.0
≥2	933	51.0
History of abortions		
Yes	203	11.1
No	1625	88.9
Parity		
Nullipara	957	52.4
≥ 1	871	47.6
Living children		
None	979	53.6
≥ 1	849	46.4
Gestational age (weeks)		
20 -27	629	34.4
28 - 31	365	20.0
32 - 36	521	28.5
≥ 37	313	17.1
Total	**1828**	**100.0**

Table II. Performance of the curve of the diameter of the umbilical cord as measured by ultrasonography (curve of Barbieri et al., 2007) to diagnose small-for-gestational-age (SGA) and large-for-gestational-age (LGA) fetuses, the gold standard being the curve established by Cecatti et al. (2000).

Diameter of the umbilical cord (Curve established by Barbieri et al., 2007)	**Estimated Fetal Weight (Curve established by Cecatti et al., 2000)**	
	SGA	Not SGA
Thin cord (< p10)	16	81
Not a thin cord (≥ p10)	176	1555
Total	192	1636
	LGA	Not LGA
Thick cord (> p90)	22	51
Not a thick cord (≤ p90)	375	1380
Total	397	1431

Performance	**SGA**		**LGA**	
	%	95% CI	%	95% CI
Sensitivity	8.3	4.8 - 13.2	5.5	3.5 - 8.3
Specificity	95.0	93.9 - 96	96.4	95.3 - 97.3
Positive predictive value	16.5	9.7 - 25.4	30.1	19.9 - 42.0
Negative predictive value	89.8	88.3 - 91.2	78.6	76.6 - 80.5

95% CI: 95% confidence interval

Table III. Performance of the curve of the area of the umbilical cord measured by ultrasonography (curve established by Barbieri et al., 2007) to diagnose small-for-gestational-age (SGA) and large-for-gestational-age (LGA) fetuses, the gold standard being the curve of estimated fetal weight established by Cecatti et al., 2001).

Area of the umbilical cord (Curve established by Barbieri et al., 2007)	**Estimated fetal weight (Curve established by Cecatti et al., 2000)**	
	SGA	**Not SGA**
Thin cord (< p10)	16	82
Not a thin cord (≥ p10)	176	1554
Total	192	1636
	LGA	**Not LGA**
Thick cord (> p90)	22	57
Not a thick cord (≤ p90)	375	1374
Total	397	1431

Performance	**SGA**		**LGA**	
	%	95% CI	%	95% CI
Sensitivity	8.3	4.8 - 13.2	5.5	3.5 - 8.3
Specificity	95.0	93.8 - 96	96.0	94.9 - 97.0
Positive predictive value	16.3	9.6 - 25.2	27.8	18.3 - 39.1
Negative predictive value	89.8	88.3 - 91.2	78.6	76.6 - 80.5

95% CI: - 95% Confidence Interval

Table IV. Performance of the curve of the area of Wharton's jelly in the umbilical cord as measured by ultrasonography (curve established by Barbieri et al., 2007) to diagnosis small-for-gestational-age (SGA) and large-for-gestational-age (LGA) fetuses, the gold standard being the curve of estimated fetal weight established by Cecatti et al., 2000.

Area of Wharton's jelly in the umbilical cord (Curve established by Barbieri et al., 2007)	**Estimated fetal weight Curve established by Cecatti et al. (2000)**	
	SGA	**Not SGA**
Thin cord (< p10)	11	82
Not a thin cord (≥ p10)	181	1553
Total	192	1636
	LGA	**Not LGA**
Thick cord (> p90)	16	43
Not a thick cord (≤ p90)	381	1387
Total	397	1431

Performance	**SGA**		**LGA**	
	%	95% CI	%	95% CI
Sensitivity	5.7	2.9 - 10.0	4.0	2.3 - 6.5
Specificity	94.9	93.7 - 95.9	97.0	96.0 - 97.8
Positive predictive value	11.7	6.0 - 20.0	27.1	16.4 - 40.3
Negative predictive value	89.6	88.0 - 91.0	78.5	76.5 - 80.4

95% CI: 95% confidence interval.

5. DISCUSSÃO

Durante muitos anos, pouco se sabia a respeito da morfologia e das funções do cordão umbilical durante a gestação. Ainda nos dias atuais, o estudo ultra-sonográfico para avaliação fetal verifica apenas o número de vasos (duas artérias e uma veia) e as medidas dos índices Dopplervelocimétricos no cordão umbilical. Estruturalmente o cordão umbilical é formado por duas artérias e uma veia, circundadas pela geléia de Wharton, a qual é composta principalmente por ácido hialurônico e proteoglicanos em uma solução aquosa de sais, metabólitos e proteínas de plasma (Klein & Meyer, 1983). A geléia de Wharton é o maior componente do cordão umbilical no segundo e terceiro trimestres da gestação.

A observação de que a presença de cordões finos ou com pouca quantidade de geléia de Wharton estava relacionada a efeitos adversos perinatais, ou ainda presença de oligoâmnio e fetos com baixo peso ao nascimento, foi inicialmente descrita na literatura através do relato de casos (Raio et al., 1999). Achados ultra-sonográficos do cordão umbilical alterados podem estar associados a anomalias fetais e cromossômicas, a restrição de crescimento fetal intra-uterino ou outras condições patológicas relacionadas com aumento da morbidade e

mortalidade fetal e neonatal. A detecção precoce destas mudanças pode ser importante para o prognóstico materno e fetal.

A identificação precoce de parâmetros que possam apresentar alterações em gestações consideradas inicialmente de baixo risco, e mesmo as de alto risco, pode ser útil na prevenção ou na observação mais cuidadosa desses casos. Uma vez estabelecida uma curva com valores que servem como parâmetro para a avaliação do cordão umbilical e de seus vasos, quaisquer diferenças encontradas podem refletir as alterações de estado fetal, pois a espessura do cordão umbilical tem participação direta e indireta no metabolismo fetal.

Com o progresso e a melhora na resolução de imagem dos aparelhos de ultra-som, o cordão umbilical passou a ser melhor estudado. Sua identificação tem início no primeiro trimestre da gestação (Dudiak et al., 1995). Com a suspeita de que o cordão umbilical pode ser mais um parâmetro na identificação precoce de fetos em situações de risco, como na restrição de crescimento intra-uterino, desenvolvimento de pré-eclâmpsia ou diabetes mellitus, alguns autores passaram a medir o diâmetro e a área do cordão umbilical. A partir de 1994 foi construída uma curva dos diâmetros do cordão umbilical e de seus vasos e da área de superfície da geléia de Wharton em gestações de baixo risco, entre 14 e 42 semanas e da área de superfície da geléia de Wharton, entre 8 e 42 semanas (Weissman et al., 1994).

No presente estudo, pela maior dificuldade em se obter imagens da área de secção transversa do cordão nas gestações mais precoces, entre 12 e 14 semanas, essas medidas foram realizadas em um corte longitudinal, enquanto as demais foram avaliadas em um corte transversal. Isso poderia talvez representar uma limitação do estudo, e conseqüentemente de seus resultados, pela diferença técnica na avaliação em idades gestacionais distintas.

Outro intervalo de referência foi construído em 1999 por Raio et al., desta vez com as medidas da área da secção transversa do cordão umbilical em gestações de baixo risco entre 10 e 42 semanas. Neste caso, foram observadas a correlação entre o diâmetro e a área da secção transversa do cordão umbilical e os parâmetros antropométricos fetais e que o aumento progressivo dessas medidas ocorre até 32 semanas de gestação, seguidas por uma redução do tamanho do cordão posteriormente (Raio et al., 1999). Segundo esses autores, a área da secção transversa do cordão é um método mais confiável, pois a medida do diâmetro do cordão sofre influência da quantidade de geléia de Wharton. Além disso, a forma da secção transversa do cordão umbilical não é totalmente circular, podendo levar a uma pequena subestimativa dos valores.

No presente estudo a análise da curva da área da secção transversa do cordão umbilical mostrou valores crescentes até 32 semanas de gestação, seguida de um platô de estabilização, de acordo com o estudo acima descrito. Em 2005, Predanic et al. publicaram um estudo retrospectivo com 650 gestantes,

correlacionando a medida do diâmetro do cordão umbilical entre 18 e 23 semanas com o peso fetal estimado e a idade gestacional (Predanic et al., 2005). A curva obtida foi comparada com a descrita por Raio et al., 1999, não havendo diferença entre elas. Já em comparação com a curva descrita por Weissman et al., em 1994, foi demonstrada uma diferença significativa entre elas, o que pode ser explicado pelas diferentes técnicas utilizadas para a medida dos vasos e do cordão umbilicais, uma vez que uma utilizou o diâmetro aferido até 0,5 cm da inserção do cordão umbilical no abdome fetal, enquanto o outro mediu a área até 2,0 cm da inserção.

Recentemente, Togni et al. também estabeleceram intervalos de referência utilizando as áreas de secção transversa do cordão umbilical e seus vasos e da quantidade de geléia de Wharton, correlacionando-os com parâmetros antropométricos fetais, em gestações de baixo risco, entre 24 e 39 semanas, demonstrando que a área da geléia de Wharton apresenta correlação estatisticamente significativa com a idade gestacional. O aumento da quantidade de geléia de Wharton, neste caso, ocorreu até 21 semanas de gestação, seguida de um platô ao redor de 35 semanas, com valores decrescentes a partir de 36 semanas (Togni et al., 2007).

Em relação à curva de crescimento das áreas das artérias e veia umbilicais, nota-se um aumento da área da veia umbilical até 34 semanas e da artéria até 36 semanas, com estabilização destes valores com 38 semanas de gestação (Weissman et al., 1994; Togni et al., 2007).

Conforme descrito anteriormente, é possível encontrar na literatura mundial vários intervalos de referência para os diâmetros e áreas do cordão umbilical e de seus componentes, em diferentes intervalos de análise da idade gestacional e com diferentes técnicas para a realização dessas medidas. Por esses motivos, este estudo se propôs à descrição de um intervalo de referência para tais parâmetros, abrangendo as gestações de baixo risco, entre 12 e 40 semanas e seguindo as técnicas mais confiáveis.

Este estudo mostrou também que há uma relação direta entre a idade gestacional e a quantidade de GW existente no cordão umbilical. Existe um aumento da área da GW em função da idade gestacional até cerca de 32 semanas, a partir de quando essas medidas permanecem praticamente estáveis até o final da gestação. Mostrou ainda que existe uma relação positiva e linear entre o peso fetal estimado e a área da GW, mas apenas até a 26ª semana de idade gestacional, visto que a partir de então a área da GW quase não varia em função do PFE. Se a área do cordão umbilical atinge seu patamar máximo em torno de 32 semanas ou 31 semanas, espera-se que a área da GW tenha um comportamento semelhante.

Nesse sentido, os resultados do presente estudo assemelham-se muito mais aos resultados apresentados por Ghezzi et al. e por Togni et al., embora com valores um pouco mais elevados. Estes últimos autores estudaram 312 gestantes entre 24 e 39 semanas e descreveram intervalos de referência das

áreas da secção transversa do cordão umbilical e de seus componentes, além da área da GW, com um aumento neste caso também em torno de 32 semanas, seguida de um platô ao redor de 35 semanas, com valores decrescentes a partir de 36 semanas.

Relativamente à correlação entre a área da Geléia de Wharton e parâmetros antropométricos (que são utilizados para a estimativa do peso fetal), de forma geral ela é baixa, como no estudo de Togni et al. que, por exemplo, encontrou uma correlação de apenas 0.240 entre a área da Geléia de Wharton e o peso fetal estimado. Entretanto, desde 2001 Ghezzi et al. já suspeitavam de que essa baixa correlação poderia ser a sobreposição de duas situações distintas em função da idade gestacional, ou seja, uma correlação boa para idades gestacionais mais precoces e uma correlação ruim para idades gestacionais maiores. Foi exatamente isso o que os resultados do presente estudo também demonstraram.

Assim, pode-se observar que desde 1994 muitos autores têm-se proposto a descrever intervalos de referência para a área do cordão umbilical e de seus componentes, além da avaliação da quantidade de GW no cordão umbilical. Possivelmente como vantagem, o presente estudo apresenta a maior casuística descrita até agora e os resultados obtidos encontram-se de acordo com os valores previamente obtidos. Tais parâmetros devem servir como referência, principalmente nos casos em que há suspeita de doenças que possam interferir com o desenvolvimento fetal, tais como diabetes mellitus, hipertensão arterial,

pré-eclâmpsia, restrição de crescimento intra-uterino, onde possa haver alterações da morfologia e da função do cordão umbilical e da quantidade de GW. Entretanto, a confirmação dessa utilidade só poderá ocorrer quando a devida validação destas curvas tiver sido realizada, o que representa um desafio de pesquisa para um futuro próximo.

A avaliação da utilização de diferentes pesquisadores para a obtenção das medidas da área da secção transversa do cordão umbilical e dos diâmetros de seus vasos internos tem como objetivo determinar a precisão do método para ser usado no rastreamento precoce de alterações que possam trazer prejuízos ao feto ou à gestação. Confiabilidade, reprodutibilidade e precisão são termos usados para descrever a extensão em que as medidas de um fenômeno estável, repetidas por pessoas e instrumentos diferentes, em momentos ou lugares diferentes, alcançam resultados semelhantes. Essa avaliação é fundamental para se ter a segurança de que uma medida possa ter algum valor preditivo.

O presente estudo mostrou, na comparação entre as medidas realizadas pelos diferentes examinadores, uma discreta tendência de superestimação do diâmetro da veia umbilical, do cordão umbilical e de sua área, e uma subestimação do diâmetro da artéria umbilical, embora não significativas. A escolha individual de cada examinador pelo local do cordão para a realização das medidas, ainda que respeitando a padronização da distância de até 2,0cm da inserção do cordão umbilical no abdome fetal e a presença de espirais ao

longo do cordão, poderia explicar em parte essas diferenças. Deve-se lembrar ainda que o cordão umbilical pode apresentar até 40 espirais à medida que seu comprimento aumenta com a idade gestacional. Se os examinadores escolhem aleatoriamente o melhor corte transversal para a realização de suas medidas, respeitando a distância padrão à inserção umbilical, em diferentes regiões do espiralamento, pequenas variações das medidas seriam até esperadas.

No caso da medida das artérias umbilicais, apenas uma teve seu diâmetro avaliado, cada examinador escolhendo aquela com melhor visualização de seus contornos. Geralmente as artérias umbilicais apresentam diâmetros de sua luz semelhantes, entretanto sabe-se que uma das artérias umbilicais pode se apresentar menor que a outra entre 0,7 e 1,4 % dos casos. Relata-se ainda uma discordância em torno de 1 a 3mm entre seus diâmetros, levando a diferenças nos parâmetros de fluxo sanguíneo e a uma maior resistência no vaso de menor calibre. Isso poderia também contribuir para as diferenças encontradas nessa medida.

Por outro lado, na avaliação das diferenças obtidas nas medidas dos vasos do cordão realizadas pelo mesmo examinador, observou-se uma tendência à superestimação da veia umbilical e subestimação dos diâmetros das artérias umbilical, do cordão umbilical e da área do cordão. Mais uma vez, essas pequenas variações não foram significativas. O coeficiente de correlação de Spearman mostrou uma boa correlação entre as medidas dos distintos examinadores para todos os parâmetros estudados, tanto na variabilidade inter

como na intra-observador, permitindo supor que estas medidas possam ser feitas com segurança por diferentes examinadores, em diferentes épocas e locais.

Muitos destes achados não estão isolados, por isso a avaliação cuidadosa do cordão umbilical nas diferentes fases da gestação pode começar a fazer parte da rotina obstétrica, através das medidas de seus vasos e do próprio cordão umbilical, não estando restrita apenas à detecção do número de vasos umbilicais, presença de cistos ou avaliação do fluxo sanguíneo pelo estudo Doppler, como na atualidade. Isso deverá permitir uma evolução qualitativa na atenção perinatal ainda durante a gestação, identificando os casos com uma maior probabilidade de apresentarem complicações maternas e feto/neonatais, para que possam ter uma melhor vigilância e talvez medidas profiláticas ou terapêuticas instituídas mais precocemente. Se os estudos futuros permitirem concluir pela capacidade preditiva destas medidas alteradas para as diversas condições patológicas associadas, o presente estudo deverá ter contribuído em mostrar que estas medidas são tecnicamente reprodutíveis.

6. CONCLUSÕES

- A variabilidade inter- e intra-observador das medidas do cordão umbilical e de seus vasos apresentou correlação, reprodutibilidade e confiabilidade elevadas.
- Foram construídos intervalos de referência da área da secção transversa do cordão umbilical, do seu diâmetro e dos vasos umbilicais em gestações de baixo risco entre 12 e 40 semanas, os quais apresentaram um aumento progressivo até 32 semanas, com estabilização posterior dos valores.
- Foi construído um intervalo de referência para a área da Geléia de Wharton, o qual mostrou também um aumento em função da idade gestacional, com tendência a estabilização a partir de 32 semanas. A área da Geléia de Wharton esteve linearmente correlacionada com o PFE apenas até 26 semanas de idade gestacional. A partir de então, a correlação entre essas medidas é praticamente nula.
- Embora exista uma correlação entre o diâmetro e a área transversal do cordão umbilical e também da área da Geléia de Wharton com o PFE, estas medidas não se mostraram úteis para predizer alterações do PFE e, portanto, não deverão ser utilizadas para rastreamento com esta finalidade.

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9. ANEXOS

9.1. Anexo 1 – Carta de aprovação do projeto pelo CEP

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA
Caixa Postal 6111, 13083-970 Campinas, SP
(0_19) 3788-8936
FAX (0_19) 3788-7187
www.fcm.unicamp.br/pesquisa/etica/index.html
cep@fcm.unicamp.br

CEP, 28/06/05.
(Grupo III)

PARECER PROJETO: N° 268/2005
CAAE: 0088.0.146.000-05

I-IDENTIFICAÇÃO:

PROJETO: **"CURVA DE NORMALIDADE DA ESPESSURA DO CORDÃO UMBILICAL E DIFERENTES IDADES GESTACIONAIS"**
PESQUISADOR RESPONSÁVEL: Cristiane Barbieri
INSTITUIÇÃO: CAISM/UNICAMP
APRESENTAÇÃO AO CEP: 08/06/2005
APRESENTAR RELATÓRIO EM: 28/06/06

II - OBJETIVOS

Avaliar a espessura do cordão umbilical, dos seus vasos e da sua área entre 12 e 40 semanas, em gestantes de baixo risco do setor de Ultra-sonografia do CAISM (UNICAMP) e da clínica privada de diagnóstico ultra-sonográfico CDE.; construir uma curva de valores normais do diâmero do cordão umbilical e de seus vasos, da superficie da área e comparar a variação do diâmetro com raça, idade e paridade.

III - SUMÁRIO

Estudo prospectivo para avaliação do diâmetro do cordão umbilical intrauterino, por ultra-sonografia em qualquer idade gestacional acima de 12 semanas. Serão avaliadas 97 gestanes para cada idade gestacional entre 12 e 40 semanas de gestação do CAISM e da Clínica CDE em exames de rotina ou pré-natal de baixo risco. Critérios de inclusão e exclusão bem definidos,assim como metodologia.

IV - COMENTÁRIOS DOS RELATORES

Estudo submetido à FAPESP, com orçamento de R$10.450,00. Termo de Consentimento simples e objetivo, faltando apenas acrescentar o telefone do CEP.

RECOMENDAÇÃO: Trabalho interessante, sem riscos para os pacientes. Solicito acrescentar o telefone do CEP no Termo de Consentimento e enviar ao CEP.

V - PARECER DO CEP

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP, após acatar os pareceres dos membros-relatores previamente designados para o presente caso e atendendo todos os dispositivos das Resoluções 196/96 e complementares, bem como ter aprovado o Termo do Consentimento Livre e Esclarecido, assim como todos os anexos incluídos na Pesquisa, resolve aprovar sem restrições o Protocolo de Pesquisa supracitado.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

VI - INFORMAÇÕES COMPLEMENTARES

O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (Res. CNS 196/96 – Item IV.1.f) e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (Item IV.2.d).

Pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou (Res. CNS Item III.1.z), exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade do regime oferecido a um dos grupos de pesquisa (Item V.3.).

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS Item V.4.). É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projeto do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial (Res. 251/97, Item III.2.e)

Relatórios parciais e final devem ser apresentados ao CEP, de acordo com os prazos estabelecidos na Resolução CNS-MS 196/96.

VII - DATA DA REUNIÃO

Homologado na VI Reunião Ordinária do CEP/FCM, em 28 de junho de 2005

Profa. Dra. Carmen Silvia Bertuzzo
PRESIDENTE DO COMITÊ DE ÉTICA EM PESQUISA
FCM / UNICAMP

9.2. Anexo 2 – Termo de consentimento livre e esclarecido

Declaro estar de acordo em participar deste trabalho de pesquisa intitulado **"Curva de normalidade da espessura do cordão umbilical em diferentes idades gestacionais"** desenvolvido na Seção Técnica de Ultra-Sonografia do CAISM. Este estudo consiste na realização de um exame de ultra-sonografia obstétrica pela Dra. Cristiane Barbieri e/ou outro membro da equipe desta Seção, com a finalidade de medir o diâmetro do cordão umbilical do meu bebê.

Fui informada que este tipo de exame não causa danos à minha saúde e nem à do bebê. Sei que minha recusa em participar, se for o caso, em nada interferirá com o meu atendimento e tratamento nesta Instituição.

Minha participação é voluntária, tendo portanto, liberdade de desistir a qualquer momento.

Também fui informada que minha identidade neste estudo manter-se-á em sigilo.

Nome_____

RG_____

HC_____

Telefone_____

Data_____

Assinatura_____

Pesquisador : Dra. Cristiane Barbieri

Seção Técnica de Ultra-sonografia do CAISM

Telefone para Esclarecimentos- (0XX19) 3788.9346

9.3. Anexo 3 – Ficha para a coleta de dados

Nome_____

HC ou identificação □□□□□□-□

Idade □□ Raça □ 1.branca 2. negra 3.parda 4. amarela 5. outras

Paridade G □□ P □□ A □ FV□

IG (DUM) □□ semanas □□ dias

IG (ECO) □□ semanas □□ dias

Diâmetro do cordão umbilical □□,□ mm

Diâmetro da artéria □□,□ mm

Diâmetro da veia □□,□ mm

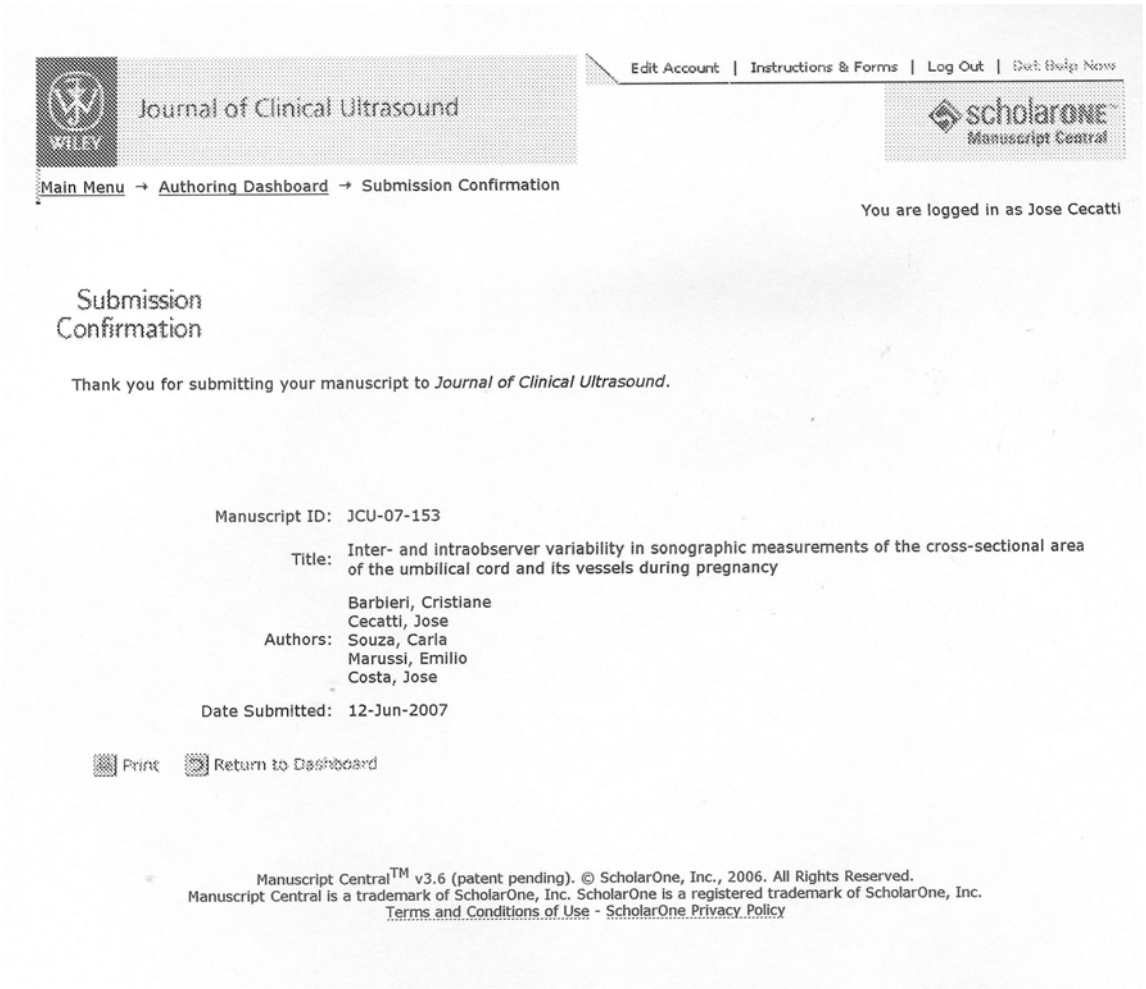
Área do cordão □□,□ mm^2

Data do Exame □□ /□□ /□□

PFE □□□□g

Telefone para contato (_ _) _ _ _ _ _ _ _ _

9.4. Anexo 4 - Carta de recebimento do artigo pela *Journal of Clinical Ultrasound*



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Authors: Barbieri, Cristiane
Cecatti, Jose
Souza, Carla
Marussi, Emilio
Costa, Jose

Date Submitted: 12-Jun-2007

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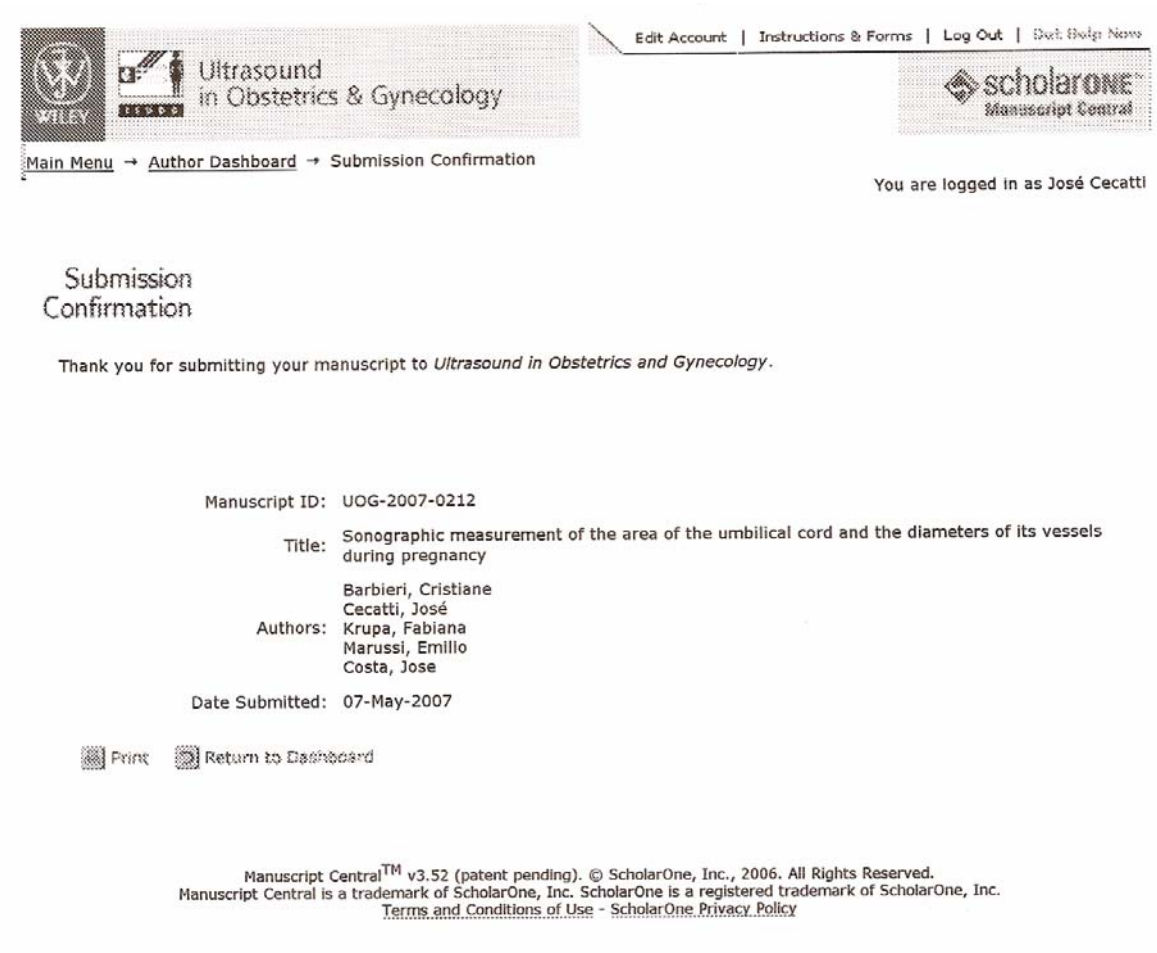
9.5. Anexo 5 – Carta de recebimento do artigo pela *Ultrasound in Obstetrics and Gynecology*



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Manuscript ID:	UOG-2007-0212
Title:	Sonographic measurement of the area of the umbilical cord and the diameters of its vessels during pregnancy
Authors:	Barbieri, Cristiane Cecatti, José Krupa, Fabiana Marussi, Emilio Costa, Jose
Date Submitted:	07-May-2007

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9.6. Anexo 6 – Carta de recebimento do artigo pelo *Acta Obstetricia et Gynecologica Scandinavica*

Acta
Obstetricia et Gynecologica Scandinavica
Founded in 1921

Dr Jose Guilherme Cecatti
P O Box 6030
13083-881 Campinas-SP
Brazil

Chief Editor:
Professor PER OLOF JANSON, MD, PhD
Dept of Obst and Gyn
Sahlgrenska University Hospital/SS
SE 413 45 GOTHENBURG
Sweden
Tel.:+46 31 342 21 41
Fax:+46 31 82 46 57
E-mail: per-olof.janson@obgyn.gu.se

Gothenburg, June 25th, 2007

Dear Dr Cecatti,

Re: Acta Obstetricia et Gynecologica Scandinavica
Our ref: AOGS7O266

The editors of the above journal extend their thanks for the submitted manuscript, which has been received for consideration:

> Area of Wharton's jelly as an estimate of the thickness of the umbilical cord and its relationship with estimated fetal weight

Please allow a couple of months for the refereeing process.

For future reference, please quote: AOGS7O266

Yours sincerely

Per Olof Janson MD PhD
Professor Chief Editor

/Eva Christina Sterner
Editorial Assistant

Publisher
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Editorial Office:
Dept of Obst and Gyn

Tel.:+46 31 342 10 00
Fax:+46 31 82 46 57

9.7 Anexo 7 – Carta de recebimento do artigo pela *Acta Obstetricia et Gynecologica Scandinavica.*

Acta
Obstetricia et Gynecologica Scandinavica
Founded in 1921

Dr Jose Guilherme Cecatti
P O Box 6081
13083-881 Campinas SP
Brazil

Chief Editor:
Professor PER OLOF JANSON, MD, PhD
Dept of Obst and Gyn
Sahlgrenska University Hospital/SS
SE 413 45 GOTHENBURG
Sweden
Tel.:+46 31 342 21 41
Fax:+46 31 82 46 57
E-mail: per-olof.janson@obgyn.gu.se

Gothenburg, June 25th, 2007

Dear Dr Cecatti,

Re: Acta Obstetricia et Gynecologica Scandinavica
Our ref: AOGS7O267

The editors of the above journal extend their thanks for the submitted manuscript, which has been received for consideration:

> Validation study of the capacity of the reference curves of ultrasonographic measurements of the umbilical cord to identify deviations in estimated fetal weight

Please allow a couple of months for the refereeing process.

For future reference, please quote: AOGS7O267

Yours sincerely

Per Olof Janson MD PhD
Professor Chief Editor

/Eva Christina Sterner
Editorial Assistant

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Tel.:+46 31 342 10 00
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