MARCOS ROBERTO CAETANO

FATORES DE PROGNÓSTICO GESTACIONAL EM MULHERES COM ABORTO ESPONTÂNEO RECORRENTE

Dissertação de Mestrado

ORIENTADOR: Prof. Dr. RICARDO BARINI

UNICAMP
2004

MARCOS ROBERTO CAETANO

FATORES DE PROGNÓSTICO GESTACIONAL EM MULHERES COM ABORTO ESPONTÂNEO RECORRENTE

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

ORIENTADOR: Prof. Dr. RICARDO BARINI

UNICAMP
2004

**FICHA CATALOGRÁFICA ELABORADA PELA
BIBLIOTECA DA FACULDADE DE CIÊNCIAS MÉDICAS
UNICAMP**

C116f

Caetano, Marcos Roberto
Fatores de prognóstico gestacional em mulheres com aborto espontâneo recorrente / Marcos Roberto Caetano. Campinas, SP : [s.n.], 2004.

Orientador : Ricardo Barini
Dissertação (Mestrado) Universidade Estadual de Campinas. Faculdade de Ciências Médicas.

1. Aborto habitual. 2. Fatores de risco. 3. Imunologia. I. Ricardo Barini. II. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. III. Título.

BANCA EXAMINADORA DA DISSERTAÇÃO DE MESTRADO

Aluno: MARCOS ROBERTO CAETANO

Orientador: Prof. Dr. RICARDO BARINI

Membros:

1.

2.

3.

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

Data: 04/08/2004

Dedico este trabalho a

Terezinha das Graças Ferreira, ***minha mãe***.

Agradecimentos

Prof. Dr. Ricardo Barini, pela orientação no trabalho
Profa Dra Egle Cristina Couto de Carvalho, pela dedicação
Prof. Dr. José Guilherme Cecatti
Prof. Dr. João Luiz Pinto e Silva
Prof. Dr. Belmiro Gonçalves Pereira
Profa. Dra. Helaine Maria Besteti Mayer Pires Milanez
Profa. Dra. Eliana Martorano do Amaral Freitas da Silva
Profa. Dra. Mary Ângela Parpinelli
Prof. Dr. Marco Fabio Prata Lima
Prof. Dr. João Ulisses Ribeiro
Prof. Dr. Eddie Murta
Prof. Paulo José Maluf
José Pio Caetano
Caroline Soares Ferreira de Sousa
Conceição Aparecida Silva Santos
Fabiana Salioni Rossato
Klesio Divino Palhares
Luzia Gonçalves de Aguiar
William Alexandre Oliveira
Sueli Chaves
Gislaine Aparecida Fonsechi Carvasan
Sirlei Siani Morais
Margarete Amado de Souza Donadon
Andréia Bognar Freitas
Leonice Saraiva Venezian
Walquiria Mascaro
Zoraide Fátima Pereira Gregório
Funcionários do SAME e Ambulatório de Esterilidade/UNICAMP

Sumário

Símbolos, Siglas e Abreviaturas

ACL	Anticorpo anticardiolipina
AL	Anticoagulante lúpico
CAISM	Centro de Atenção Integral à Saúde da Mulher
DNA	Ácido desoxirribonucléico
DRVVT	*Dilute Russell Viper Venom Time*
ELISA	*Enzyme Linked Immunosorbent Assay*
EPI INFO	*Epidemiological Information*
ERA	Aborto espontâneo recorrente
Et al.	E outros (as)
HIV	Vírus da imunodeficiência humana
IC	Intervalo de confiança
IgG	Imunoglobulina G
IgM	Imunoglobulina M
IP	Índice de pulsatilidade
KCT	*Kaolin Clotting Time*
LA	*Lupus* anticoagulante
Mg	Miligrama(s)
Ml	Mililitro(s)

NK	*Natural Killer*
NPH	*Neutral Protamine Hagedorn*
OR	*Odds ratio*
PC	Prova cruzada
PTU	*Propiltiuracil*
SIDA	Síndrome da imunodeficiência adquirida
T4L	Tiroxina livre
TP	Tempo de protrombina
TSH	Hormônio tiroestimulante
TTPA	Tempo de tromboplastina parcialmente ativado
Unicamp	Universidade Estadual de Campinas
%	Percentual

Resumo

O aborto espontâneo é uma ocorrência freqüente na vida reprodutiva dos casais. Quando ocorre por três ou mais vezes subseqüentes é chamado aborto espontâneo recorrente, sendo relacionado com alterações genéticas, anatômicas, hormonais, infecciosas, imunológicas e outras. Os fatores imunológicos vêm sendo amplamente estudados, com bons resultados gestacionais após o tratamento. Contudo, mesmo com avanços no diagnóstico, muitos casos de aborto espontâneo recorrente continuam como de causa desconhecida. Novos fatores ou associações podem influenciar o resultado gestacional. O objetivo deste estudo foi identificar as possíveis causas do aborto espontâneo recorrente, isoladas ou associadas, que poderiam predizer o prognóstico gestacional em mulheres submetidas a um protocolo de investigação e tratamento. Para isso, foi realizado um estudo de caso-controle aninhado em uma coorte histórica, através da revisão de 246 prontuários médicos de mulheres com três ou mais perdas espontâneas sucessivas atendidas no Ambulatório de Perdas Gestacionais do Centro de Atenção Integral à Saúde da Mulher da Universidade Estadual de Campinas entre março de 1994 e julho de 2003. Todas foram submetidas a um protocolo de investigação e

tratamento. Foram avaliados dados relativos à idade, antecedentes obstétricos, possíveis etiologias para a recorrência do aborto, tratamentos realizados e resultados gestacionais. A análise estatística envolveu o*dds ratio* e análise por Regressão Logística. Das 246 mulheres incluídas no estudo, 17 não possuíam registros de resultados neonatais e foram excluídas, perfazendo o total de 229 mulheres estudadas. A maior parte delas (86%) tinha entre 25 e 39 anos. Após a aplicação do protocolo, e de acordo com o resultado da gestação, as mulheres foram divididas em dois grupos: 170 evoluíram para parto (Grupo 1) e 59 evoluíram para aborto (Grupo 2). O fator imunológico, principalmente o aloimune, foi o mais freqüentemente encontrado (93,9%). Mulheres com fator aloimune isolado obtiveram melhores resultados gestacionais do que aquelas com a associação de outros fatores. Não foi encontrada associação entre o número de abortos prévios ao tratamento e o resultado gestacional. Mulheres com 40 anos ou mais apresentaram a mais alta taxa de aborto espontâneo e o pior prognóstico gestacional (OR 5.83 95% CI 1.12-30.40). Concluímos que a idade acima de 40 anos, a presença de fatores imunológicos e a associação de dois ou mais fatores conferiram o pior prognóstico gestacional às mulheres avaliadas.

Summary

Spontaneous abortion is a common occurrence during reproductive life. It is called recurrent spontaneous abortion (RSA) when occurs three or more consecutive times and is associated with several etiologic factors, such as genetic, anatomic, hormonal, infectious and immunologic ones. Immunologic factors have been largely investigated. When treated, good gestational results have been obtained. Despite advances in diagnosis, several cases of RSA remain unclear. New factors or their associations can influence gestational results. The objective of this study was to identify possible single or associated causes of RSA, which could predict gestational prognosis for investigated and treated women. A nested case-control study included the evaluation of 246 medical records of women with RSA from Recurrent Abortion Outpatient Clinic at CAISM/ UNICAMP, from March 1994 to July 2003. All of them were submitted to an investigation and treatment protocol. Data concerning age, obstetrical history, possible etiology for recurrent abortion, treatments and pregnancy outcomes were evaluated. Statistical analysis was performed using odds ratio and logistic regression analysis. From 246 women who were initially included in the study,

17 had no records of gestational outcomes and were excluded, performing 229 studied women. Most of them (86%) were 25 to 39 years old. After application of the protocol and according to pregnancy results, studied women were divided in two groups: 170 delivered (Group 1) and 59 aborted (Group 2). The most frequently found etiologic factors were immunologic, mainly the alloimmune (93,9%). Women with single alloimmune factor had better gestational results that with other associated factors. No association was found between the number of abortions prior to treatment and pregnancy results. Women aged 40 or older presented the highest rate of spontaneous abortion and worst gestational prognosis (OR 5.83 95% CI 1.12-30.40). We conclude that age, immunologic factors and two or more concomitant factors were associated with poor gestational outcomes in studied women.

1. Introdução

O aborto espontâneo é a complicação mais comum da gravidez. Ocorre em 10% a 15% das gestações clinicamente detectadas (UNITED NATIONS, 1954). A perda espontânea e sucessiva de três ou mais gestações até 20 semanas é chamada de aborto espontâneo recorrente (AER), situação que ocorre em 1% a 2% das mulheres em idade reprodutiva (COULAM, 1986; REGAN *et al*., 1998). Estimativas mostram que 2% a 5% dos casais americanos sem filhos passaram pela experiência de AER e referem-na como um grande desgaste emocional em suas vidas (COULAM, 1992).

No passado era possível detectar a causa do AER na minoria dos casais. Ao longo dos anos procurou-se estudar as possíveis causas, em busca de alternativas que pudessem produzir melhores resultados gestacionais. Em estudo de THO *et al.* (1979) foi identificada a possível etiologia em 63% dos casais que tinham antecedente de dois ou mais abortos. Com a evolução da ciência, entretanto, não foram encontradas porcentagens superiores a esta que

explicassem a ocorrência do AER. Em praticamente metade dos casos a etiologia permanecia desconhecida (COSTA *et al.*, 1993).

Inicialmente, a maioria das séries dirigia-se para a investigação genética do casal e do produto do aborto. O fator genético sempre foi bastante relacionado com a ocorrência do aborto e sua repetição posterior. As anomalias cromossômicas fetais foram responsabilizadas por 50% a 60% dos abortos espontâneos de primeiro trimestre (BOUÉ *et al.,* 1975). Destas anomalias, as numéricas foram as mais freqüentes: 50% a 60% eram trissomias, 20% a 25% poliploidias e 15% a 25% monossomias do cromossomo X. A ocorrência de trissomia no cariótipo fetal aumentaria a chance de sua repetição na próxima gestação. Para GLASS e GOLBUS (1978), a identificação do cariótipo fetal foi importante, pois quando anormal no produto do primeiro aborto indicaria chance de 80% de ocorrer cromossomopatia na próxima gestação, enquanto o resultado normal no primeiro aborto reduziria a chance de cromossomopatia para 50% na gravidez subseqüente. Os autores sugeriram também a realização de amniocentese para diagnóstico pré-natal nas gestações de mulheres com cariótipo anormal em gestação passada.

Além das alterações numéricas, destacam-se as estruturais, como translocação e mosaicismo. A translocação é a quebra de um fragmento de um cromossomo e sua adição a outro cromossomo. A translocação balanceada foi identificada em 7,2% dos casos por BOUÉ (1998). A presença de mosaicismo no cariótipo materno ou paterno responderia também por uma parcela de abortos recorrente, comparável àquela por translocações balanceadas (SACHS *et al.,* 1985). Quatro a oito por cento dos casais com AER têm anomalias

cromossômicas (HARGER *et al.*, 1983). Entretanto, há variantes normais na população geral que não implicam etiologia para AER.

Alguns estudos foram dirigidos para a avaliação da qualidade do sêmen nos casais. A inativação do cromossomo X foi encontrada em 14% das mulheres com AER de causa desconhecida (ROBINSON *et al.*, 2001). HILL *et al.* (1994), estudando casais com AER, não notaram grandes anormalidades na morfologia ou alterações em outros parâmetros qualitativos do sêmen. Relataram aumento de linfócitos CD4 e CD8 no sêmen dos maridos dessas mulheres. A porcentagem encontrada de aneuploidias não mostrou associação com os parâmetros de qualidade do sêmen, mas com o aumento da fragmentação do DNA no esperma. Cogita-se, como explicação para os abortos, que esta alteração produziria embrião de má qualidade e, conseqüentemente, defeito na modulação da implantação (CARRELL *et al.*, 2003).

Os defeitos anatômicos do útero foram relacionados ao AER em 15% a 27% dos casos (HARGER *et al.*, 1983; STRAY-PETERSEN e STRAY-PETERSEN, 1984), utilizando-se diferentes técnicas e critérios de diagnóstico. Incluem-se nesse grupo os úteros bicorno, unicorno e didelfo (BUTTRAM e GIBBONS, 1979; HOMER *et al.,* 2000), as sinéquias uterinas (COSTA, 1994), os miomas que fazem saliência para a cavidade uterina e a insuficiência cervical (KURUP e GOLDKRAND, 1999). De 509 mulheres com AER investigadas ecograficamente, foram encontradas alterações uterinas em 23,8%. A distorção da anatomia uterina foi a mais severa anomalia congênita encontrada (SALIM *et al.*, 2003). Na insuficiência cervical, uma das alterações anatômicas mais estudadas nos

casos de AER, sugere-se a circlagem cervical como método preventivo à recorrência dos abortos (CECATTI *et al.,* 1989; SURICO *et al.*, 2000).

As perdas gestacionais precoces foram também associadas à insuficiência de corpo lúteo (JONES, 1949; CSAPO *et al.,* 1973). O mecanismo sugerido foi a baixa produção de progesterona, levando à maturação endometrial insuficiente para suportar a nidação e o desenvolvimento do ovo (BOTELLA, 1962). A prevalência dos defeitos da fase lútea nos casos de AER varia de 5,1% a 60% (THO *et al.,* 1979; DUDLEY e BRANCH, 1989). O diagnóstico é sugerido através da realização de biópsia endometrial dois a três dias antes da menstruação. Contudo, nem sempre este método é preciso; por isso, muitos autores, em casos de dois ou mais abortos, têm usado rotineiramente a administração de progesterona. As ações miorrelaxante e imunossupressora deste hormônio têm sido relatadas como adjuvantes na manutenção da gestação inicial (REIN, 1991).

Além da deficiência de progesterona, outras alterações hormonais foram observadas em mulheres com AER. O *diabetes mellitus* foi citado como responsável por abortos (SUTHERLAND e PRITCHARD, 1986). Entretanto, MILLS (1983) não encontrou diferença nas taxas de perdas entre mulheres diabéticas insulino-dependentes e mulheres saudáveis.

As endocrinopatias têm sido relacionadas à ocorrência de óbito fetal (PRITCHARD *et al.,* 1985; COSLOVSKY e WAISSMAN, 1987; THOMAS e REID, 1987). Com relação à tireóide, JONES e DELFS (1951) encontraram

63,5% de alterações funcionais em mulheres com AER, e aumento da incidência de aborto em mulheres com hipotireoidismo**.** Recentemente, os estudos concentraram-se na investigação dos anticorpos antitireoidianos. ESPLIN *et al.* (1998) concluíram não existir associação entre estes anticorpos e AER. Entretanto, outros estudos discordam desta conclusão e referem alta freqüência destes anticorpos em mulheres com história de AER. A revisão da literatura mostrou que a maioria das publicações (67%) confirma esta relação (DENDRINOS *et al*., 2000; AMBRAMSON e STAGNRO-GREEN, 2001).

As infecções maternas por *Chlamydia trachomatis, Brucella abortus, Citomegalovirus, Toxoplasma gondii, Herpes simplex, Mycoplasma hominis e Listeria monocytogenes* já foram citadas como responsáveis por aborto. O *Ureaplasma urealyticum* foi relacionado ao AER, mas os resultados da literatura permanecem inconclusivos (STRAY-PETERSEN *et al.,* 1978). Não há evidências que comprovem a importância das infecções citadas na gênese do AER (CARP *et al*., 1990).

Os fatores imunológicos têm sido evidenciados nos estudos envolvendo mulheres com AER nos últimos anos. Nos casos em que a etiologia dos abortos era desconhecida, os fatores auto e/ou aloimunes foram encontrados em mais de 80% (MCINTYRE *et al.,* 1989).

O interesse na etiologia autoimune foi despertado com a descoberta da associação entre anticorpos antifosfolípides e a ocorrência de AER (COWCHOCK *et al.,* 1986). Um estudo tentou resumir a prevalência dos anticorpos antifosfolípides

em diferentes populações: 5,3% em pacientes obstétricas normais, 24% em mulheres submetidas a vários ciclos de fertilização *in vitro*, 37% em mulheres com *lupus* eritematoso sistêmico e 28% em mulheres com AER (KANERIA e VISHWANATHAN, 1999). Outro trabalho tentou categorizar o risco de um novo aborto em 203 casais. Oligomenorréia, idade acima de 30 anos, número de abortos superior a quatro e títulos elevados do anticorpo anticardiolipina foram responsabilizados pela diminuição da taxa de sucesso na gestação subseqüente (QUENBY e FARQUHARSON, 1993).

O anticorpo anticardiolipina (ACL) e/ou o anticoagulante lúpico (AL) foram encontrados em 15% das mulheres com dois abortos anteriores e em 18,5% das mulheres com três ou mais perdas (MACLEAN *et al.*, 1994). Foi observado que 95% das gestantes portadoras de AL e não tratadas evoluíram para aborto espontâneo ou morte fetal. O mecanismo sugerido foi o que, devido à presença do AL, estas mulheres apresentariam um estado de hipercoagulabilidade, levando à trombose placentária com vasculopatia decidual, e conseqüente infarto placentário e perda fetal (SCOTT *et al.,* 1987). Estudos recentes confirmaram a associação entre a presença de anticorpos antifosfolípides e AER. O uso combinado de aspirina e heparina em tais casos mostrou-se eficiente, reduzindo a chance de um novo aborto em até 54%. Outros fatores autoimunes que podem estar associados com aborto espontâneo ainda estão em estudo (EMPSON *et al.*, 2002; KUTTEH, 2002).

O fator aloimune tem sido amplamente estudado, e sua verdadeira atuação na etiologia do AER e formas de tratamento tem gerado polêmica.

Estima-se que esteja presente em 40% a 60% dos casais com AER de causa desconhecida. O termo aloimunidade refere-se a diferenças imunológicas entre indivíduos da mesma espécie. Na histocompatibilidade materno-fetal, o evento inicial é uma anormalidade que impede a mãe de desenvolver respostas imunológicas essenciais para a sobrevivência do concepto geneticamente estranho (OBER *et al.*, 1999).

Em mulheres com AER parece haver um decréscimo no número de células supressoras na decídua e uma atividade aumentada das células *natural killer* (NK), que acabam por agredir o concepto instalado, levando ao aumento na incidência de abortos precoces e, conseqüentemente, ao pior prognóstico reprodutivo (AOKI *et al.*, 1995). A proposta terapêutica de utilizar linfócitos do parceiro ou doador surgiu da observação de pacientes submetidos a transplantes renais que, após várias transfusões sangüíneas, apresentavam menor taxa de rejeição (SOLLINGER *et al.*, 1984). A imunização com linfócitos tem por objetivo suprimir a atividade das células NK, possibilitando o desenvolvimento do concepto (CAUCHI *et al.,* 1991; FRASER *et al.,*1993; KWAK *et al.*, 1998). Mulheres que receberam injeção intradérmica de linfócitos do parceiro apresentaram melhores resultados gestacionais do que as que não receberam esse tratamento (BARINI *et al.*,1998; RAMHORST *et al.*, 2000). MAKINO (2002) estudou 473 mulheres que foram submetidas a um protocolo de investigação para AER, sem detectar qualquer etiologia. Ofereceu aos casais o tratamento com injeção de linfócitos do marido. As que aceitaram utilizar as injeções

tiveram 79,6% de sucesso gestacional, e as que não fizeram qualquer tratamento abortaram em 64,1% dos casos.

A associação entre AER e o antecedente de trombose venosa profunda também foi descrita (HARRIS *et al.*, 1986; TRIPLETT, 1992; ARTHURS *et al.* 1994). A trombofilia, termo aplicado à tendência a desenvolver trombose em idade precoce ou com recorrência freqüente, tem sido relacionada com a ocorrência de aborto de repetição, falhas de implantação nos casos de fertilização *in vitro* e outras complicações durante a gravidez. Tal estado parece interferir no desenvolvimento do sistema vascular útero placentário, tornando-o ineficaz (GLUECK *et al.*, 2000). São descritos como fatores responsáveis por trombofilia hereditária a deficiência das proteínas C e S, da antitrombina III, as mutações fator V de Leiden, G20210A no gene da protrombina e C677T no gene da enzima metileno tetrahidrofolato redutase (CARVALHO, 2001).

Avaliando a vascularização uterina através de dopplerfluxometria, HABARA *et al.* (2002) notaram que as mulheres com AER apresentaram maior índice de pulsatilidade (IP), e que este índice foi inversamente proporcional aos níveis de progesterona. Sugeriram o uso da dopplerfluxometria em mulheres com AER de causa desconhecida para avaliar possível circulação uterina alterada.

Fatores como idade, número prévio de abortos, infertilidade e aspectos emocionais também têm sido relatados como possíveis etiologias para AER. Em estudo com 472 mulheres, a taxa de recorrência de aborto nas que tinham três abortos prévios foi de 47% (POLAND *et al.*, 1977). Na avaliação de

300.000 gestações na população geral, o risco de ocorrência de aborto foi de 11,3%. Este risco aumentou para 15,9%, 25,1%, 45% e 54,3%, respectivamente, para mulheres que tinham antecedente de um, dois, três ou quatro abortos (KNUDSEN *et al.*, 1991). Em outro estudo, a chance de um casal ter um filho vivo diminuiu em torno de 15% quando apresentou três a cinco abortos prévios. Nessa mesma linha, para CLIFFORD *et al.* (1997), o risco de um novo aborto após três consecutivos girou em torno de 29%, aumentando pra 53% quando a história incluía seis ou mais.

Com relação à idade materna, foi encontrado maior percentual de aborto recorrente em faixas etárias consideradas inferiores a 30 ou superiores a 40 anos (COWCHOCK e SMITH, 1992). Analisando essas mesmas faixas etárias, CLIFFORD *et al.* (1997) encontraram taxa de 25% de repetição do aborto nas mulheres com 30 anos ou menos, e de 52% nas mulheres com 40 anos ou mais.

O Ministério da Saúde (BRASIL, 2004) estima pouco mais de 58 milhões de mulheres em idade fértil. Supondo que 1% a 2% destas tenham como complicação o AER clínico, mais de um milhão de casais poderiam estar envolvidos com esta ocorrência. Para melhorar esse contexto são necessários novos estudos.

Muitas são as possíveis etiologias citadas como responsáveis por AER, algumas bem estudadas e definidas, outras ainda a ser exploradas. A etiologia imunológica pode ser encontrada com alta freqüência nas mulheres com AER

de causa desconhecida. O diagnóstico e tratamento específico podem aumentar para até 78% a taxa de bons resultados (MOWBRAY *et al.*, 1985).

Contudo, algumas mulheres tratadas de forma regular, incluindo tanto a aloimunidade quanto a autoimunidade, continuam a apresentar recorrência de aborto. É provável que existam outros fatores complicadores em alguns grupos ou associações de várias causas que contribuam para o insucesso.

Este estudo buscou identificar e corrigir estes fatores, isolados e/ou associados, avaliando o resultado da gestação. Pretendeu-se conhecer as características das mulheres que mais se beneficiariam com a propedêutica de avaliação e tratamento para AER, além das características daquelas com menor chance de sucesso gestacional.

2. Objetivos

2.1. Objetivo geral

Identificar as causas presumíveis de aborto espontâneo recorrente que, isoladas ou associadas, poderiam predizer o prognóstico gestacional.

2.2. Objetivos específicos

- Identificar as causas presumíveis de AER na coorte estudada.
- Comparar o resultado da primeira gravidez após tratamento, em mulheres com diferentes causas presumíveis de AER.
- Identificar, dentre as causas presumíveis de AER, aquelas que, associadas entre si, conferem maior risco de um novo aborto espontâneo.

3. Publicação

Artigo enviado para: São Paulo Medical Journal
Revista – Paulista de Medicina

Gestational prognostic factors in women with recurrent spontaneous abortion

Fatores de prognóstico gestacional em mulheres com aborto espontâneo recorrente

Artigo original

Marcos Roberto Caetano[1]

Egle Couto[2]

Renato Passini Junior[2]

Renata Zaccaria Simoni[3]

Ricardo Barini[2]

Department of Gynecology and Obstetrics– Universidade Estadual de Campinas – Brazil

[1] – Post graduated student – Universidade Estadual de Campinas

[2] – MD, PhD, Universidade Estadual de Campinas

[3] – MD, Universidade Estadual de Campinas

Running title: Prognostic factors in spontaneous abortion.

Correspondence:

Marcos Roberto Caetano

Rua Uruguai, 164, Bairro Fabricio, Uberaba, MG, CEP 13067-160

Phone: +55 19 37889477 / Fax: +55 19 37889304

e-mail: mrcaetano@itelefonica.com.br; egle@unicamp.br

Gestational prognostic factors in women with recurrent spontaneous abortion

Summary

Background: Spontaneous abortion is a common ocurrence during reproductive life. It is called recurrent spontaneous abortion (RSA) when occurs three or more consecutive times and is associated with several etiologic factors, such as genetic, anatomic, hormonal, infectious and immunologic ones. Immunologic factors have been largely investigated. When treated, good gestational results have been obtained. Despite advances in diagnosis, several cases of RSA remain unclear. New factors or their associations can influence gestational results. The objective of this study was to identify possible single or associated causes of RSA, which could predict gestational prognosis for investigated and treated women.

Patients and Methods: A nested case-control study included the evaluation of 246 medical records of women with RSA from Recurrent Abortion Outpatient Clinic at CAISM/ UNICAMP, from March 1994 to July 2003. All of them were submitted to an investigation and treatment protocol. Data concerning to age, obstetrical history, possible etiology for recurrent abortion, treatments and pregnancy outcomes were evaluated. Statistical analysis was performed using odds ratio and logistic regression analysis.

Results: From 246 women who were initially included in the study, 17 had no records of gestational outcomes and were excluded, performing 229 studied women. Most of them (86%) were 25 to 39 years old. After application of the protocol and according to pregnancy results, studied women were divided in two groups: 170 delivered (Group 1) and 59 aborted (Group 2). The most frequently found etiologic factors were immunologic, mainly the alloimmune (93,9%). Women with single alloimmune factor had better gestational results that with

other associated factors. No association was found between the number of abortions prior to treatment and pregnancy results. Women aged 40 or older presented the highest rate of spontaneous abortion and worst gestational prognosis (OR 5.83 95% CI 1.12-30.40).

Conclusion: Age, immunologic factors and two or more concomitant factors were associated with poor gestational outcomes in studied women.

Keywords: recurrent abortion, risk factors, immunology

Background

The mechanisms involving human reproduction are highly complex, especially the role played by immunologic factors. It is known that 10% to 15% of clinically diagnosed pregnancies end in spontaneous abortion (1). Recurrent spontaneous abortion (RSA) is defined as three or more consecutive spontaneous pregnancy losses before the 20^{th} week of gestation, a situation occurring in 1% to 2% of women of reproductive age (2,3).

During many years, the unexpected termination of pregnancy has been a subject of study. Some factors have already been confirmed as possible causes of RSA. Chromosomal abnormalities, for example, are mentioned as being responsible for 50% to 60% of spontaneous abortions in the first trimester (4). Numerical aberrations, translocations and mosaicism are the most frequently found genetic modifications (5). Furthermore, qualitative semen evaluation is currently suggested. These studies have demonstrated a 14% rate of chromosome X inactivation in partners of women with RSA (6).

Uterine anatomic defects, synechiae, myomas that protrude from the uterine cavity and an incompetent cervix predispose a women to reproductive difficulties, such as abortion in the first and second trimesters, premature delivery and anomalous fetal presentation. It has been mentioned that these alterations account for 15% to 27% of RSA cases (5,7).

Changes in hormonal patterns have also been implicated in the cause of recurrent abortion. Low progesterone production, due to insufficient corpus luteum, renders endometrial maturation inadequate to the point that nidation and egg development are no longer sustained (8,9). Other hormonal alterations were observed in women diagnosed with RSA, such as an increased rate of abortion in women with hypothyroidism (10).

Maternal infections due to *Chlamydia trachomatis*, *Brucella abortus*, Cytomegalovirus, *Toxoplasma gondii* and Herpes simplex have already been mentioned as causes of abortion; however, most studies do not confirm this etiology for RSA (11).

Immunologic alterations were cited as being responsible for more than 80% of RSA cases of unknown etiology (12). The discovery of an association between antiphospholipid antibodies and RSA was a major step towards preventing the occurrence of this complication. It was observed that pregnancies of 95% of untreated women with lupus anticoagulant ended in spontaneous abortion or fetal death (13).

Similarly, it has been estimated that the alloimmune factor is present in 40% to 60% of couples suffering from RSA of unknown etiology (14). Alloimmunity refers to the immunologic differences existing between individuals of the same species. The initial event in these cases is an abnormality that prevents a mother from developing an immunologic response essential to a genetically foreign conceptus (15). In women with RSA, there seems to be a decreased number of suppressor cells in the decidua and an increased activity of natural killer cells (NK). These cells end up attacking the implanted conceptus, increasing the incidence of early

abortions and therefore producing a worse gestational prognosis (16). The therapy proposed was to use the partner's or donor's lymphocytes to suppress NK cell activity, making it possible for the conceptus to develop (17).

It has been reported that other factors, such as age, number of previous abortions, infertility and emotional aspects are causes of RSA. However, immunologic factors appear to occur most frequently in women with RSA of unknown etiology. Diagnosis and specific treatment may increase viable pregnancy rates up to 78% (18).

Nevertheless, until now, some women who are regularly treated continue to experience recurrent abortions. Other complicating factors may exist in some groups or a combination of multiple causes can contribute to an unsuccessful outcome. This study sought to identify and correct these single or combined factors, evaluating pregnancy outcome. It enabled us to gain an understanding of the characteristics of women who benefited from the investigation and treatment of recurrent spontaneous abortion.

Patients and Methods

A nested case-control study included the evaluation of 246 medical records of women with RSA from Gestational Loss Ambulatory at Campinas University, from March 1994 to July 2003. All studied women had history of three or more consecutive spontaneous abortions and who had become pregnant after completing the evaluation and treatment. The study was approved by Research and Ethical Committees.

To minimize the action of cohort effect on results, a minimum protocol of investigation and treatment included genetic, hormonal, infectious, anatomic and immunologic factors, as can be seen:

The genetic factor was evaluated by the couple's karyotype. Those with abnormal results were evaluated by a clinical geneticist.

The hormonal factor was investigated by measuring serum progesterone and/or performing endometrial biopsy three to five days before menstruation. In women who were advised to attempt conception, the use of natural progesterone (100 mg/day beginning at the second phase of the menstrual cycle) was recommended. The dose was doubled when pregnancy was confirmed and was maintained until the 16th week of pregnancy. Assessment of thyroid function was performed by measuring serum thyroid-stimulating hormone (TSH) and free thyroxine (FT4). Once diagnosis of hypothyroidism or hyperthyroidism was made, thyroid replacement or antithyroid drug therapy was used respectively. Diabetes mellitus (DM) was investigated by measuring fasting plasma glucose level and performing a glucose tolerance test. Treatment of DM was achieved by diet follow-up or use of insulin when necessary.

Maternal infections were evaluated by culture (*mycoplasma* and gonococci) and immunofluorescence and/or immunoenzymatic studies (Chlamydia, syphilis and toxoplasmosis). When infection was confirmed, it was treated by well-known antibiotic or antiparasitic drugs.

Uterine anatomic defects were evaluated by clinical and gynecologic examination and/or ultrasound and/or hysterosalpingography. When deemed necessary, the investigation was supplemented by hysteroscopy and/or laparoscopy, performing the possible and necessary surgical corrections.

The autoimmune factor was investigated by ELISA (Enzyme-Linked Immunosorbent Assay) to screen IgG and/or IgM anticardiolipin antibodies (aCL). A positive kaolin clotting time (KCT) and/or Russel viper venom time higher than 1.2 (and confirmed by a test diluted to 50%) detected lupus anticoagulant activity (LAC). Immunofluorescence was used to investigate

antinuclear and anti-DNA antibodies. Treatment was based on the use of acetylsalicylic acid (ASA) and low molecular weight heparin. Investigation of alloimmune factor was performed by crossmatch using the microlymphocyte toxicity test. The alloimmune factor was considered when the crossmatch test between the female serum and the male partner's lymphocytes was negative. In these cases, serum tests for hepatitis B and C and for the human acquired immunodeficiency virus (HIV), syphilis and Chagas disease were performed in both partners before treating this factor. If a diagnosis was made, immunization therapy was prepared with a concentration of $80x10^6$ /ml of the male partner's lymphocytes intradermally injected into the female on two occasions separated by a four to six weeks interval. Crossmatch was repeated and women who had demonstrated a partial response received two booster immunizations. A new crossmatch was performed to evaluate the response. Women who showed no response to immunization by their partner's lymphocytes (crossmatch remained negative) or who did not present a change in partial response after two booster shots were submitted to two immunizations with lymphocytes of a non-related donor together with husband lymphocytes. After a positive crossmatch, patients were advised to attempt pregnancy.

The analysis of the association of morphologic criteria and final diagnosis was performed using estimated odds ratio values, with 95% confidence intervals (CI). The analysis of the association of morphologic criteria to predict final diagnosis was also performed by logistic regression models and results were expressed by odds ratio values, with 95% confidence intervals (CI). Statistics were calculated using SAS 8.2 (SAS Institute, Cary, NC).

Results

From 246 women who were initially included in the study, 17 had no records of gestational outcomes and were excluded, performing 229 studied women. One hundred and fifty eight women had history of three abortions, 55 had four or five,

and 16 had six or more previous abortions. After application of the protocol and according to pregnancy results, studied women were divided in two groups. In Group 1 included 170 women (74.2%) who went into labor and group 2 included 59 women (25.8%) whose pregnancy ended in spontaneous abortion. Of group 1, 148 women (87%) had a full-term pregnancy, 22 women (13%) had preterm birth and 6 women had fetal death. In group 2, 51 women (86.4%) had one abortion and 8 (13.6%) had two abortions after treatment. Most of the studied women (86%) were 25 to 39 years old; mean age in group 1 was 31.6 ± 4.8 years (19 to 42) and in group 2 it was 32 ± 4.9 years (20 to 43).
Table 1 shows the occurrence of studied factors and pregnancy outcomes. Alloimmune factor was found in 215 women, anatomic in 41 women and autoimmune in 23. Ten women had no identified etiologic factor.

A variety of combinations of etiologic factors was detected. Genetic, non immunologic, several factors associations and no identified factors were classified as “Others”. Single alloimmune factor was seen in 137 women (59.8%) and associated to other factors in 78 women (34%). Women with single alloimmune factor had better pregnancy outcomes (delivery) than women with association of alloimmune and other factors. These women and those from “Others” group showed higher risk for abortion, as can be seen in Table 2.

When the women studied were divided into groups according to age, it was observed that those aged 40 or older had a higher chance of spontaneous abortion (OR 5.83 95% CI 1.12-30.40), with a negative influence on pregnancy outcome. This is shown in Table 3

When evaluating the number of abortions prior to treatment, group 1 had an average of 3.5 ± abortions (3 to10) and group 2 had an average of 3.9 (3 to 10) abortions. After correcting the factors identified during the investigation, pregnancy outcome was not influenced by the number of abortions prior to treatment, as may be observed in Table 4.

For a better significative results visualization in multivariate analysis, decision trees were constructed to identify the sequence of events for evaluating the possible causes of RSA that might indicate a better or worse pregnancy outcome. The decision trees adjusted were based on these criteria and the outcome was arranged in a hierarchical order of odds ratio. Graph 1 shows the outcome after “pruning” decisions that were supported by desired clinical events. In agreement with the results of logistic regression it was observed that the variable autoimmune factor was at the forefront of the decision levels. This criterion was followed by the alloimmune factor at the second level of decision. It was also noted that when the autoimmune factor was present, the percentage of deliveries decreased and thus the number of abortions increased.

In the absence of this factor, the alloimmune factor was the next factor shown by statistical significance. At the second level of decision, the percentage of deliveries increased when the first factor was absent and the second factor was present.

A new multivariate analysis was performed now observing the groups or combination of factors that were formed and related to RSA (alloimmune alone, alloimmune and autoimmune, alloimmune and hormonal, alloimmune and uterine, non immunologic and others).

The tree resulting from this new arrangement demonstrated the development of a decision level with groups of factors that, if present, worsened the outcome (more abortions) whereas others improved it (more deliveries). In these conditions, as observed at the only level of this decision tree, women with an alloimmune factor alone or an alloimmune factor combined with hormonal or uterine factors had a better outcome than all other possible combinations. Graph 2.

Discussion

CAISM/UNICAMP is a referral center for the investigation and treatment of women with pregnancy losses, primarily those with RSA. The pregnancy outcome in our institution, i.e. 74.2% of deliveries and 25.8% of abortions, is similar to that found in other medical services specialized in this type of care (21). We used an investigation and treatment protocol for the main factors admitted as possible causes of RSA. The immunologic factor was the most commonly found and data indicate its importance in the context of female reproductive health. Pregnancy outcome was better when women presented a single alloimmune factor or alloimmune factor combined with hormonal or anatomic factors. Women with hormonal factors received specific treatment for each case. Thus, women with thyroid disorders who were diagnosed before or during the investigation received adequate therapy, which usually improved pregnancy outcome (5, 7, 26). None of the women evaluated presented abnormal serum glucose levels. In cases of luteal phase deficiency, due to technical difficulties in obtaining an accurate diagnosis, all women who were advised to atttempt conception received natural micronized progesterone. Therefore, all hormonal factors diagnosed were adequately treated, which may have contributed to the better outcomes seen in the group of alloimmune combined with hormonal factors. Likewise, anatomic factors were evaluated. The influence of these factors on the origin of RSA is still an object of discussion in the literature (5, 7). Nevertheless, when synechiae, uterine septums, polyps, myomas or cervical incompetence were diagnosed, treatment was given. Some studies showed an improvement in pregnancy outcome in certain types of treatments for anatomic factors (27, 28) It is possible that these corrections may have contributed to a better pregnancy outcome in women with combined alloimmune and anatomic factors.

On the other hand, worse outcomes were obtained in women who had combined alloimmune and autoimmune factors, non immunologic and others (genetic, several associations and no identified factor).

Current studies confirm an association between antiphospholipid antibodies and RSA. (29, 12). Furthermore, they refer that the combined use of aspirin and heparin has proven to be very efficient, and may reduce the chance of a new abortion in 54% of the cases (18, 19, 20). Pregnancy outcomes related to the autoimmune factor found in this study are quite similar to those presented in the literature, i.e. slightly more than 50% of the women went into labor. The alloimmune factor was found in 93.3% of the cases and when corrected, 77.7% of the women went into labor. Perhaps this frequency is higher than that found by other authors (13,14) because our institution is the only public health service in Brazil that investigate and treat women with RSA of alloimmune cause. In agreement with other studies, we believe that the alloimmune factor is one of the main factors responsible for RSA described as “unknown etiology” (21, 22). On the other hand, women who presented alterations in other sites studied besides the immunologic, had an increased risk of abortion and the rate of a successful pregnancy fell to 58.8%. It is clear that when other factors besides the immunologic are involved, new treatments need to be added and consequently the risks will be higher. When the factors are grouped, their influence on the pathophysiology of RSA is expressed in a different manner. The combination of alloimmune and non-immunologic factors also did not produce good pregnancy outcome, only happening in three cases. The group “Others” was complex as it involved women with no identified factors or several associations of them, including genetic one. This complexity certainly contributed to the worst outcomes in this group, as a specific treatment could not be instituted and genetic therapy is not available in our country.

Our results also indicated a higher risk of RSA in women aged 40 years or more, data concordant with most authors in the literature (18, 23). While analysing the

prior number of abortions, in contrast to most authors, we found that this data had no influence on pregnancy outcome. When reconsidering the alloimmune debate, we observed that these studies did not use this factor to evaluate the causes of RSA. In his series, Poland and co-workers (24) did not have this technique available and other authors (23, 25) presented results without treating abnormalities, including the alloimmune alteration, which in most cases was never investigated.

Conclusion

Age over 40, immunologic factors, mainly autoimmune, and two or more concomitant etiologic factors were associated with poor gestational outcomes in studied women.

References

1. United Nations, Departament of Social Affairs, Fetal, Infant, And Early Childhood Mortality I. The Statistics. United Nations, New York, 1954.

2. Coulam CB. Recurrent pregnancy loss. Clin Obstet Gynecol 1986;29:863.

3. Regan CL, Mcadam BF, McParland P, Boylan PC, Fitzgerald GA, Fitzgerald DJ. Reduced fetal exposure to aspirin using a novel controlled-release preparation in normotensive and hypertensive pregnancies. Br J Obstet Gynecol 1998;105:732-8.

4. Boué J, Boué A, Lazar P. Retrospective and prospective epidemiological studies of 1500 karyotyped spontaneous human abortions. Teratol 1975;12:11.

5. Harger JH, Archer DF, Marchese SG, Muracca-Clements M, Garver KL. Etiology of recurrent pregnancy losses and outcome of subsequent pregnancies. Obstet Gynecol 1983;62:574-81.

6. Robinson WP, Beever C, Brown CJ, Stephenson MD. Skewed X inactivation and recurrent spontaneous abortion. Semin Reprod Med 2001;19:175-81.

7. Stray-Petersen B, Stray-Petersen S. Etiologic factors and subsequent reproductive performance in 195 couples with prior history of habitual abortion. Am J Obstet Gynecol 1984;148:140.

8. Botella TJ. The endometrium in repeated abortion. Int J Fertil1962;*7*:147-9.

9. Csapo AI, Pulkkinen MO,Wiest WG. Effecs of luteectomy and progesterone replacement therapy in early pregnancy patients. Am J Obstet Gynecol 1973;115:759-65.

10. Jones GES, Delfs E. Endocrine patterns in term pregnancies following abortion. JAMA 1951;146:1212.

11. Carp HJA, Toder V, Mashiach S, Nebel L, Serr DM. Recurrent miscarriage: a review of current concepts, immune mechanisms, and results of treatment. Obstet Gynecol Surv 1990;45:657-69.

12. McIntyre JA, Coulam CB, Faulk WP. Recurrent spontaneous abortion. A J Reprod Immunol 1989;21:100-4.

13. Ober CL, SimpsoN JL, Radvany R. Increased male-to-female sex rations among HLA-DR compatible pregnancies. Lancet 1985;30:761-5.

14. Ober C, Karrison T, Odem RR et al. Mononuclear-cell immunization in prevention of recurrent miscarriage. Fertil Steril 1999;75:916-9.

15. Aoki K,.Kajiura S, Matsumoto Y, Ogasawara M, Okada S,Yagam Y, Gleicher N. Preconcepcional natural-killer cell activity as a predictor of miscarriage. Lancet 1995;27:1340-2.

16. Kwak JYH, Gilman-Sachs A, MorettI M, Beaman KD, Beer AE. Natural killer cell cytotoxity and paternal lynphocyte immunization in women with recurrent spontaneous abortion. Am J Reprod Immunol 1998;40:352-8.

17. Mowbray JF, Gibbings C, Liddell H, Reginald PW, Underwood JL, Beard RW. Controlled trial of treatment of recurrent spontaneous abortion by immunisation with paternal cells. Lancet 1985;27:941-3.

18. Cowchock FS, Smith JB. Predictors for live birth after unexplained spontaneous abortions: Correlations between imunologic test results, obstetric histories, and outcome of next pregnancy without treatment. Am J Obstet Gynecol 1992;167:1208.

19 Kutteh WH. Autoimmune factors in assisted reproduction. Minerva Gynecol 2002;54(3):217-24.

20. Empson M, Lasser M, Craig JC, Scott JR. Recurrent pregnancy loss with antifhospholipid antibody: a systematic review of therapeutic trials. Obstet Gynecol 2002,99:135-44.

21. Makino T. Recurrent reproductive wastage and immunologic factors. Am J Reprod Immunol 2002; 48:266-68.

22. Ramhorst R, Agriello E, Zittermann S et al. Is the paternal mononuclear cells' immunization a successful treatment for recurrent spontaneous abortion? Am J Immunol 2000; 44:129-35.

23. Clifford K, Rai R, Regan L. Future pregnancy outcome In unexplained recurrent first trimester miscarriage. Hum Reprod 1997;12:387-89.

24. Poland BJ, Miller JR, Jones DC, Trimble BK. Reprodutive counseling in patients who have had a spontaneous abortion. Am J Obstet Gynecol 1977;127:685-91.

25. Knudsen UB, Hansen V, Juul S, Secher NJ. Prognosis of a new pregnancy following previous spontaneous abortions. Eur J Obstet Reprod Biol 1991;39:31-36.

26. Bussen S, Sütterlin M, Steck T. Endocrine abnormalities during the follicular phase in women with recurrent spontaneous abortion. Hum Reprod 1999;14(1):18-20.

27. Dudley DJ, Branch DW. New approaches to recurrent pregnancy loss. Clin Obstet Gynecol 1989;32:520-32.

28. Salim R, Regan L, Woelfer B, Backos M, Jurkovic D. A comparative study of the morphology of congenital uterine anomalies in women with and without a history of recurrent first trimester miscarriage. Hum Reprod 2003;18:162-66.

29. Cowchock S, Smith JB, Gocial B. Antibodies to phospholipid and nuclear antigens in patients with repetead miscarriages. Am J Obstet Gynecol 1986;155:1002-10.

Table 1

Percentage distribution of women according to factors investigated and first pregnancy outcome after treatment

Factors	Abortion (n=59)		Delivery (n=170)		Total
Alloimmune	53	89.8	162	95.3	215
Autoimmune	10	16.9	13	7.6	23
Hormonal	5	8.5	12	7.1	17
Genetic	3	5.0	1	0.6	4
Infective	6	10.2	7	4.1	13
Uterine	11	18.6	30	17.6	41
No factors	6	10.2	4	2.3	10

Table 2

Percentage distribution of women according to groups of factors investigated and first pregnancy outcome after treatment

Groups	Abortion (n=59)		Delivery (n=170)		Crude OR (95% CI)	Age-adjusted OR (95% CI)
Single Alloimmune	28	47.5	109	64.1	Ref.	Ref
Alloimmune and Autoimmune	7	11.9	9	5.3	3.03 (1.04 to 8.84)	4.30 (1.36 to 13.63)
Alloimmune and Hormonal	3	5.1	8	4.7	1.46 (0.36 to 5.86)	1.41 (0.34 to 5.96)
Alloimmune and Uterine	5	8.5	22	12.9	0.89 (0.31 to 2.54)	1.13 (0.37 to 3.44)
Non Immunologic	2	3.4	1	0.6	7.78 (0.68 to 88.95)	9.02 (0.70 to 116.5)
Others	14	23.7	21	12.4	2.60 (1.17 to 5.74)	2.79 (1.21 to 6.45)

Table 3

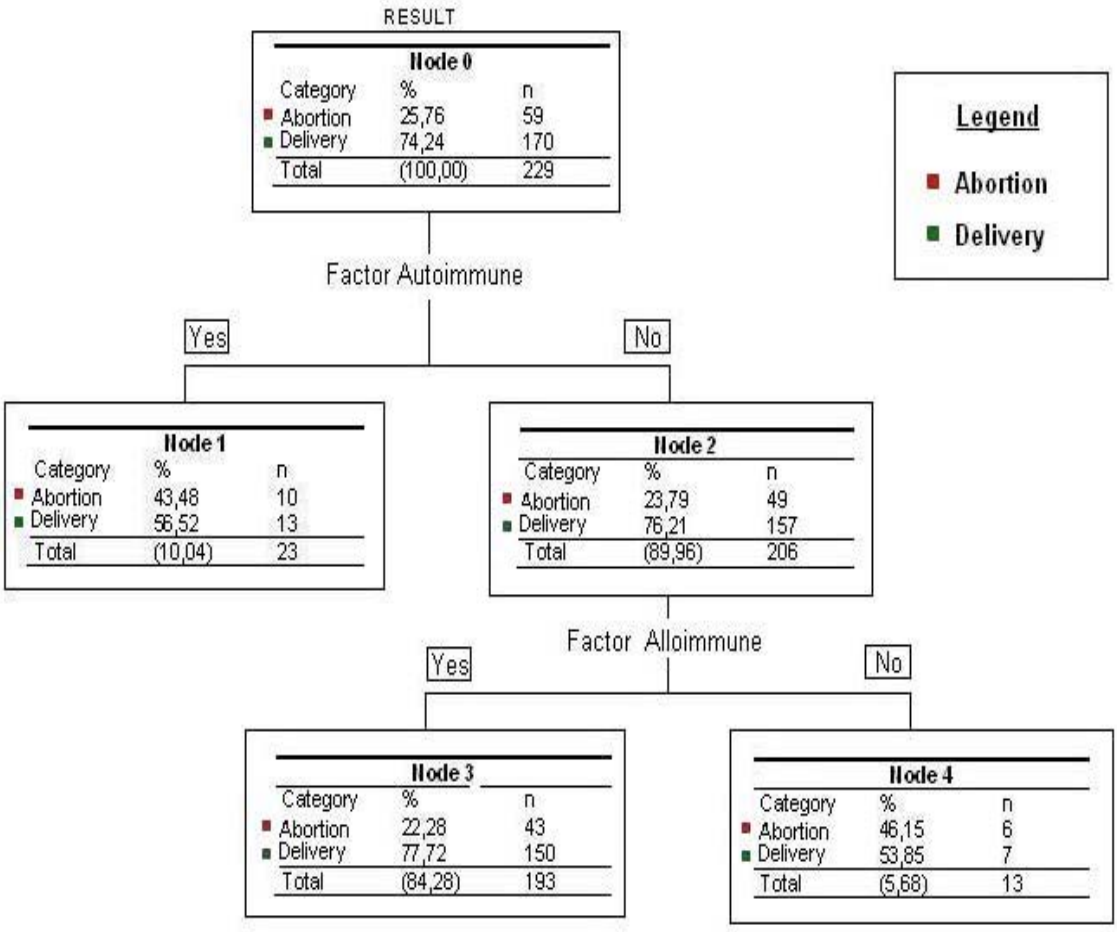
Percentage distribution of women according to age at the beginning of treatment and first pregnancy outcome after treatment

Age	Abortion (n=59)		Delivery (n=170)		Crude OR (95% CI)
Until 24 years	3	5.1	15	8.2	Ref
25 to 29 years	14	23.7	40	23.5	1.75 (0.44 to 6.96)
30 to 34 years	28	47.5	67	39.4	2.09 (0.56 to 7.79)
35 to 39 years	7	11.9	42	24.7	0.83 (0.19 to 3.64)
40 years or more	7	11.9	6	3.5	5.83 (1.12 to 30.40)

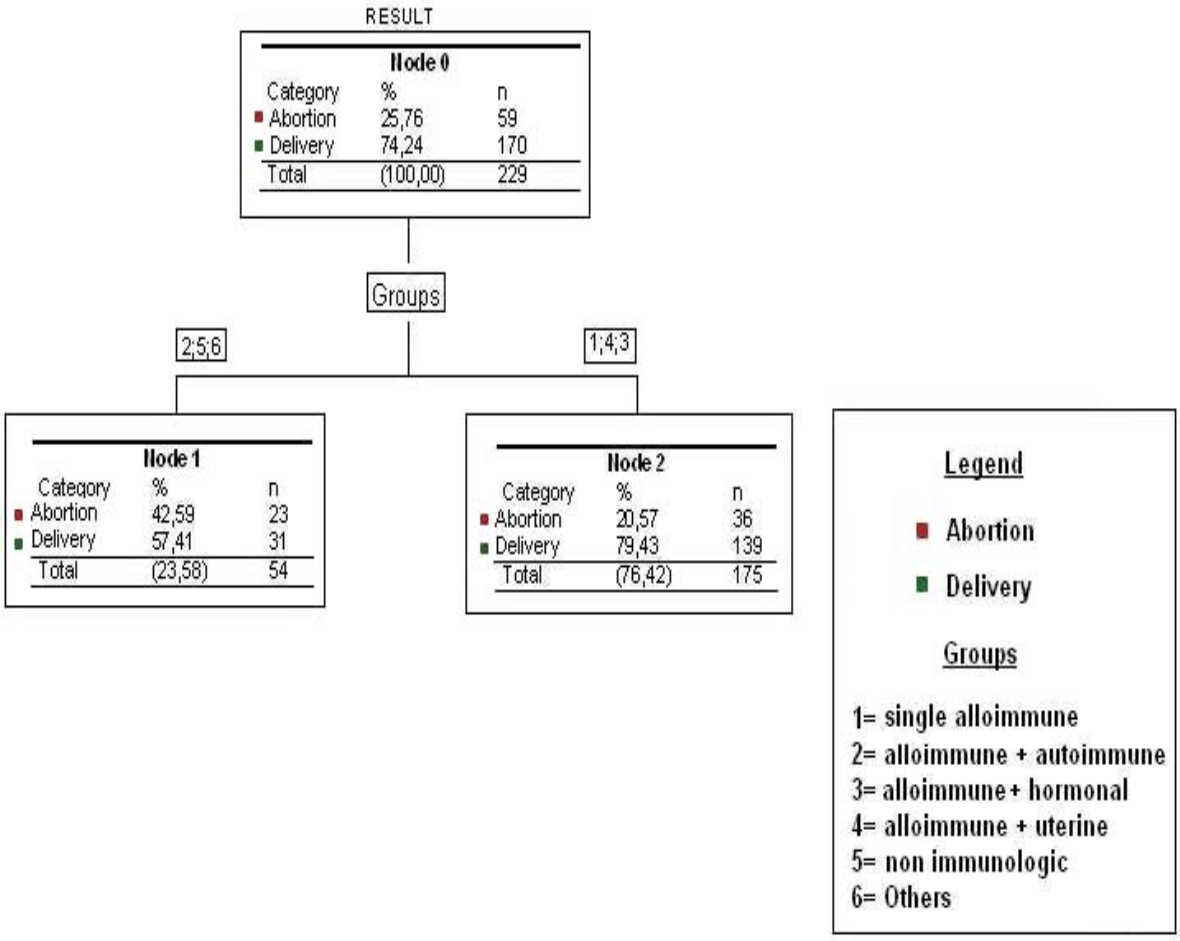
Table 4

Percentage distribution of women according to number of abortions prior to treatment and first pregnancy outcome after treatment

Nº Abortions	Abortion (n=59)		Delivery (n=170)		Crude OR (95% CI)	Age-adjusted OR (95% CI)
3	48	81.4	152	89.4	Ref	ref
4 to 5	9	15.3	15	8.8	0.94 (0.46 to 1.94)	0.96 (0.45 to 2.03)
>6	2	3.4	3	1.2	2.37 (0.83 to 6.79)	2.66 (0.86 to 8.19)



Graph 1. Decision tree regarding immunologic, alloimmune and autoimmune factors, in which the left branches represent decisions made when the factor in question was found, and the right branches represent decisions made when this factor was absent.



Graph 2. Decision tree regarding groups of factors investigated and grouped according to frequency.

4. Conclusões

- Idade, presença de fatores imunológicos (autoimune e aloimune) e ocorrência de vários fatores associados foram as principais causas de AER identificadas.

- Independentemente do número prévio de abortos, após o tratamento, as mulheres em que identificamos fator aloimune isolado apresentaram melhor resultado (parto) que qualquer outra associação de fatores.

- Mulheres com 40 anos ou mais de idade, presença de fator autoimune e/ou identificação de vários fatores associados conferem maior risco de ocorrência de um novo aborto.

5. Referências Bibliográficas

ABRAMSON, J.; STAGNARO-GREEN A; Thyroid antibodies an fetal loss: an evolving story. ***Thyroid***, 11:57-63, 2001.

AOKI, K.; KAJIURA, S.; MATSUMOTO, Y.; OGASAWARA, M.; OKADA, S.;YAGAMI, Y. et al. Preconcepcional natural-killer cell activity as a predictor of miscarriage. ***Lancet***, 27:1340-2, 1995.

ARTHURS, M.; MORGAN, O.S.C.; DECEULAER, K; BROWNE, B. Primary antiphospholipid syndrome. A case report. ***West Indian Med,*** 43:27-9,1994.

BARINI, R.; COUTO, E.; RIBEIRO, S.T.; LEIBER, S.R.; BATISTA, S.C.; SILVA, J.L.P. Abortamento recorrente de causa imunológica; avaliação de um protocolo de investigação e tratameto. ***RBGO***, 20:83-9, 1998.

BOTELLA, T.J. The endometrium in repeated abortion. ***Int J Fertil***, *7*:147-9, 1962.

BOUÉ, J.; BOUÉ, A.; LAZAR, P. Retrospective and prospective epidemiological studies of 1500 karyotyped spontaneous human abortions. ***Teratology***, 12:11. 1975.

BOUÉ, A. Spontaneous abortions and cytogenentic abnormalities. In: BEHRMAN, S. J.; KISTNER, R.W.; PATTON, G.W. (eds.) - **Progress in infertility**. 3ª ed., Little, Brown and Company; 1998. p.783-95.

BRASIL. Ministério da Saúde. IBGE – Censos Demográficos e Contagem Populacional e MS/SE/Datasus, a partir de totais populacionais fornecidos pelo IBGE, para os anos intercensitários. 2004.

BUSSEN, S.; SÜTTERLIN, M.; STECK, T. Endocrine abnormalities during the follicular phase in women with recurrent spontaneous abortion. ***Hum Reprod***, 14:18-20, 1999.

BUTTRAM, V.C.; GIBBONS, W.E. Mullerian anomalies: a proposed classification (an analysis of 144 cases). ***Fertil Steril***, 32:40, 1979.

CARP, H.J.A.; TODER, V.; MASHIACH, S.; NEBEL, L.; SERR, D. M.Recurrent miscarriage: a review of current concepts, immune mechanisms, and results of treatment. ***Obstet Gynecol Surv***, 45:657-69, 1990.

CARRELL, D.T.; WILCOX, A.L.; LOWY, L.; PETERSON, C.M.;JONES, K.P.; ERICKSON, L. et al. Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. ***Obstet Gynecol***, 101:1229-35, 2003.

CARVALHO, E.C.C. Estudo comparativo da frequência de fatores trombogênicos entre mulheres com aborto espontâneo recorrente e mulheres férteis. Campinas, 2001 [Tese - Doutorado - Faculdade de Ciências Médicas/ UNICAMP].

CAUCHI, M.N.; LIM, D.; YOUNG, D.E.; KLOSS, M.; PEPPERELL, R.J. Treatament of recurrent aborters by immunisation with paternal cells – controlled trial. ***Am J Reprod Immunol***, 25:10-5. 1991.

CECATTI, J.G.; AMARAL, E.M.F.S.; BAHAMONDES, L.G; BARINI, R. Avaliação dos resultados de circlagem pela técnica de Espinosa modificada. ***Rev Bras Ginecol Obstet***, 12:232-7, 1989.

CLIFFORD, K.;RAI, R.; REGAN, L. Future pregnancy outcome in unexplained recurrent first trimester miscarriage. ***Hum Reprod***,12:387-89.1997.

COSLOVSKY, S.; WAISSMAN, I. As doenças intercorrentes no ciclo grávido-puerperal - diabete, In: REZENDE J. (ed.) **Obstetrícia**. 5ª ed., Rio de Janeiro: Guanabara; 1987. p.313-9.

COSTA, H.L.F.F.; MOURA, M.D,; FERRIANI, R.A.; ANCESCHI, M.I.S.; BARBOSA, J.E. Prevalence of anti-cardiolipin antibody in habitual aborters. ***Gynecol Obstet Invest***, 36:221-5, 1993.

COSTA, H.L.F.F. Autoimunidade e perda conceptual. **Tese de doutorado**. Ribeirão Preto, 1994. [Tese – Doutorado – Faculdade de Medicina -USP].

COULAM, C.B. Recurrent pregnancy loss. ***Clin Obstet Gynecol***, 29:863-7, 1986.

COULAM, C.B. Immunologic tests in the evaluation of reproductive disorders:A critical review. ***Am J Obstet Gynecol***, 167:1844-51, 1992.

COWCHOCK, S.; SMITH, J.B.; GOCIAL, B. Antibodies to phospholipid and nuclear antigens in patients with repetead miscarriages. ***Am J Obstet Gynecol***, 155:1002-0, 1986.

COWCHOCK, F.S.; SMITH, J.B.; Predictors for live birth after unexplained spontaneous abortions: Correlations between imunologic test results, obstetric histories, and outcome of next pregnancy without treatment. ***Am J Obstet Gynecol***, 167:1208-12, 1992.

CSAPO, AI; PULKKINEN, M.O.;WIEST, W.G. Effecs of luteectomy and progesterone replacement therapy in early pregnancy patients. ***Am J Obstet Gynecol***, 115:759-65,1973.

DENDRINOS, S.; PAPASTERIADES, C.; TARASSI, K.; CHRISTODOULAKOS, G.; PRASINOS, G.; CREATSOS, G. Thyroid autoimmunity in patients with recurrent spontaneous miscarriages. ***Gynecol Encocrinol***, 14:270-4, 2000.

DUDLEY, D.J.; BRANCH, D.W. New approaches to recurrent pregnancy loss. ***Clin Obstet Gynecol***, 32:520-32, 1989.

EMPSON, M.; LASSER, M.; CRAIG, J.C.; SCOTT, J.R. Recurrent pregnancy loss with antifhospholipid antibody: a systematic review of therapeutic trials. ***Obstet Gynecol***, 99:135-44, 2002.

ESPLIN, M.S.; BRANCH, D.W.; SILVER, R.; STAGNARO-GREEN A. Thyroid autoantibodies are not associated with recurrent pregnancy loss. ***Am J Obstet Gynecol***, 179:1583-6, 1998.

FRASER, E.J.; GRIMES, D.A.; SCHULZ, K.F. Imumunization as therapy for recurrent spontaneous abortion: A review and meta-analysis. ***Obstet Gynecol***, 82:854-8, 1993.

GLASS, R.H.; GOLBUS, M.S. Habitual abortion. ***Fertil Steril***, 29:257-65,1978.

GLUECK, C.J.; AWADALLA, S.G.; PHILLIPS, H.; CAMERON, D.; WANG, P.; FONTAINE, R.N. Polycystic ovary syndrome, infertility, familial thrombophilia, familial hypofibrinolysis, recurrent loss of in vitro fertilized embryo, and miscarriage. ***Fertil Steril***, 74:394-7, 2000.

HABARA, T.;NAKATSUKA, M.; KONISHI, H.; ASAGIRI, K.; NOGUEHI, S.; KUDO, T. Elevated blood flow resistance in uterine arteries of women with unexplained recurrent pregnancy loss. ***Hum Reprod***, 17:190-4, 2002.

HARGER, J.H.; ARCHER, D.F.; MARCHESE, S.G.; MURACCA-CLEMENTS, M.; GARVER, K.L. Etiology of recurrent pregnancy losses and outcome of subsequent pregnancies. ***Obstet Gynecol***, 62:574-81, 1983.

HARRIS, E.N.; PHIL, M.; CHAN, J.K.H; ASHERSON, R. A.;ABER, V.R.; GHARAVI, A. E. et al. Thrombosis, recurrent fetal loss and thrombocytopenis: predictive value of the anticardiolipin antibody test. ***Arch Intern Med***, 146:2153-6, 1986.

HILL, J.A.; ABBOTT, A.F.; POLITCH, J.A. Sperm morphology and recurrent abortion. ***Fertil Steril***, 61:776-8, 1994.

HOMER, H.A.; LI, T.C.; COOKE, L.D. The septate uterus: a review of management and reprodutive outcome. ***Fertil Steril***, 73:1-14, 2000.

JONES, G.S. Some newer aspects of the management of infertility. ***J Am Med Ass***, 141:1123-6, 1949.

JONES, G.E.S.; DELFS, E. Endocrine patterns in term pregnancies following abortion. ***JAMA***, 146:1212-6, 1951.

KANERIA, M.V.; VISHWANATHAN, C. A preliminary study of antiphospholipid antibodies in 50 cases of bad obstetric history. ***J Assoc Physicians India***, 47:665-7, 1999.

KNUDSEN, U.B.; HANSEN, V.; JUUL, S.; SECHER, N. J. Prognosis of a new pregnancy following previous spontaneous abortions. ***Eur J Obstet Reprod Bio***, 39:31-6, 1991.

KURUP, M.; GOLDKRAND, J.W. Cervical incompetence: Eletive, emergent, or urgent cerclage. ***Am J Obstet Gynecol***, 181:240-4, 1999.

KUTTEH, W.H. Autoimmune factors in assisted reproduction. ***Minerva Ginecol***, 54:217-24, 2002.

KWAK, J.Y.H.; GILMAN-SACHS, A.; MORETTI, M.; BEAMAN, K.D.; BEER, A.E. Natural killer cell cytotoxity and paternal lynphocyte immunization in women with recurrent spontaneous abortion. ***Am J Reprod Immunol***, 40:352-8, 1998.

MACLEAN, M.A.; CUMMING, G.P.; McCALL, F.; WALKER, I.D.; WALKER, J.J. The prevalence of lupus anticoagulant and anticardiolipin antibodies in women with a history of first trimester miscarriages. ***Br J Obstet Gynecol***, 101:103-6, 1994.

MAKINO, T. Recurrent reproductive wastage and immunologic factors. ***Am J Reprod Immunol***, 48:266-8, 2002.

MCINTYRE, J.A.; COULAM, C.B.; FAULK,W.P. Recurrent spontaneous abortion. ***A J Reprod Immunol***, 21:100-4, 1989.

MILLS, J.L. A prospective study of fetal losses in diabetic and control pregnancies from the third week post conception. ***Diabetes***, 34:46-9, 1983.

MOWBRAY, J.F.; GIBBINGS, C.; LIDDELL, H.; REGINALD, P.W.; UNDERWOOD, J.L.; BEARD, R.W. Controlled trial of treatment of recurrent spontaneous abortion by immunisation with paternal cells. ***Lancet***, 27:941-3, 1985.

OBER, C.L.; SIMPSON, J.L.; RADVANY, R. Increased male-to-female sex rations among HLA-DR compatible pregnancies. ***Lancet***, 30:761-5, 1985.

OBER, C.; KARRISON, T.; ODEM, R.R.; BARNES, R.B.; BRANCH, D.W.; STEPHENSON, M.D. et al. Mononuclear-cell immunization in prevention of recurrent miscarriage. ***Fertil Steril***, 75:916-9, 1999.

POLAND, B.J; MILLER, J.R.;JONES, D.C; TRIMBLE, B.K. Reprodutive counseling in patients who have had a spontaneous abortion. ***Am J Obstet Gynecol***, 127:685-91, 1977.

PRITCHARD, J.A.; McDONALD, P.C.; GANT, N.F. Hipertensive disorders in pregnancy. In: PRITCHARD, J.A.; McDONALD, P.C.; GANT, N.F. (eds.) **Williams obstetrics**. 17ª ed., Appleton-Century-Crafts; 1985. p.525-60, 1985.

QUENBY, S.M.; FARQUHARSON, R.G. Predicting recurring miscarriage: hat is important? ***Obstet Gynecol***, 82:132-8, 1993.

RAMHORST, R.; AGRIELLO, E.; ZITTERMANN, S.; PANDO, M.; LARRIBA, J.; IRIGOYEN, M.; et al. Is the paternal mononuclear cells' immunization a successful treatment for recurrent spontaneous abortion? ***Am J Immunol*****,** 44:129-35, 2000.

REGAN, C.L.; McADAM, B.F.; MCPARLAND, P.; BOYLAN, P.C.; FITZGERALD, G.A.; FITZGERALD, D.J. Reduced fetal exposure to aspirin using a novel controlled-release preparation in normotensive and hypertensive pregnancies. ***Br J Obstet Gynecol***, 105:732-8, 1998.

REIN, M.S. Luteal phase defect and recurrent pregnancy loss. ***Infertil Reprod Clin Borth Am***, 2:121-4, 1991.

ROBINSON, WP; BEEVER, C; BROWN, CJ; STEPHENSON, MD. Skewed X inactivation and recurrent spontaneous abortion. ***Semin Reprod Med***, 19:175-81, 2001.

SACHS, E.S.; JOHODA, M.G.J.;VAN HEMEL, J.O. Chromosome studies of 500 couples with two or more abortions. ***Obstet Gynecol***, 65:375-8, 1985.

SALIM, R; REGAN,L; WOELFER, B; BACKOS, M; JURKOVIC, D. A comparative study of the morphology of congenital uterine anomalies in women with and without a history of recurrent first trimester miscarriage. ***Hum Reprod***, 18:162-6, 2003.

SCOTT, J. R.; ROTE, N. S.;BRANCH, D.W. Immunologic aspects of recurrent abortion and fetal death. ***Obstet Gynecol***, 70:645-56, 1987.

SOLLINGER, H.W.; BURLIGHAM, W.J; SPARKS, E.M.F; GLASS, N.R; BELZER, F.O. Donor specific transfusions in unrelated and related HLA mismatched donor recipient combinations. ***Transplantation***, 38:612-4. 1984.

STRAY-PETERSEN, B.; ENG, J.; REIKVAN, T.M. Uterine T-Mycoplasma colonization in reprodutive failure. ***Am J Obstet Gynecol***, 130:307-11, 1978.

STRAY-PETERSEN, B.; STRAY-PETERSEN, S. Etiologic factors and subsequent reproductive performance in 195 couples with prior history of habitual abortion. ***Am J Obstet Gynecol***, 148:140-4, 1984.

SURICO, N.; RIBALDONE, R.; ARNULFO, A.; BAJ, G. Uterine malformations and pregnancy losses: is cervical cerclage effective? ***Clin Exp Obstet Gynecol***, 27:147-9, 2000.

SUTHERLAND, H. W.; PRITCHARD, C.W. Increased incidence of spontaneous abortion in pregnancies complicated by maternal diabetes mellitus. ***Am J Obstet Gynecol***, 155:135-8, 1986.

THO, P.T.;BYRD, J.R.; McDONOUGH, P.G. Etiologies and subsequent reproductive performance of 100 couples with recurrent abortion. ***Fertil Steril***, 32:389-95, 1979.

THOMAS, R.; REID, R.L. Thyroid disease and reproductive disfunction: a review. ***Obstet Gynecol***, 70:789-98, 1987.

TRIPLETT, D.A. Obstetrical complications associated with antiphospholipid antibodies. In: COULAM, C.B.; FAULK, W.P.; McINTYRE, J.A. (eds). **Immunological obstetrics**. London: Norton Medical Books; 1992. p.377-403.

UNITED NATIONS, Departament of Social Affairs, Foetal, Infant, And Early Childhood Mortality - I. **The statistics**. New York: United Nations; 1954.

6. Bibliografia de Normatizações

FRANÇA, J.L.; BORGES, S.M.; VASCONCELLOS, A.C.; MAGALHÃES, M.H.A. – **Manual para normatização de publicações técnico-científicas**. 4ª ed., Editora UFMG, Belo Horizonte, 1998. 213p.

Normas e procedimentos para publicação de dissertações e teses. Faculdade de Ciências Médicas, UNICAMP. Ed. SAD – Deliberação CCPG-001/98 (alterada 2002).

7. Anexos

7.1. Anexo 1 – PROTOCOLO PARA INVESTIGAÇÃO DE CASOS DE AER

A. Avaliação uterina

Exame ginecológico completo

Ultra-sonografia

Hiterossalpingografia/histeroscopia

B. Estudo genético

Cariótipo do casal

C. Estudo microbiológico

Culturas aeróbicas vaginais

Culturas para gonococo e *Mycoplasma* nas secreções cervicais

Sorologia para *Chamydias,* toxoplasmose e sífilis

D. Avaliação endócrina

Glicemia de jejum e GTT

Função tireodiana (T4, TSH)

Avaliação da fase lútea

- hormonal (progesterona plasmática nos dias 4, 7 e 10 pós ovulação)
- biópsia de endométrio (dia 24-26 do ciclo menstrual)

E. Avaliação imunológica

- Tipagem HLA, A, B, C, DR e DQ do casal
- Ensaio para atividade bloqueadora

  Em culturas mistas de linfócitos compara-se a atividade bloqueadora do soro materno fresco e do inativado por calor: atividade menor do que 50% indica ausência de anticorpos bloqueadores.
- Rastreamento de fatores auto-imunes/reprodução

  Tempo de tromboplastina parcial ativada (TTPA)

  Anticoagulante lúpico

  Anticardiolipina (IgG e IgM)

  Anticorpos anti-DNA

FICHA PARA LEVANTAMENTO DE DADOS DOS CASOS AER

1. FICHA Nº

2 . IDADE

3. PARTOS

4. ABORTOS

4.1. PRIMÁRIO

4.2.SECUNDÁRIO

5. FILHOS VIVOS

6. CAUSA ALOIMUNE
- 1 - Presente
- 2 - Ausente

7. FATOR AUTOIMUNE
- 1 - Sim
- 2 - Não (Passe para o item 9)

8. QUAL?
- 1 - Presente
- 2 - Ausente

8.1 ANTI-DNA

8.2 ACL

8.3 AL

8.4 ANTI-LA

8.5 ANTI-RO

8.6 TTPA

9. CAUSA HORMONAL
- 3 - Sim
- 4 - Não (Passe para o item 11)

10. QUAL?

10.1 PROGESTERONA
- 5 - Normal
- 6 - Anormal

10.2 BIOP. ENDOMET.
- 5 - Normal
- 6 - Anormal

10.3 FUNC. TIREOIDE
- 5 - Normal
- 7 - Hipotiroidismo
- 8 - Hipertiroidismo

10.4 DIABETES
- 1 - Presente
- 2 - Ausente

11. CAUSA GENÉTICA
- 1 - Presente
- 2 - Ausente

12. CAUSA INFECCIOSA
- 1 - Presente
- 2 - Ausente (Passe para o item 14)

13. QUAL?
- 1 - Presente
- 2 - Ausente

13.1 LUES

13.2 TOXO

13.3 CLAMÍDIA

13.4 GONOCOCO

13.5 OUTRO

14. CAUSA LOCAL
- 5 - NORMAL
- 9 - IIC
- 10 - DEFEITO CORPO UTERINO
- 11 - DEFEITO CORPO E CAVIDADE

15. TRATAMENTO ALOIMUNE

15.1 IMMPAT ☐
1 - Presente
2 - Ausente

15.2 Nº DE REFORÇOS ☐

15.3 IMZ COM DOADOR ☐
1 - Presente
2 – Ausente

16. OUTROS TRATAMENTOS ☐
1 - Presente
2 - Ausente

16.1 CIRCLAGEM ☐

16.2 CIRC. CORPO E/OU CAV. UTERINA ☐

16.3 USO HEPARINA ☐

16.4 USO DE AAS ☐

16.5 CORREÇÃO DE FATOR HORMONAL ☐

16.6 TRAT. INFECCIOSO ☐

17. RESULTADO DOS TRATAMENTOS

17.1 PARTO
12 - Termo
13 – Pré-Termo

17.2 ABORTO ☐
14 – Primário
15 – Secundário
16 – Ignorado

Outros_____

18. DADOS DO RN

18.1 PESO ☐☐☐☐

✂.....
.....

PRONT.: _____ FICHA N°: _____

NOME: _____ TELEFONE: _____