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Editorial

The Individual Progress Test of Gynecology and Obstetrics Residents (TPI-GO): The Brazilian Experience by FEBRASGO

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The great expansion of Medical Residency Programs in Brazil in recent decades has made it difficult to properly evaluate the trained professionals and the quality of training offered.

Today, ~53,776 physicians are registered at the National Medical Residency Commission enrolled in 4,862 Medical Residency Programs offered by 809 institutions. Only in Gynecology and Obstetrics (Ob-gyn), there are 312 Medical Residency Programs. Despite the recommendation that resident physicians undergo quarterly theoretical and practical evaluations by the programs, this has not been happening regularly. Therefore, there is no information about the performance of residents during their training and the quality of training programs. Evidently, this knowledge should be based on information obtained during ongoing evaluations of the programs, with visits, audits and reports, although this has not happened in practice.

According to current legislation, specialists graduated from Medical Residency Programs approved by the National Medical Residency Commission automatically receive the specialist certificate recognized by the Ministry of Education and the Federal Council of Medicine without any evaluation process of the students' competences at the end of the program.

In view of the fragility of the system, the Medical Specialties Societies in Brazil, with special authorization from the Brazilian Medical Association, started to grant the title of specialist for graduates of residency programs after an evaluation through a flexible exam, depending on the Society granting it. In this sense, the Title of Specialist in Gynecology and Obstetrics (Portuguese acronym: TEGO) given by the Brazilian Federation of Gynecology and Obstetrics Associations (FEBRASGO) is only granted upon evaluation of the candidate through theoretical and practical tests carefully prepared by the National TEGO Commission of FEBRASGO.

In this evaluation process, important failures in the training of new specialists who completed the residency program have been observed, which has raised the failure rates for the TEGO. Thus, considering the need to qualify the training of Ob-gyn specialists in Brazil and understanding that the Individual Progress Test (IPT) represents a great reference for the self-assessment and improvement of resident physicians and Medical Residency Programs, as of 2018, FEBRASGO has implemented the Individual Progress Test for Ob-Gyn Residents (IPT-GO). Although the IPT is widely used internationally, in Brazil there is little experience reported on its use in Medical Residency.

The IPT is a comprehensive assessment that preferably uses multiple choice questions and is periodically applied to all students of the same curriculum or program,[1] aligned with modern constructivist education and promotes long-term knowledge. The longitudinality of this modality of assessment provides a unique and demonstrable measure of students cognitive progression.[2]

The functional purpose of the IPT is to provide reliable information for self-assessment of candidates and service providers of Medical Residency Programs hence, it is a formative assessment. For resident physicians who take the test, the IPT provides an accurate measure of their level of knowledge in relation to their peers and in relation to the final objectives of the specialty training, according to the Gynecology and Obstetrics Competence Matrix.[2,3]

Furthermore, through the performance in serial evaluations, the individual progress of the cognitive component can be evaluated. After each assessment, it is possible to reaffirm

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and consolidate knowledge and identify learning gaps and points to be improved.[4]

For preceptors and supervisors of Medical Residency Programs, the IPT-GO provides information on the performance and progression of residents who have taken the exam. Through this information, the profile of residents admitted to each service, the added knowledge of residents throughout the training program and the level of knowledge of graduates in relation to the national average and in relation to the objectives of the Competence Matrix can be assessed. It also allows the identification of strengths and points of improvement or areas requiring reinforcement for learning.[4]

Since 2018, the IPT began to be offered annually to all resident physicians in the first (R1), second (R2) and third years (R3) of training regularly enrolled in Medical Residency Programs recognized by the Ministry of Education.

In 2018 and 2019, the test was applied in person and simultaneously in 11 Brazilian cities in regions with the highest concentration of Ob-Gyn Residency Programs. In 2020, due to the Covid-19 pandemic and in compliance with sanitary requirements, the IPT could not be applied in person. Faced with this new challenge, the choice was to apply the test online, since this measure is supported by the literature.[5]

The theoretical test for obtaining the TEGO, applied annually to newly graduated specialists in the field, is a comprehensive assessment of the skills provided for in the Gynecology and Obstetrics Competence Matrix[3] and used as a reference to the final level of Residency Programs. For these reasons, this test model was chosen to be applied in the IPT-GO.[6]

Criteria ranging from a bonus of points to exemption from the TEGO theoretical test in the year following the completion of the residency program were established as a form of encouraging residents' participation in the IPT-GO. These criteria are based on adherence and individual performance on the test. To be entitled to bonuses, it is an essential condition that the resident participates in all three versions of the IPT as R1, R2 and R3, with a progressive minimum performance established in accordance with the competition notice published each year.[7]

Individual Feedback to Candidates

Feedback on the results of each candidate's performance in the IPT-GO is provided confidentially through a password-protected online system with personal access. The aim of this measure is to avoid embarrassment, discrimination or disqualification of candidates with unsatisfactory performance. Therefore, this evaluation is not intended to rank candidates or services. The online system presents graphs where candidates can assess the progression of their individual performance and compare it to their peers through the median (Me), 30th percentile (P30) and 60th percentile (P60) of the overall performance. For candidates completing residency programs (R3), in addition to the evaluation of the performance level in the triennium, information on whether or not they have received a bonus for TEGO of the following year is also provided.

Feedback to Services and Residency Programs

For Medical Residency Programs, feedback is provided by FEBRASGO directly to preceptors or responsible persons. The information provided corresponds to the median performance of each category of residents (R1, R2 and R3) of the service compared with the Median, P30 and P60 values of the overall performance without identifying the residents.

Analysis of Results

In 2020, the IPT-GO completed its third edition, making it possible to assess the performance of the first complete cohort of residents who took the test in the three consecutive years of Ob-Gyn Residency Programs in Brazil (►**Table 1**).

Among candidates, female participation was 85.6%, 85.9% and 85.5% respectively in 2018, 2019 and 2020, in a clear demonstration that Ob-Gyn have become a predominantly women's specialty. The total number has increased over the three years, particularly due to R1 increment, since a decrease in R2 and R3 was observed. Some factors should be mentioned in this "balance" of gains and losses in these numbers. Undoubtedly, the bonus and exemption opportunities in the TEGO theoretical test have contributed to encourage longitudinal participation of residents. However, a large part of the discontinuity may be attributed to the poor performance of some candidates in the first versions of the test, resulting in dropouts due to the impossibility of bonus or exemption from the TEGO theoretical test. Another noteworthy factor that justifies non-adherence a considerable part of the residents would be the difficulty of program coordinators in releasing candidates on the day of the exam, considering the resident's need for work given the composition of the teams on duty at the respective hospital. As demonstrated, in all editions of the IPT-GO there was a progressive improvement in performance among candidates from the first (R1), second (R2) and third (R3) years of the Ob-Gyn Residency Program. This reflects the knowledge increase occurring throughout the training period in most programs. It also reflects the quality of the test, which consists of a comprehensive, valid and reliable assessment of all

Table 1 Number of participants in the three Residency levels over the three years of application of the Individual Progress Test

Level	2018 n (%)	2019 n (%)	2020 n (%)
R1	497(41)	568(43)	628(44)
R2	360(30)	457(35)	480(34)
R3	345(29)	289(22)	314(22)
Total	1202	1314	1422
Increment		9.3	7.6

Table 2 Median (Me), 30th (P30) and 60th (P60) percentiles of the grades assigned to residents at different levels who took the Individual Progress Test-GO in 2018, 2019 and 2020

	Progress Test - Performance of Gynecology and Obstetrics residents by level of treatment								
	2018			2019			2020		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
P30	4.8	5.2	5.5	5.5	5.8	6.1	5.1	5.4	5.8
Me	5.2	5.6	5.9	5.9	6.2	6.6	5.5	5.8	6.3
P60	5.5	5.8	6.1	6.1	6.4	6.8	5.6	6.0	6.5

knowledge expected by those completing the program (R3) (►**Table 2**).

Performance of the Cohort of Residents from 2018 to 2020

The performance evaluation of 314 resident physicians who started the IPT-GO in 2018 as R1 and completed the last version in 2020 deserves attention. This cohort of candidates represents the first group that underwent the three versions of the IPT-GO since its implementation, and serves as a reference for various analyzes and considerations.

The median grades of performance of this group in 2018, 2019 and 2020 were respectively 5.2(R1) 6.2(R2) and 6.3(R3). A progression in the performance of the same group of residents from the beginning to the end of their training period can be observed, reflecting the acquisition of knowledge during the residency program. These results are consistent with a well-structured IPT, which is comprehensive and focused on the content expected for graduates of the Program.[2]

The smaller increase in performance between years 2019 and 2020 may somehow be a reflection of the Covid-19 pandemic period that certainly affected the performance of all residents indistinctly, given the huge restrictions imposed on in-person activities, particularly in outpatient and surgical practices.[8]

In conclusion, the implementation of the IPT for Ob-Gyn residents across the country was a great experience in several aspects.

The wide dissemination of the IPT on social networks, on FEBRASGO's institutional Web site, at scientific events in the specialty and through direct mail to all Ob-Gyn residents and Medical Residency Programs in the country allowed FEBRASGO to get closer to residents, improve and update the registration of Medical Residency Programs, preceptors and other relevant information. The number of residents associated with FEBRASGO has increased considerably in the last two years.

The form of performance feedback to program coordinators allowed for the comparison of results with similar services throughout the country and motivated reflections and internal debates among coordinators, preceptors and residents, and drew attention to the need for improvement in many programs. Note that institutional commitment through the support of program directors, coordinators and preceptors is key, as there is a need to release residents from the shift schedule on the day of the test.

Information from the Individual Progress Test can be used consistently for diagnostic, prognostic and corrective learning through self-assessment and structured feedback. When compared with final summative evaluations, the IPT provides greater support and security in making high-impact decisions such as approval, failure and progression of the student. Additionally, longitudinal data can serve as a measure of the quality and transparency of programs and achievement of curriculum objectives by educational institutions.

Considering the immense difficulties encountered by government agencies to audit Medical Residency Programs on a permanent basis, this model may be adopted and validated by the National Medical Residency Commission as one of the parameters for evaluating Medical Residency Programs through partnerships with the Medical Specialties Societies. It is worth mentioning that all expenses arising from the application of the test were made with FEBRASGO's own resources so to not burden the National Medical Residency Commission.

Conflicts of Interest
None to declare.

References

1 van der Vleuten C, Freeman A, Collares CF. Progress test utopia. Perspect Med Educ. 2018;7(02):136–138. Doi: 10.1007/s40037-018-0413-1
2 Wrigley W, van der Vleuten CP, Freeman A, Muijtjens A. A systemic framework for the progress test: strengths, constraints and issues: AMEE Guide No. 71. Med Teach. 2012;34(09): 683–697. Doi: 10.3109/0142159X.2012.704437
3 da Educação M. Secretaria de Educação Superior. Comissão Nacional de Residência Médica. Resolução No. 3, de 8 de abril de 2019. Dispõe a matriz de competências dos Programas de Residência Médica em Ginecologia e Obstetrícia. Diário Oficial da União [Internet]. 2019 Abr. 11 [cited 2019 May 7];Sect. 1:192. Available from: https://www.in.gov.br/web/guest/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/71090760
4 Dijksterhuis MG, Scheele F, Schuwirth LW, Essed GG, Nijhuis JG, Braat DD. Progress testing in postgraduate medical education. Med Teach. 2009;31(10):e464–e468. Doi: 10.3109/01421590902849545

5 Karay Y, Reiss B, Schauber SK. Progress testing anytime and anywhere - Does a mobile-learning approach enhance the utility of a large-scale formative assessment tool? Med Teach. 2020;42 (10):1154–1162. Doi: 10.1080/0142159X.2020.1798910
6 Romão GS, Fernandes CE, Sá MF. Teste de Progresso Individual do residente em GO: relato da experiência do primeiro ano de implantação no Brasil. Femina. 2019;47(05):282–287
7 Federação Brasileira das Associações de Ginecologia e Obstetrícia. Edital do Teste de Progresso Individual para residentes em ginecologia e obstetrícia - TPI-GO [Internet] 2020 [cited 2020 Dec 10]. Available from: https://www.febrasgo.org.br/images/arquivos/TEGO/2020/EDITAL-TPI-Corrigido.pdf
8 Silva de Sá MF. Shortcomings in the training program of medical residency during the COVID-19 pandemic in Brazil. How will they be repaired? Rev Bras Ginecol Obstet. 2021;43(03):155–157. Doi: 10.1055/s-0041-1728694

Evaluation of the Blood Level of Adiponectin in Pregnant Adolescents

Avaliação dos níveis séricos de adiponectina em gestantes adolescentes

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Abstract

Objective To evaluate serum levels of adiponectin in pregnant adolescents between 30 and 36 weeks of gestation.

Method: A prospective cross-sectional study enrolled 67 normal pregnant women between 30 and 36 weeks of gestation and eutrophic (body mass index [BMI]: 18.5–25 kg/m^2), of which 36 were adolescents ($<$ 20 years old) and 31 adults ($\geq$ 20 years old). Serum adiponectin levels were determined by enzyme-linked immunosorbent assay (ELISA). The *t*-student or Mann-Whitney tests were used for intergroup comparison.

Results Pregnant adolescents showed significantly higher serum adiponectin concentrations compared with pregnant adults ($p = 0.04$). No differences were observed in adiponectin levels in younger pregnant adolescents ($<$ 16 years old) compared with older pregnant adolescents ($\geq$ 16 years old). Adiponectin values were divided into 3 subgroups: $<$ 3,000 ng/mL, between 3,000 and 5,000 ng/mL, and $>$ 5,000 ng/mL. Birthweight was significantly higher in women $>$ 5,000 ng/mL when compared with $<$ 3,000 ng/mL in the adolescent group. No association between pregestational adiponectin levels and BMI, gestational weight gain, and gestational age was observed; however, there was a positive relation with birthweight ($p = 0.0239$).

Conclusion Serum adiponectin values in pregnant adolescents between 30 and 36 weeks of gestation were higher compared with pregnant adults; however, no differences between younger and older pregnant adolescents were observed.

Keywords
- adiponectin
- adolescence
- gestation
- inflammation

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Resumo

Objetivo Avaliar os níveis séricos de adiponectina em gestantes adolescentes entre 30 e 36 semanas de gestação.

Métodos Estudo prospectivo e transversal incluindo 67 gestantes normais entre 30 a 36 semanas e eutróficas (índice de massa corporal [IMC]: 18,5–25 kg/m^2), sendo 36 adolescentes ($<$ 20 anos) e 31 adultas ($\geq$ 20 anos). Os níveis séricos de adiponectina foram avaliados por teste imunoenzimático (ELISA, na sigla em inglês). Para a comparação entre os grupos, utilizou-se os testes t-Student ou Mann-Whitney.

Resultados As gestantes adolescentes apresentaram significativamente maiores concentrações séricas de adiponectina do que as adultas ($p = 0{,}04$). Não houve diferenças nos níveis de adiponectina quando comparadas as gestantes adolescentes precoces ($<$ 16 anos) às tardias ($\geq$ 16 anos). Os valores de adiponectina foram subdivididos em 3 grupos: $<$ 3.000 ng/mL, entre 3.000 e 5.000 ng/mL e $>$ 5.000 ng/mL. O peso do recém-nascido foi significantemente maior nas mulheres com $>$ 5.000 ng/mL, quando comparadas as com $<$ 3.000 ng/mL no grupo das adolescentes. Não foi observada associação entre os níveis de adiponectina e o IMC pré-gestacional, ganho de peso gestacional e a idade gestacional, porém houve relação positiva com o peso do recém-nascido ($p = 0{,}0239$).

Conclusão Os valores séricos de adiponectina em gestantes adolescentes entre 30 e 36 semanas de gestação foram maiores do que os das gestantes adultas; contudo, sem diferenças entre gestantes adolescentes precoces e tardias.

Palavras-chave

- adiponectina
- adolescência
- gestação
- inflamação

Introduction

Adiponectin is a polypeptide hormone abundantly produced and secreted by adipose tissue that regulates metabolism by interfering in insulin resistance in hepatic and cellular territories.[1] It stimulates glucose uptake by adipocytes and myocytes and directly activates adenosine monophosphate activated protein kinase (AMPK), acting as an insulin sensitizer. The main metabolic effects of adiponectin include glucose and lipid metabolism regulation through fatty acid oxidation stimulation, suppression of hepatic glucose production, and increased insulin sensitivity in liver and muscle tissue.[2,3] In contrast to other hormones secreted by the adipose tissue, its serum level decreases as adiposity increases and are negatively correlated with obesity, insulin resistance, and metabolic syndrome.[1,4] Furthermore, it presents other roles, presenting antihyperglycemic, antiatherogenic and anti-inflammatory properties.[5,6]

Adiponectin is produced abundantly by adipose tissue and circulates at high concentration, in contrast to other adipokines. Although it is secreted by adipocytes, plasma adiponectin concentration is paradoxically lower in patients with type 2 diabetes mellitus, cardiovascular diseases, obesity, and in smokers.[7] Weight reduction in obese individuals is accompanied by an increase in plasma adiponectin concentration, suggesting that adipose tissue can exert a negative feedback on adiponectin production and secretion.[8,9]

Serum adiponectin levels differ according to gender, being higher in women compared with men, even after matching for weight and body mass index (BMI).[10] In adolescents, no change in serum adiponectin concentration is observed between genders. However, studies showed that adiponectin levels, similar to what is observed in adults, are lower in obese adolescents and in pubescents. These values relate negatively to age and are significantly lower in puberty compared with the prepuberal period. Puberty is associated with decreased insulin sensitivity and changes in serum adiponectin concentrations.[11,12]

Age effects in adiponectin production is still controversial, but many studies could observe differences in adiponectin levels between ages. It seems that adiponectin levels increase with advancing age,[13–17] and an experimental study indicated that estrogens have the ability to inhibit adiponectin production.[18] There are still few studies about adiponectin and puberty, but Lausten-Thomsen et al.[19] showed that, in adolescent women, adiponectin levels increase with increasing age and demonstrated how age- and sex-specific reference curves for adipokines are still necessary.

During pregnancy, there is a hypothesis that adiponectin may also play an important role in insulin resistance.[20] Lower concentrations of adiponectin have been consistently reported in patients with gestational diabetes mellitus (GDM) when compared with patients with a healthy pregnancy.[21,22]

Since adiponectin has an essential role in insulin metabolism and that glucose and insulin are crucial for fetal growth, maternal adiponectin may play an important role in fetal development; however, the literature results about this association are still controversial.[23–25]

The aim of the present study was to evaluate serum adiponectin concentration in pregnant adolescents between 30 and 36 weeks of gestation.

Methods

A prospective cross-sectional study was conducted with eutrophic (BMI between 18.5 and 25 kg/m^2) pregnant adolescents (< 20 years old) and adults (≥ 20 years old), between 30 and 36 weeks of gestation. The subjects were selected from the Ambulatory of Prenatal Physiology of the Department of Obstetrics of Universidade Federal de São Paulo (UNIFESP, in the Portuguese acronym). The exclusion criteria were multiple pregnancies or chronic maternal diseases, such as arterial hypertension, pregestational diabetes mellitus or systemic lupus erythematosus. The present study was approved by UNIFESP's Ethics Committee (nº 1714/10), and all subjects signed the informed consent form.

A blood sample of 8 mL was drawn by venipuncture from the pregnant subject in a sterile and dry tube with separation gel (BD Diagnostics, Franklin Lakes, NJ, USA). The sample collected was centrifuged after clot retraction, and the obtained serum was aliquoted and stored in sterile microtube in a freezer at -80° C. Serum adiponectin levels were determined by enzyme-linked immunosorbent assay (ELISA) capture method using Quantikine-Human Adiponectin/Acrp30 DuoSet (R&D [R&D Systems Inc., Minneapolis, MN, USA]) commercial kit. This is an immunoenzymatic assay based on the sandwich technique performed according to the instructions of the manufacturer. Adiponectin sensitivity was 62.5 pg/mL.

Descriptive statistics with mean, median, minimum and maximum values and standard deviation (SD) was performed for all quantitative variables, and frequency analysis was performed for qualitative variables. The Kolmogorov-Smirnov or Shapiro-Wilk tests and Skewness and Kurtosis values were used to evaluate distribution for quantitative variables. Intergroup comparisons of quantitative variables were made using the *t*-Student test when distribution was normal and the Mann-Whitney test when distribution was non-normal.

Factorial analysis of variance (ANOVA) was used to compare the means of categorical variables by adiponectin concentration (ng/mL) and gestational group (adolescents and adults). When significant differences were observed by F statistic, the post-hoc Fisher LSD test was used to determine those differences.

Linear regression analysis was used to assess the association between adiponectin concentration and the other independent variables, with a 95% confidence interval (CI). Comparisons between adolescent and adult regressions were made using the *t*-Student test, and in the presence of significant differences, regression analyses were performed to define the better adjustment of curves.

To determine the influence of the variables study group, age, race, BMI, systolic and diastolic blood pressures, gestational age, pregnancy numbers, and birthweight on adiponectin concentration, a Stepwise Forward linear regression analysis was performed. Intergroup comparisons of qualitative variables were made using the chi-squared test, Fischer exact tests or G-tests.

The analyses were performed using GraphPad Prism 5.0 statistical package (GraphPad Software, San Diego, CA, USA). A statistical significance of $p < 0.05$ was adopted.

Results

A total of 143 pregnant adolescents were followed-up, and blood samples were collected from 60 of them. In accordance with the exclusion criteria, 24 pregnant subjects were excluded, and, therefore, 36 were included (►**Fig. 1**). Thirty-one pregnant adults were selected, all with the same gestational period, absence of chronic maternal diseases and within the same BMI interval.

The age for pregnant adolescents varied from 13 to 19 years old, average of 16.53 years old, whereas in adults the age varied from 20 to 38 years old, average of 28.06 years old. Regarding marital status, the majority of the adolescents was single (63.9%), whereas adults were married (35.5%) or in a stable union (41.9%). ►**Table 1** presents the sociodemographic characteristics of the study population.

In relation to the number of pregnancies and parturition, there was a significant intergroup difference, with fewer pregnancies ($p < 0.0001$) and lower numbers of children ($p < 0.0001$) in adolescents compared with adults. When we assessed gestational age at the time of collection and birthweight, no significant difference was observed between both groups (►**Table 1**). Serum adiponectin concentrations were significantly higher in pregnant adolescents compared with adults ($p = 0.04$) (►**Fig. 2**).

In the younger adolescent group (< 16 years old), the age varied from 13 to 15 years old, with an average of 14.25 years old. In turn, in the older adolescent group (≥ 16 years old), the average age was 17.67 years old, varying from 16 to 19 years old. Older adolescents had more years of study (10.96 years) compared with the younger group (8.83 years). Regarding race, marital status and salaried work, there was no difference between groups (►**Table 2**).

Serum adiponectin concentrations showed no significant differences in both pregnant adolescent subgroups ($p = 0.57$). We categorized adiponectin levels in 3 groups: < 3,000 ng/mL, between 3,000 ng/mL and 5,000 - ng/mL, and > 5,000 ng/mL; and evaluated the intergroup

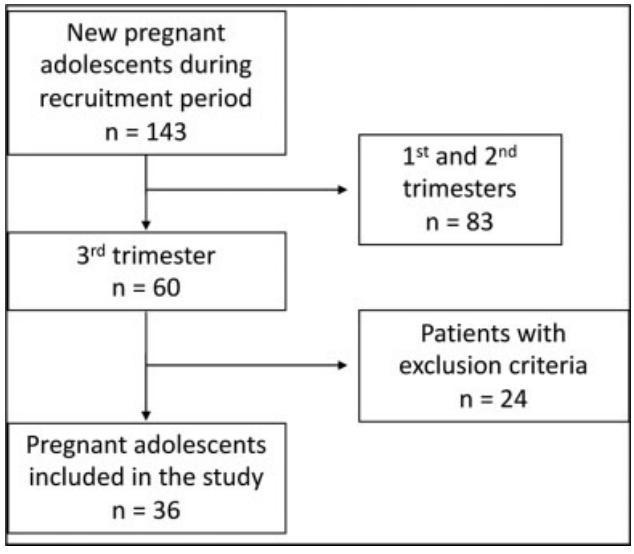

Fig. 1 Flow chart of the selection of pregnant adolescents.

Table 1 Sociodemographic, obstetric and perinatal characteristics of pregnant adolescents and adults

Variable	Adolescents $n = 36$	Adults $n = 31$	*p-value*
Age (years old)[a]	16.53 (1.98)	28.06 (4.99)	$< 0.0001^{*}$
Race[b]			0.19^{**}
White	13 (36.1%)	13 (41.9%)	
Multiracial	15 (41.7%)	16 (51.6%)	
Black	8 (22.2%)	2 (6.5%)	
Marital status[b]			0.003^{**}
Single	23 (63.9%)	6 (19.4%)	
Married	5 (13.9%)	11 (35.5%)	
Stable union	8 (22.2%)	13 (41.9%)	
Divorced	0 (0%)	1 (3.2%)	
Years of study (years)[a]	10.25 (1,96)	10.89 (2,13)	0.22^{*}
Salaried work[b]			$< 0.0001^{\dagger}$
Yes	8 (22.2%)	22 (71.0%)	
No	28 (77.8%)	9 (29.0%)	
Smoking[b]			$0.02^{\dagger}$
Yes	1 (2.8%)	7 (22.6%)	
No	35 (97.2%)	24 (78.4%)	
Alcohol abuse[b]			$0.002^{\dagger}$
Yes	1 (2.8%)	10 (32.3%)	
No	35 (97.2%)	21 (67.7%)	
BMI (Kg/m^2)[a]	21.59 (2.20)	21.25 (1.62)	0.48^{*}
GA at collection (weeks) [a]	32.45 (1.58)	33.19 (1.51)	0.055^{*}
Number of pregnancies[b]			$< 0.0001^{\dagger}$
1	35 (97.2%)	11 (35.5%)	
≥ 2	1 (2.8%)	20 (64.5%)	
Parturition[b]			$< 0.0001^{**}$
0	35 (97.2%)	14 (45.2%)	
1	1 (2.8%)	12 (38.7%)	
≥ 2	0 (0%)	5 (16.1%)	
Birthweight (grams)[a]	3103 (570.5)	3065 (245.0)	$0.72^{\S}$

Abbreviations: BMI, body mass index; GA, gestational age.
[a]mean (standard deviation).
[b]absolute number (percentage).
[*]*t*-Student Test.
[**]Chi-squared Test.
[†]Fisher Test.
[§]Welch adjusted *t*-Student Test.

difference and association with the independent variables. Factorial ANOVA was used to compare means ($\pm$SD) of categorical variables by adiponectin concentration (ng/mL) and gestational group (adolescents and adults). When significant differences were observed by F statistic, the post-hoc Fisher LSD test was used to determine those differences. It was possible to observe that adiponectin categories were

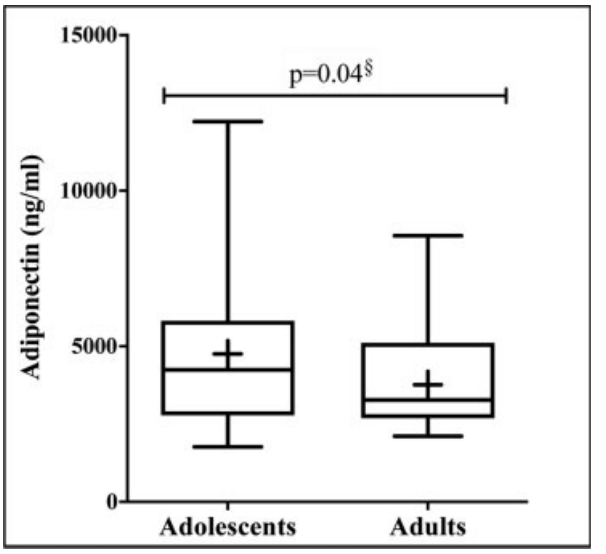

Fig. 2 Serum adiponectin levels in pregnant adolescents ($n = 36$) and adults ($n = 31$)
[§]Welch's adjusted *t*-Student Test.

Table 2 Sociodemographic characteristics of younger and older adolescents

Variable	Adolescents <16 $n = 12$	Adolescents ≥16 $n = 24$	*p-value*
Age (years old)[a]	14.25 (0.75)	17.67 (1.27)	$< 0.0001^{*}$
Race[b]			0.82^{**}
White	5 (41.7%)	8 (33.3%)	
Multiracial	5 (41.7%)	10 (41.7%)	
Black	2 (16.6%)	6 (25.0%)	
Marital status[b]			0.13^{**}
Single	6 (50.0%)	17 (70.8%)	
Married	1 (8.3%)	4 (16.7%)	
Stable union	5 (41.7%)	3 (12.5%)	
Years of study (years) [a]	8.83 (1.40)	10.96 (1.83)	0.001^{*}
Salaried work[b]			$0.22^{\dagger}$
Yes	11 (91.7%)	17 (70.8%)	
No	1 (8.3%)	7 (29.2%)	
Smoking[b]			$0.33^{\dagger}$
Yes	1 (8.3%)	0 (0%)	
No	11 (91.7%)	24 (100%)	
Alcohol abuse[b]			$1.00^{\dagger}$
Yes	0 (0%)	1 (4.2%)	
No	12 (100%)	23 (95.8%)	
BMI (Kg/m^2) [a]	20.95 (1.97)	21.91 (2.28)	0.22^{*}

Abbreviation: BMI, body mass index.
[a]mean (standard deviation).
[b]absolute number (percentage).
[*]*t*-Student Test.
[**]Chi-square Test.
[†]Fisher Test.

Table 3 Influence of independent variables on adiponectin concentration (ng/mL) according to pregnancy group

	Adolescents			Adults			Statistic F		
	< 3,000 (*n* = 10)	3,000–5,000 (*n* = 12)	> 5,000 (*n* = 14)	< 3,000 (*n* = 12)	3,000–5000 (*n* = 11)	> 5,000 (*n* = 8)	$F_{(1, 66)}$; *p-value**	$F_{(2, 66)}$; *p-value***	$F_{(2, 66)}$; *p-value****
Gestational age (days)	224.20 (11.16)	230.50 (11.67)	226.43 (10.54)	230.83 (9.76)	233.91 (14.09)	230.63 (9.16)	2.91; 0.09	1.08; 0.34	0.12; 0.88
Weight gain (kg)	13.45 (4.42)	13.47 (4.69)	13.34 (5.02)	10.85 (4.66)	10.47 (4.30)	11.94 (4.28)	4.15; 0.05	0.12; 0.89	0.17; 0.84
BMI (kg/m^2)	21.26 (1.68)	21.53 (2.66)	21.89 (2.21)	20.83 (1.75)	21.16 (1.26)	22.00 (1.81)	0.22; 0.64	1.12; 0.33	0.12; 0.89
SBP (mm Hg)	104.00 (13.50)	105.83 (14.43)	107.14 (9.14)	90.00 (11.28)	102.73 (11.04)[a,§]	95.00 (10.69)[§]	11.17; < 0.01	2.15; 0.13	1.38; 0.26
DBP (mm Hg)	65.00 (10.80)	70.00 (11.28)	69.29 (11.41)	54.17 (9.00)[§]	65.45 (11.28)[a]	58.75 (12.46)[§]	10.00; < 0.01	3.06; 0.05	0.58; 0.56
Birth weight (g)	2828.00 (564.88)	3037.58 (525.60)	3355.71 (539.18)[a]	2950.83 (210.00)	3056.36 (210.00)	3248.13 (310.11)	0.01; 0.91	5.01; 0.01	0.39; 0.68

Abbreviations: BMI, body mass index; DBP: diastolic blood pressure; SBP, systolic blood pressure.
*Adolescents and adults.
**Categories of adiponectin concentration.
***Interaction between 'adolescents and adults' and 'categories of adiponectin concentration'.
[a]Significant difference in adiponectin concentration < 3,000 ng/mL.
[§]Significant difference for adolescents.

related to birth weight, regardless of the pregnancy group (►**Table 3**).

The association of serum adiponectin levels with the independent variables (gestational age, weight gain, BMI, systolic and diastolic blood pressures, and birthweight) was also evaluated using univariate linear regression. A positive relation between adiponectin levels and birthweight was observed in all assessed pregnant subjects ($p = 0.0239$).

To determine the influence of variables on adiponectin concentration, a Stepwise Forward linear regression analysis was performed. In a first analysis of the main components that might influence adiponectin concentration in pregnant subjects, it was observed that some variables (race, pregnancy numbers, gestational age, and systolic and diastolic blood pressures) were not important to the model, considering the modification they produced together in the model (R^2 variation = 0.31%), absence of statistical significance ($F_{(9,57)} = 1.56$; $p = 0.15$), and lack of model adjustment ($R^2 = 0.20$; $R^2_{adjusted} = 0.07$).

A second analysis was performed. When just the "birthweight" variable was included, it resulted in a model statistically significant ($F_{(1,65)} = 5.35$; $p = 0.02$), but with poor association (R = 0.28). When "Birth weight" and "Age" variables were included, it resulted in a statistically significant model ($F_{(2,64)} = 4.75$; $p = 0.01$), but with a weak association (R = 0.36). Next, when the "birthweight," "age" and "group (adolescents and adults)" variables were included, it resulted in a statistically significant model ($F_{(3,63)} = 4.46$; $p = 0.01$), but still with weak association (R = 0.42). Then, when the "birthweight," "age," "group (adolescents and adults)" and "BMI" variables were included, it resulted in a model with moderate association (R = 0.44), statistically significant ($F_{(4,62)} = 3.74$; $p = 0.01$), and with a better adjustment ($R^2 = 0.20$; $R^2_{adjusted} = 0.15$).

Discussion

In the present study, it was observed that pregnant adolescents showed higher serum adiponectin levels compared with pregnant adults. Rasmussen-Torvik et al.[26] assessed serum adiponectin concentrations in male and female adolescents from 15 to 22 years old and observed higher levels in those with an average age of 15 years old compared with adolescents between 19 and 22 years old. In this study, the BMI was higher in older adolescents, from 19 to 22 years old, displaying greater abdominal circumference. Fifteen-year-old subjects showed a lower BMI, smaller abdominal circumference, and higher adiponectin concentration. The authors concluded that insulin sensitivity in younger adolescents was related to visceral fat, whereas adiponectin was associated with subcutaneous fat.[26] Among adult women, serum adiponectin levels tend to decrease as weight increases, in relation to an increase in adiposity, causing BMI changes.[16]

Our results revealed higher adiponectin values in pregnant adolescents regardless of the age group. When younger adolescents (< 16 years old) were analyzed, no differences in serum adiponectin levels were observed when compared with older adolescents (≥ 16 years old). Both groups were highly heterogeneous regarding social, clinical, and obstetric characteristics, but with no differences in adiponectin levels between them. A possible explanation would be the small number of younger adolescents, in which higher adiponectin levels are expected. All adolescents had already experienced their respective menarche, so despite of separating younger from older adolescents, the hormonal variations probably responsible for the changes in blood adiponectin levels were not so evident in these groups.

Another important aspect of the present study was the racial balance between groups. Adiponectin values are strongly hereditary and are linked to genes that can be changed by race-dependent polymorphisms. Genetic load interferes with the prevalence of overweight or obesity in the studied population.[27] A significant association was observed between adiponectin values and single nucleotide polymorphism of the gene coding this protein. These changes are observed mainly in white women, but not among black women, reinforcing the difference observed between races.[28]

Another factor that could interfere in adiponectin concentration would be weight gain during pregnancy and, again, there was no association in our study. Some studies revealed a negative correlation between adiponectin and maternal BMI;[29,30] however, in relation to our study, other studies did not attain the same results.[31,32]

Adipokines not only influence maternal metabolism during pregnancy but may also affect fetal growth.[33] Our study showed a positive association between adiponectin levels and birthweight in all pregnant subjects evaluated. When the groups are studied separately, this association is demonstrated only in pregnant adolescents.

Pregnant adolescents with adiponectin levels $> 5{,}000$ ng/mL seem to give birth to more babies with adequate weight ($\sim 3{,}000$ g) compared with subjects with adiponectin values $< 3{,}000$ ng/mL that had insufficient average birthweight ($< 3{,}000$ g). Our findings are similar to those observed by Mazaki-Tovi et al.,[34] who showed that maternal adiponectin levels are decreased when newborns present lower birthweight ($< 2{,}999$ g), described as insufficient.

The exact mechanism of how maternal adiponectin levels can affect birthweight still deserves more investigation. While there are studies that did not find this association,[25,35] other authors observed a negative association.[36] However, our result corroborates with another recent study that showed a positive association between maternal adiponectin and birthweight.[24] The association between maternal serum and umbilical cord adiponectin levels has been investigated, but the results are also conflicting. While some authors described an association between maternal and umbilical cord adiponectin,[23] others observed the opposite.[25] Aye et al.[37] proposed a mechanism by how adiponectin could affect birthweight, indicating that maternal adiponectin decreases placental insulin-signaling in the placenta, inhibiting fetal growth.

Conclusion

In summary, we observed that serum adiponectin values were higher in pregnant adolescents than in pregnant adults; however, with no differences between younger and older pregnant adolescents. In addition to that, a significant difference in birthweight was observed when the categories of serum adiponectin concentration $> 5{,}000$ ng/mL and $< 3{,}000$ ng/mL were compared in pregnant adolescents.

Contributors
All authors were involved in the design and interpretation of the analyses, contributed to the writing of the manuscript, read and approved the final manuscript.

Conflict of Interests
The authors have no conflict of interests to declare.

References

1 Catalano PM, Hoegh M, Minium J, Huston-Presley L, Bernard S, Kalhan S, et al. Adiponectin in human pregnancy: implications for regulation of glucose and lipid metabolism. Diabetologia. 2006;49(07):1677–1685. Doi: 10.1007/s00125-006-0264-x
2 Scherer PE. Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes. 2006;55(06):1537–1545. Doi: 10.2337/db06-0263
3 Hada Y, Yamauchi T, Waki H, Tsuschida A, Hara K, Yago H, et al. Selective purification and characterization of adiponectin multimer species from human plasma. Biochem Biophys Res Commun. 2007;356(02):487–493. Doi: 10.1016/j.bbrc.2007.03.004
4 Mazaki-Tovi S, Romero R, Vaisbuch E, Erez O, Mittal P, Chaiworapongsa T, et al. Maternal serum adiponectin multimers in gestational diabetes. J Perinat Med. 2009;37(06):637–650. Doi: 10.1515/JPM.2009.101
5 Mazaki-Tovi S, Romero R, Kusanovic JP, Erez O, Vaisbuch E, Gotsch F, et al. Adiponectin multimers in maternal plasma. J Matern Fetal Neonatal Med. 2008;21(11):796–815. Doi: 10.1080/14767050802266881
6 Low CF, Mohd Tohit ER, Chong PP, Idris F. Adiponectin SNP45TG is associated with gestational diabetes mellitus. Arch Gynecol Obstet. 2011;283(06):1255–1260. Doi: 10.1007/s00404-010-1548-4
7 Zavalza-Gómez AB, Anaya-Prado R, Rincón-Sánchez AR, Mora-Martínez JM. Adipokines and insulin resistance during pregnancy. Diabetes Res Clin Pract. 2008;80(01):8–15. Doi: 10.1016/j.diabres.2007.12.012
8 Gimeno RE, Klaman LD. Adipose tissue as an active endocrine organ: recent advances. Curr Opin Pharmacol. 2005;5(02):122–128. Doi: 10.1016/j.coph.2005.01.006
9 Hutley L, Prins JB. Fat as an endocrine organ: relationship to the metabolic syndrome. Am J Med Sci. 2005;330(06):280–289. Doi: 10.1097/00000441-200512000-00005
10 Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, et al. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem. 2003;278(41):40352–40363. Doi: 10.1074/jbc.M300365200
11 Andersen KK, Frystyk J, Wolthers OD, Heuck C, Flyvbjerg A. Gender differences of oligomers and total adiponectin during puberty: a cross-sectional study of 859 Danish school children. J Clin Endocrinol Metab. 2007;92(05):1857–1862. Doi: 10.1210/jc.2006-2310
12 Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics. 2005;115(03):e290–e296. Doi: 10.1542/peds.2004-1808
13 Staiger H, Tschritter O, Machann J, Thamer C, Fritsche A, Maerker E, et al. Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. Obes Res. 2003;11(03):368–372. Doi: 10.1038/oby.2003.48
14 Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Retzlaff BM, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia. 2003;46(04):459–469. Doi: 10.1007/s00125-003-1074-z

15 Koh SJ, Hyun YJ, Choi SY, Chae JS, Kim JI Y, Sungha Park, et al. Influence of age and visceral fat area on plasma adiponectin concentrations in women with normal glucose tolerance. Clin Chim Acta. 2008;389(1-2):45–50. Doi: 10.1016/j.cca.2007.11.017
16 Lecke SB, Morsch DM, Spritzer PM. Leptin and adiponectin in the female life course. Braz J Med Biol Res. 2011;44(05):381–387
17 Mancuso P, Bouchard B. The impact of aging on adipose function and adipokine synthesis. Front Endocrinol (Lausanne). 2019; 10:137. Doi: 10.3389/fendo.2019.00137
18 Combs TP, Berg AH, Rajala MW, Klebanov S, Iyengar P, Jimenez-Chillaron JC, et al. Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. Diabetes. 2003;52(02):268–276. Doi: 10.2337/diabetes.52.2.268
19 Lausten-Thomsen U, Lund MAV, Frithioff-Bøjsøe C, Hedley PL, Pedersen O, Hansen T, et al. Reference values for leptin/adiponectin ratio in healthy children and adolescents. Clin Chim Acta. 2019;493:123–128. Doi: 10.1016/j.cca.2019.03.004
20 Leung KC, Xu A, Craig ME, Martin A, Lam KS, O'Sullivan AJ. Adiponectin isoform distribution in women–relationship to female sex steroids and insulin sensitivity. Metabolism. 2009;58 (02):239–245. Doi: 10.1016/j.metabol.2008.09.020
21 Hara K, Yamauchi T, Kadowaki T. Adiponectin: an adipokine linking adipocytes and type 2 diabetes in humans. Curr Diab Rep. 2005;5(02):136–140. Doi: 10.1007/s11892-005-0041-0
22 Georgiou HM, Lappas M, Georgiou GM, Marita A, Bryant VJ, Hiscock R, et al. Screening for biomarkers predictive of gestational diabetes mellitus. Acta Diabetol. 2008;45(03):157–165. Doi: 10.1007/s00592-008-0037-8
23 Ballesteros M, Simón I, Vendrell J, Ceperuelo-Mallafré V, Miralles RM, Albaiges G, et al. Maternal and cord blood adiponectin multimeric forms in gestational diabetes mellitus: a prospective analysis. Diabetes Care. 2011;34(11):2418–2423. Doi: 10.2337/dc11-0788
24 Ma L, Lu Q, Ouyang J, Huang J, Huang S, Jiao C, et al. How are maternal dietary patterns and maternal/fetal cytokines associated with birth weight? A path analysis. Br J Nutr. 2019;121(10): 1178–1187. Doi: 10.1017/S0007114519000382
25 Fazeli Daryasari SR, Tehranian N, Kazemnejad A, Razavinia F, Tork Tatari F, Pahlavan F. Adiponectin levels in maternal serum and umbilical cord blood at birth by mode of delivery: relationship to anthropometric measurements and fetal sex. BMC Pregnancy Childbirth. 2019;19(01):344. Doi: 10.1186/s12884-019-2460-y
26 Rasmussen-Torvik LJ, Pankow JS, Jacobs DR Jr, Steinberger J, Moran A, Sinaiko AR. Development of associations among central adiposity, adiponectin and insulin sensitivity from adolescence to young adulthood. Diabet Med. 2012;29(09):1153–1158. Doi: 10.1111/j.1464-5491.2012.03726.x
27 Rasmussen-Torvik LJ, Wassel CL, Ding J, Carr J, Cushman M, Jenny N, et al. Associations of body mass index and insulin resistance with leptin, adiponectin, and the leptin-to-adiponectin ratio across ethnic groups: the Multi-Ethnic Study of Atherosclerosis (MESA). Ann Epidemiol. 2012;22(10):705–709. Doi: 10.1016/j.annepidem.2012.07.011
28 Cohen SS, Gammon MD, North KE, Milikan RC, Lange EM, Williams SM, et al. ADIPOQ, ADIPOR1, and ADIPOR2 polymorphisms in relation to serum adiponectin levels and BMI in black and white women. Obesity (Silver Spring). 2011;19(10):2053–2062. Doi: 10.1038/oby.2010.346
29 Fuglsang J, Skjaerbaek C, Frystyk J, Flyvbjerg A, Ovesen P. A longitudinal study of serum adiponectin during normal pregnancy. BJOG. 2006;113(01):110–113. Doi: 10.1111/j.1471-0528.2005.00792.x
30 Gueuvoghlanian-Silva BY, Torloni MR, Mattar R, Oliveira LS, Scomparini FB, Nakamura MU, et al. Profile of inflammatory mediators in gestational diabetes mellitus: phenotype and genotype. Am J Reprod Immunol. 2012;67(03):241–250. Doi: 10.1111/j.1600-0897.2011.01090.x
31 Mazaki-Tovi S, Kanety H, Pariente C, Hemi R, Wiser A, Schiff E, et al. Maternal serum adiponectin levels during human pregnancy. J Perinatol. 2007;27(02):77–81. Doi: 10.1038/sj.jp.7211639
32 Kajantie E, Kaaja R, Ylikorkala O, Andersson S, Laivuori H. Adiponectin concentrations in maternal serum: elevated in preeclampsia but unrelated to insulin sensitivity. J Soc Gynecol Investig. 2005;12(06):433–439. Doi: 10.1016/j.jsgi.2005.04.006
33 Briana DD, Malamitsi-Puchner A. The role of adipocytokines in fetal growth. Ann N Y Acad Sci. 2010;1205:82–87. Doi: 10.1111/j.1749-6632.2010.05650.x
34 Mazaki-Tovi S, Romero R, Vaisbuch E, Erez O, Mittal P, Chaiworapongsa T, et al. Maternal serum adiponectin multimers in patients with a small-for-gestational-age newborn. J Perinat Med. 2009;37(06):623–635. Doi: 10.1515/JPM.2009.128
35 Zhang ZQ, Lu QG, Huang J, Jiao CY, Huang SM, Mao LM. Maternal and cord blood adiponectin levels in relation to post-natal body size in infants in the first year of life: a prospective study. BMC Pregnancy Childbirth. 2016;16(01):189. Doi: 10.1186/s12884-016-0978-9
36 Dawczynski K, de Vries H, Beck JF, Schleußner E, Wittig S, Proquitté H Adiponectin serum concentrations in newborn at delivery appear to be of fetal origin. J Pediatr Endocrinol Metab. 2014;27(3-4):273–278. Doi: 10.1515/jpem-2013-0218
37 Aye IL, Powell TL, Jansson T. Review: Adiponectin–the missing link between maternal adiposity, placental transport and fetal growth? Placenta. 2013;34(Suppl):S40–S45. Doi: 10.1016/j.placenta.2012.11.024

Risk Factors for Intrapartum Cesarean Section Delivery in Low-risk Multiparous Women Following at Least a Prior Vaginal Birth (Robson Classification 3 and 4)

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Abstract

Objective The aim of the present study was to evaluate the risk factors for cesarean section (C-section) in low-risk multiparous women with a history of vaginal birth.

Methods The present retrospective study included low-risk multiparous women with a history of vaginal birth who gave birth at between 37 and 42 gestational weeks. The subjects were divided into 2 groups according to the mode of delivery, as C-section Group and vaginal delivery Group. Risk factors for C-section such as demographic characteristics, ultrasonographic measurements, smoking, weight gain during pregnancy (WGDP), interval time between prior birth, history of macrosomic birth, and cervical dilatation at the admission to the hospital were obtained from the charts of the patients. Obstetric and neonatal outcomes were compared between groups.

Results The most common C-section indications were fetal distress and macrosomia (33.9% [$n = 77$ and 20.7% [$n = 47$] respectively). A bivariate correlation analysis demonstrated that mothers aged > 30 years old (odds ratio [OR]: 2.09; 95% confidence interval [CI]: 1.30–3.34; $p = 0.002$), parity >1 (OR: 1.81; 95%CI: 1.18–2.71; $p = 0.006$), fetal abdominal circumference (FAC) measurement > 360 mm (OR: 34.20; 95%CI: 8.04—145.56; $p < 0.001$)) and < 345 mm (OR: 3.06; 95%CI: 1.88–5; $p < 0.001$), presence of large for gestational age (LGA) fetus (OR: 5.09; 95%CI: 1.35–19.21; $p = 0.016$), premature rupture of membranes (PROM) (OR: 1.52; 95%CI: 1–2.33; $p = 0.041$), and cervical dilatation < 5cm at admission (OR: 2.12; 95%CI: 1.34–3.34; $p = 0.001$) were associated with the group requiring a C-section.

Conclusion This is the first study evaluating the risk factors for C-section in low-risk multiparous women with a history of vaginal birth according to the Robson classification 3 and 4. Fetal distress and suspected fetal macrosomia constituted most of the C-section indications.

Keywords
- cesarean section
- vaginal delivery
- risk factors
- fetal abdominal circumference
- cervical dilatation

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Introduction

Increasing cesarean section (C-section) rates are becoming a concern especially in countries with higher C-section rates. The C-section rates have increased gradually in recent years, especially in middle- and high-income countries, without any increase in indications or strict medical reasons.[1] Cesarean section rates > 15% are not recommended by the World Health Organization (WHO).[2] In Turkey, the C-section rate among all deliveries has increased from 21% in 2012 to 53% in 2015.[3] Various reasons for why mothers and obstetricians prefer C-section have been postulated for this increase, including prior C-section deliveries, advanced maternal age, systemic diseases such as hypertension and diabetes mellitus, multiple pregnancies, fetal distress, macrosomic fetus, malpresentation of fetus, cephalopelvic disproportion, prolonged labor, and insufficient supplementary health network.[4]

In recent years, the rate of incidences requiring a C-section is steadily increasing all over the world. Advanced maternal age, chronic health problems, multiple pregnancies as a result of the development of assisted reproductive technologies, and an insufficient supplementary health network can be considered as the reasons why mothers and obstetricians prefer a C-section.[5] Notwithstanding, C-section includes some short and long-term risks, such as reduction in fertility, increased risk of maternal mortality and morbidity, poor obstetric outcomes, requirement of treatment in an intensive care unit (ICU), and need for blood transfusion due to the risky surgical procedure. Women who delivered vaginally are much more likely to have a subsequent vaginal birth.[6] Determination of the risk factors that we can change in multiparous women for subsequent C-section may help to reduce unintended primary C-sections. There are previous reports on risk factors of intrapartum C-sections in multiparous women in limited patient groups; however, a detailed analysis including a large cohort have not been reported according to our knowledge. So, we aimed to demonstrate the risk factors for intrapartum C-section in low-risk women with a history of vaginal birth.

Methods

The present retrospective case-control study included low-risk multiparous women with a history of at least 1 prior vaginal birth who gave birth at between 37 and 42 gestational weeks in the University of Health Sciences, Zekai Tahir Burak Woman's Health and Research Hospital, between January 2017 and July 2017. The project was approved by the Institutional Review Board of the hospital (No: 41/2018, February 2018). The women were divided into 2 groups according to the mode of delivery, as Cesarean section (C-section) Group and vaginal delivery Group (Control group). Deliveries were included in the study according to the Robson classification 3 and 4.[6] Women who underwent intrapartum C-section with a history of vaginal birth were enrolled into the study group. The control group was randomly constituted by women who gave birth vaginally. Demographic characteristics, parity, ultrasonographic measurements including estimated fetal weight (EFW), biparietal diameter (BPD), fetal abdominal circumference (FAC), smoking, weight gain during pregnancy (WGDP), interval time between prior birth, history of macrosomic birth, cervical dilatation at the admission to the hospital, obstetric and neonatal outcomes were obtained from the charts and electronic database of the patients. The exclusion criteria included multiple gestation, nonmedical oxytocin induction, previous uterine scarring, maternal fever, gestational diabetes, pregnancy-induced hypertension, oligohydramnios, and any history of chronic systemic disease.

Gestational age was determined by the reported last menstrual period and dating of first-trimester ultrasound measurements. The body mass index (BMI) was calculated as weight divided by height in m^2. Premature rupture of membranes (PROM) was defined as the rupture of membranes before the onset of labor.[7] Infants were classified by gestational age and birthweight into small-for-gestational-age (SGA), appropriate for gestational age (AGA), and large-for gestational age (LGA) categories.[8] Ultrasonography was performed on all patients within $\sim$ 24 hours before delivery to assess the presentation of fetuses, EFW, BPD, FAC, placental site, and amniotic fluid volume. The FAC was measured at the level where the umbilical vein passes through the liver. The BPD was measured as a transverse image of the head with the cursors placed from the leading edge to leading edge of the skull bones. Formulas have been calculated to estimate the fetal weight using combinations of BPD, HC, FL, and AC. The Hadlock formula was used for EFW.[8] According to the Bishop score, oxytocin infusion or in the presence of an unfavorable cervix, a vaginal insert containing 10 mg timed-release dinoprostone (PGE2) was used in cases of medical indications such as ineffective contractions accompanying cervical dilatation and effacement, decreased fetal movements, nonreassuring fetal heart rate, prolonged PROM, and/or post-term pregnancy.[9]

Statistical analyses were performed using SPSS Statistics for Windows, version 17 (SPSS Inc., Chicago, IL, USA). The distribution of the parameters was analyzed by the Kolmogorov-Smirnov and Shapiro-Wilk tests. The continuous variables with normal distribution were presented by means $\pm$ standard deviation (SD) and were compared by the independent samples *t*-test. Nonparametric variables without normal distribution were tested by the Mann-Whitney U test. The chi-squared and the Fisher exact tests were used for categorical data.

For the multivariate analysis, possible risk factors identified in the univariate analyses were further entered into the binary logistic regression analysis to determine independent predictors of C-section. The significance boundary was set at 0.05. In the post-hoc power analysis, the power of the study was found to be between 0.80 and 1 (for age, FAC, cervical dilatation at admission, SGA, PROM, and LGA, the power of the study was 0.80. 0.80, 0.80, 0.84, 0.91, and 1, respectively), with a 0.5 effect size and a 0.05 error rate for 500 participants consisting of 227 subjects in the C-section group and 273 subjects in the Control group (Newton.stat.ubc.ca).

Results

During the study period, 2,268 healthy low-risk multiparous women with a history of vaginal birth who met the inclusion criteria at between 37 and 42 gestational weeks gave birth in our hospital. Of these, 10% of the patients ($n = 227$) had given birth through a C-section. The control group ($n = 228$) was chosen randomly from women giving birth by the vaginal route in the same cohort. The age of the mothers, parity, gestational age at delivery, rate of post-term pregnancy, BMI, birthweight, WGDP, and macrosomia were significantly higher in the C-section Group. The rate of a history of macrosomic birth was higher for the Control group, and the difference was statistically significant: 34 (12%) versus 15 (6%); $p = 0.020$). The incidence of newborns with Apgar 1st minute score < 7 was significantly higher in the C-section group ($p = 0.006$). Also, the rate of neonatal intensive care unit (NICU) admission was significantly higher in the C-section group(7 [2.6%] versus 15 [6.6%]; $p = 0.048$)]. There were no other significant differences between the groups. Demographic, obstetrics and neonatal characteristics are listed in ►**Table 1**. ►**Table 1** also shows the ultrasonographic and labor characteristics of the two groups. The EFW, rate of EFW ≥ 4,000 g, FAC, and rate of PROM were significantly higher in the C-section Group. The cervical dilatation at admission, the requirement of induction, and meconium-stained amnion were higher in the Control group, with a statistically significant difference. The C-section indications were fetal distress (33.9%; $n = 77$), macrosomia (20.7%; $n = 47$), cephalopelvic disproportion (16.3%; $n = 37$),

Table 1 Demographic, obstetrics and neonatal characteristics

Variable	Control group	C-Section group	*p-value*
	(*n* = 273)	(*n* = 227)	
Age (years old) (mean ± SD)	30.24 ± 5.56	32.86 ± 6.38	< 0.001*
Gravidity, median (min-max)	3 (2–7)	3(2–8)	0.051
Parity, median (min-max)	1.2 ± 1.1	2.3 ± 1.4	0.032*
Abortion, median (min-max)	0 (0–2)	0 (0–2)	0.921
BMI (kg/m^2) (mean ± SD)	31.79 ± 3.73	32.59 ± 4.64	0.032*
Gestational age at delivery (weeks) (mean ± SD)	39.06 ± 1.28	39.39 ± 1.25	0.004*
Post-term pregnancy (> 41 weeks) (n, %)	35 (12%)	47 (20%)	0.021*
Birthweight (g) (mean ± SD)	3333 ± 374	3561 ± 588	< 0.001*
SGA, n (%)	14 (5%)	21 (12%)	0.019*
LGA, n (%)	26 (10%)	57 (33%)	< 0.001*
Birthweight > 4,000 g, median (min-max)	13 (5%)	52 (23%)	< 0.001*
Apgar scores n (%)			
1st minute < 7	5 (1.8%)	16 (7%)	0.006*
5th minute < 7	1 (0.4%)	3 (1.3%)	0.334
NICU (n, %)	7 (2.6%)	15 (6.6%)	0.048*
Smoking n (%)	40 (14%)	46 (20%)	0.121
Birthweight of previous child (g) (mean ± SD)	3,448 ± 372	3,387 ± 361	0.065
History of macrosomic birth	34 (12%)	15 (6%)	0.020*
Time interval between previous birth (years) Median (min-max)	4 (2–13)	4 (2–8)	0.735
WGDP (kg) median (min-max)	15 (10–24)	17 (8–28)	0.005*
Estimated fetal weight (g) (mean ± SD)	3351 ± 333	3639 ± 558	< 0.001*
Estimated fetal weight ≥ 4,000 g (n, %)	18 (6.6%)	65 (28.6%)	< 0.001*
BPD (mm) median (min-max)	95 (86–105)	95 (82–100)	0.188
FAC (mm) median (min-max)	339 (228–371)	349 (318–385)	< 0.001*
Cervical dilatation (cm) median (min-max)	4 (2–10)	3 (2–8)	< 0.001*
Requirement of induction (n, %)	134 (35%)	66 (22%)	0.002*
PROM (n, %)	83 (30%)	94 (45%)	0.011*
Meconium stained amnions (n, %)	17 (6%)	5 (2%)	< 0.030*

Abbreviations: BMI, body mass index; BPD, biparietal diameter; FAC, fetal abdominal circumference; LGA, Large for gestational age; NICU, requirement of neonatal intensive care unit; PROM, premature rupture of membranes; SGA, small for gestational age; WGDP, weight gain during pregnancy.
*$p < 0.05$, significant.

Table 2 Result of binary logistic regression analysis for risk of C-section

Variable	Wald	OR (95%CI)	*p-value*
Age > 30 years old	9.522	2.09 (1.30–3.34)	0.002*
Parity > 1	7.408	1.81 (1.18–2.71)	0.006*
BMI > 30 kg/m^2	0.693	1.19 (0.76–1.86)	0.437
LGA	5.794	5.09 (1.35–19.21)	0.016*
SGA	9.641	0.32 (0.15–0.65)	0.002*
PROM	3.833	1.52 (1–2.33)	0.041*
EFW ≥ 4,000 g	0.004	1.04 (1.27–3.89)	0.951
FAC > 360 mm	22.859	34.20 (8.04—145.56)	< 0.001*
FAC < 345 mm	20.172	3.06 (1.88–5)	< 0.001*
Post-term pregnancy (> 41 weeks)	0.509	0.80 (0.44–1.45)	0.475
WGDP >15 kg	2.711	0.70 (0.46–1.07)	0.100
Cervical dilatation < 5 cm	10.525	2.12 (1.34–3.34)	0.001*

Abbreviations: BMI, body mass index; BPD, biparietal diameter; EFW, estimated fetal weight; FAC, fetal abdominal circumference; LGA, large for gestational age; PROM, premature rupture of membrane; SGA, small for gestational age; WGDP, weight gain during pregnancy.
*$p < 0.05$, significant.

malpresentation (14.5%; $n = 33$), failure to progress in labor (12.3%; $n = 28$), and others (2.2%; $n = 5$).

►**Table 2** shows the results of the binary logistic regression analysis. A bivariate correlation analysis demonstrated that mothers aged > 30 years old (odds ratio [OR]: 2.09; 95% confidence interval [CI]: 1.30–3.34; $p = 0.002$), parity >1 (OR: 1.81; 95%CI: 1.18–2.71; $p = 0.006$), fetal abdominal circumference (FAC) measurement > 360 mm (OR: 34.20; 95%CI: 8.04—145.56; $p < 0.001$)) and < 345 mm (OR: 3.06; 95%CI: 1.88–5; $p < 0.001$), presence of large for gestational age (LGA) fetus (OR: 5.09; 95%CI: 1.35–19.21; $p = 0.016$), premature rupture of membranes (PROM) (OR: 1.52; 95%CI: 1–2.33; $p = 0.041$), and cervical dilatation < 5cm at admission (OR: 2.12; 95%CI: 1.34–3.34; $p = 0.001$) were associated with the group requiring a C-section.

Discussion

In the present study, we evaluated the risk factors for C-section in low-risk women with a history of at least one prior vaginal birth. Previous studies demonstrated that the demand for a C-section was associated with a fear of childbirth, previous C-Section, and unfavorable delivery experience.[9] The decision to perform a C-section depends, at least in part, on the presence of several evolving conditions, such as pre-eclampsia, premature PROM, fetal growth restriction, and maternal chronic medical condition in multiparous women.[10] We excluded these parameters in our study. Besides, nowadays, women are older when they give birth, and their BMIs have increased.[11] Ennen et al.[12] showed that advanced maternal age and high BMI increased the possibility of C-section. In addition, the increase in the number of gravidity and parity increases the likelihood of many adverse pregnancy outcomes. In a population-based analysis using an Italian region data including Robson classification 3 and 4, the authors found that increased maternal age was an independent risk factors for C-section.[13] We demonstrated that increased mother's age, gravidity, parity, and BMI were significantly higher in the C-section Group.

In our study, less cervical dilatation at admission was another important risk factor for C-section in multiparous women. Some authors suggested that the increased C-section rate was associated with unfavorable cervix but unaffected by labor induction.[14] Some studies proposed a decrease in C-section delivery with admission at higher cervical dilatation.[15,16] Recent studies showed that the active phase of labor may not start until 6 cm of cervical dilatation; this is consistent with the results of our study.[17] Some retrospective studies have demonstrated the relationship between cervical dilatation upon admission and C-section rates. Holmes et al.[18] showed that C-section rates were significantly higher in women who were admitted with between 0 and 3 cm of cervical dilatation when compared with women who were admitted with between 4 and 10 cm of cervical dilatation among multiparous women (5.7 versus 1.3%; OR: 4.73; 95%CI: 2.64–8.49). Bailit et al.[19] demonstrated that cervical examination with ≤ 4 cm dilatation at admission was associated with significantly increased C-section rates in multiparous women (3.1 versus 1.4%; $p < 0.001$). Recently, a prospective cohort study by Wood et al.[20] found that, especially in multiparous women, lower cervical dilatation at admission was a modifiable risk factor for C-section. Similar to previous studies, we found that women with cervical dilatation < 5 cm at admission were 2 times more likely to undergo a C-section. Fetal distress has been shown to contribute to increase C-section rates. With results similar to ours, Çelik et al.[21] conducted a study in Turkey showing that fetal distress was the most common C-section indication in multiparous women. Intrapartum hypoxia is a condition linked between maternal and neonatal morbidity. Uterine contractions during labor are associated with a reduction in uterine blood flow by up to 60%, which may lead to fetal decompensation, particularly in the presence of inadequate placental function.[22] We found that FAC < 345 mm and PROM were dependent risk factors for C-section delivery after vaginal birth. Also, newborns with 1st minute Apgar score < 7 and requirement of admission to the NICU were higher in the C-section group, and this was consistent with our findings. These conditions may predispose to intrapartum hypoxia, which is clinically associated with fetal heart rate abnormalities.

Fetal macrosomia has potentially serious effects that may result in a traumatic birth for newborns and mothers. Although the cause is unknown in many LGA cases, these factors associated with this condition include maternal diabetes, history of macrosomic delivery, multiparity, pre-pregnancy maternal obesity, excessive WGDP, and post-term pregnancy.[23] Weiner et al.[24] found that the rate of C-section in fetuses estimated ultrasonographically as weighing ≥

4,000 g was 2 times higher than in controls (50.7 versus 24.9%; $p < 0.05$)(37). Some authors support that adverse outcomes such as hemorrhage, shoulder dystocia, brachial plexus injury, and asphyxia during vaginal delivery caused by macrosomia can be prevented by elective C-section or early induction of labor.[24] Also, medicolegal problems that may occur as a result of complications after vaginal delivery may play a role in the preference by part of physicians for C-section.[24] In the regression analysis, we demonstrated that advanced maternal age, increased parity, WGPD, and FAC $>$ 362 mm were a significant factor for C-section.

The main limitation of the present study is its retrospective design. To the best of our knowledge, this is the first study evaluating the risk factors for C-section in low-risk multiparous women with a history of vaginal birth according to Robson classification 3 and 4. A total of 10% of patients had given birth through a C-section. We found that increased maternal age, parity, presence of LGA fetus, FAC $>$ 360 mm or $<$ 345 mm, PROM, and decreased cervical dilatation at admission $<$ 5 cm were significant risk factors for C-section delivery in low-risk multiparous women with history of prior vaginal birth. If we look at the indications, 55% of C-section indications were fetal distress and suspected fetal macrosomia in our study population. Especially low-risk multiparous women with PROM and unfavorable cervical dilatation at admission should be followed-up carefully for the risk of fetal distress. On the other hand, although antenatal suspected macrosomia is associated with a marked increase in C-sections, these cannot provide a significant reduction in the incidence of shoulder dystocia or of birth trauma.[25] Therefore, the management of suspected fetal macrosomia requires clear contact and decision-making between the woman and her physician. Although our study was retrospectively designed, the number of patients was quite sufficient. However, further randomized prospective research is needed for the management of labor in low-risk multiparous women with a history of vaginal birth.

Contributors

All authors were involved in the design and interpretation of the analyses, contributed to the writing of the manuscript, read and approved the final manuscript.

Conflict of Interests

The authors have no conflict of interests to declare.

References

1 Vercellini P, De Giorgi O, Pisacreta A, Pesole AP, Vicentini S, Crosignani PG. Surgical management of endometriosis. Best Pract Res Clin Obstet Gynaecol. 2000;14(03):501–523. Doi: 10.1053/beog.1999.0088

2 Khooshide M, Mirzarahimi T, Akhavan Akbari G. The impact of physiologic and non-physiologic delivery on the mother and neonate outcomes; a comparative study on the primi gravid mothers. J Family Reprod Health. 2015;9(01):13–18

3 Betran AP, Torloni MR, Zhang JJ, Gülmezoglu AMWHO Working Group on Caesarean Section. WHO Statement on Caesarean Section Rates. BJOG. 2016;123(05):667–670. Doi: 10.1111/1471-0528.13526

4 Ministry of Health. Turkey Health Statistics Yearbook 2015. Ankara: General Directorate for Health Research (SAGEM); 2015

5 Ferrari AP, Carvalhaes MA, Parada CM. Association between prenatal and parturition in the supplementary health network and elective cesarean section. Rev Bras Epidemiol. 2016;19(01):75–88. Doi: 10.1590/1980-5497201600010007

6 Betrán AP, Vindevoghel N, Souza JP, Gülmezoglu AM, Torloni MR. A systematic review of the Robson classification for caesarean section: what works, doesn't work and how to improve it. PLoS One. 2014;9(06):e97769. Doi: 10.1371/journal.pone.0097769

7 Practice Bulletins No. Practice bulletins No. 139: premature rupture of membranes. Obstet Gynecol. 2013;122(04):918–930. Doi: 10.1097/01.AOG.0000435415.21944.8f

8 March MI, Warsof SL, Chauhan SP. Fetal biometry: relevance in obstetrical practice. Clin Obstet Gynecol. 2012;55(01):281–287. Doi: 10.1097/GRF.0b013e3182446e9b

9 Mazzoni A, Althabe F, Liu NH, Bonotti AM, Gibbons L, Sánchez AJ, et al. Women's preference for caesarean section: a systematic review and meta-analysis of observational studies. BJOG. 2011;118(04):391–399. Doi: 10.1111/j.1471-0528.2010.02793.x

10 Chiofalo B, Laganà AS, Vaiarelli A, La Rosa VL, Rossetti D, Palmara V, et al. Do miRNAs play a role in fetal growth restriction? A fresh look to a busy corner. BioMed Res Int. 2017;2017:6073167. Doi: 10.1155/2017/6073167

11 Lindstad Løvåsmoen EM, Nyland Bjørgo M, Lukasse M, Schei B, Henriksen L. Women's preference for caesarean section and the actual mode of delivery - Comparing five sites in Norway. Sex Reprod Healthc. 2018;16:206–212. Doi: 10.1016/j.srhc.2018.04.009

12 Ennen CS, Bofill JA, Magann EF, Bass JD, Chauhan SP, Morrison JC. Risk factors for cesarean delivery in preterm, term and post-term patients undergoing induction of labor with an unfavorable cervix. Gynecol Obstet Invest. 2009;67(02):113–117. Doi: 10.1159/000166307

13 Gerli S, Favilli A, Franchini D, De Giorgi M, Casucci P, Parazzini F. Is the Robson's classification system burdened by obstetric pathologies, maternal characteristics and assistential levels in comparing hospitals cesarean rates? A regional analysis of class 1 and 3. J Matern Fetal Neonatal Med. 2018;31(02):173–177. Doi: 10.1080/14767058.2017.1279142

14 Ben-Haroush A, Yogev Y, Bar J, Glickman H, Kaplan B, Hod M. Indicated labor induction with vaginal prostaglandin E2 increases the risk of cesarean section even in multiparous women with no previous cesarean section. J Perinat Med. 2004;32(01):31–36. Doi: 10.1515/JPM.2004.005

15 Rahnama P, Ziaei S, Faghihzadeh S. Impact of early admission in labor on method of delivery. Int J Gynaecol Obstet. 2006;92(03):217–220. Doi: 10.1016/j.ijgo.2005.12.016

16 Kauffman E, Souter VL, Katon JG, Sitcov K. Cervical dilation on admission in term spontaneous labor and maternal and newborn outcomes. Obstet Gynecol. 2016;127(03):481–488. Doi: 10.1097/AOG.0000000000001294

17 Zhang J, Troendle J, Mikolajczyk R, Sundaram R, Beaver J, Fraser W. The natural history of the normal first stage of labor. Obstet Gynecol. 2010;115(04):705–710. Doi: 10.1097/AOG.0b013e3181d55925

18 Holmes P, Oppenheimer LW, Wen SW. The relationship between cervical dilatation at initial presentation in labour and subsequent intervention. BJOG. 2001;108(11):1120–1124. Doi: 10.1111/j.1471-0528.2003.00265.x

19 Bailit JL, Dierker L, Blanchard MH, Mercer BM. Outcomes of women presenting in active versus latent phase of spontaneous labor. Obstet Gynecol. 2005;105(01):77–79. Doi: 10.1097/01.AOG.0000147843.12196.00

20 Wood AM, Frey HA, Tuuli MG, Caughey AB, Odibo AO, Macones GA, et al. Optimal admission cervical dilation in spontaneously laboring women. Am J Perinatol. 2016;33(02):188–194. Doi: 10.1055/s-0035-1563711

21 Çelik HG, Bestel A, Çelik E, Aydın AA. Why do multiparous women with a history of vaginal delivery give birth by cesarean section? J Turk Ger Gynecol Assoc. 2016;17(04):209–213. Doi: 10.5152/jtgga.2016.16119
22 Parer JT, Livingston EG. What is fetal distress? Am J Obstet Gynecol. 1990;162(06):1421–1425, discussion 1425–1427. Doi: 10.1016/0002-9378(90)90901-i
23 Yli BM, Kjellmer I. Pathophysiology of foetal oxygenation and cell damage during labour. Best Pract Res Clin Obstet Gynaecol. 2016; 30:9–21. Doi: 10.1016/j.bpobgyn.2015.05.004
24 Weiner Z, Ben-Shlomo I, Beck-Fruchter R, Goldberg Y, Shalev E. Clinical and ultrasonographic weight estimation in large for gestational age fetus. Eur J Obstet Gynecol Reprod Biol. 2002; 105(01):20–24. Doi: 10.1016/s0301-2115(02)00140-9
25 Weeks JW, Pitman T, Spinnato JA II. Fetal macrosomia: does antenatal prediction affect delivery route and birth outcome? Am J Obstet Gynecol. 1995;173(04):1215–1219. Doi: 10.1016/0002-9378(95)91356-4

Prevalence and Determinants of Adequate Compliance with Antenatal Care in Peru

Prevalência e determinantes da conformidade adequada à atenção pré-natal no Peru

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Abstract

Objective To determine the adequacy of compliance with antenatal care (ANC) by pregnant women in Peru and to identify the associated factors.

Methods An analytical cross-sectional study of data from the 2019 Peruvian Demographic and Family Health Survey (Encuesta Demográfica y de Salud Familiar, ENDES, in Spanish) was conducted. The dependent variable was adequate compliance with ANC (provided by skilled health care professionals; first ANC visit during the first trimester of pregnancy; six or more ANC visits during pregnancy; ANC visits with appropriate content) by women aged 15 to 49 years in their last delivery within the five years prior to the survey. Crude and adjusted prevalence ratios and their 95% confidence intervals were calculated using a log-binomial regression model.

Results A total of 18,386 women were analyzed, 35.0% of whom adequately complied with ANC. The lowest proportion of compliance was found with the content of ANC (42.6%). Sociodemographic factors and those related to pregnancy, such as being in the age groups of 20 to 34 years and 35 to 49 years, having secondary or higher education, belonging to a wealth quintile of the population other than the poorest, being from the Amazon region, not being of native ethnicity, having a second or third pregnancy, and having a desired pregnancy, increased the probability of presenting adequate compliance with ANC.

Conclusion Only 3 out of 10 women in Peru showed adequate compliance with ANC. Compliance with the content of ANC must be improved, and strategies must be developed to increase the proportion of adequate compliance with ANC.

Keywords
- prenatal care
- health surveys
- cross-sectional studies
- quality of health care
- maternal health
- maternal health services
- Peru

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Resumo

Objetivo Determinar a adequação do cumprimento dos cuidados pré-natais (CPN) por mulheres grávidas no Peru e identificar os fatores associados.

Métodos Foi realizado um estudo analítico transversal dos dados da Pesquisa Demográfica e de Saúde da Família Peruana de 2019 (Encuesta Demográfica y de Salud Familiar, ENDES, em espanhol). A variável dependente foi conformidade adequada com a CPN (fornecida por profissionais de saúde qualificados; primeira visita CPN durante o primeiro trimestre de gravidez; seis ou mais visitas CPN durante a gravidez; visitas CPN com conteúdo apropriado) por mulheres de 15 a 49 anos em seu último parto nos cinco anos anteriores à pesquisa. Os índices de prevalência bruta e ajustada e seus intervalos de confiança de 95% foram calculados usando um modelo de regressão log-binomial.

Resultados Foi analisado um total de 18.386 mulheres, das quais 35,0% cumpriram adequadamente o CPN. A menor proporção de conformidade foi encontrada com o conteúdo de ANC (42,6%). Fatores sociodemográficos e aqueles relacionados à gravidez, como estar na faixa etária de 20 a 34 anos e 35 a 49 anos, ter educação secundária ou superior, pertencer a um quintil de riqueza da população que não a mais pobre, ser da região da selva, não ser de etnia nativa, ter um segundo ou terceiro gravidez, e tendo uma gravidez desejada, aumentou a probabilidade de apresentar conformidade adequada com CPN.

Conclusão Apenas 3 em cada 10 mulheres no Peru mostraram conformidade adequada com o CPN. O cumprimento do conteúdo do CPN deve ser melhorado, e estratégias devem ser desenvolvidas para aumentar a proporção de cumprimento adequado com o CPN.

Palavras-chave

- cuidado pré-natal
- inquéritos epidemiológicos
- estudos transversais
- qualidade da assistência à saúde
- saúde materna
- serviços de saúde materna
- Peru

Introduction

Maternal mortality still occurs in different parts of the world, despite a marked reduction from 385 to 216 deaths per 100 thousand births between 1990 and 2015.[1] Therefore, the United Nations (UN) Sustainable Development Goals (SDGs) are a blueprint established with the aim of reducing global maternal mortality to a figure lower than 70 per 100 thousand births, especially in low and middle-income countries, which account for almost all deaths.[2]

Antenatal care (ANC) is considered vital to reduce maternal and neonatal morbidity and mortality.[3,4] According to the World Health Organization (WHO), ANC includes the treatment of pregnancy symptoms, nutritional consultations, evaluations of the mother and fetus, and improvement in health care services directed to the mother and the fetus.[3] Nonetheless, the literature consulted shows differences among countries regarding the number of ANC visits and compliance with ANC.[5–7]

Latin America and the Caribbean are among the regions with the highest maternal mortality.[1] However, the number of deaths in these regions has decreased in recent decades (from 124 to 69 per 100 thousand live births in Latin America, and from 276 to 175 per 100 thousand births in the Caribbean).[1] Nevertheless, no country in this region has reached the goal of reducing maternal mortality by 75%.[1] In the case of Peru, maternal mortality has decreased from 265 to 68 per 100 thousand live births between 1990 and 2015.[1] Despite this clear reduction, in 2019, the maternal mortality rate in Peru was 56.1 per 100 thousand births,[8] indicating a scenario in which pregnancy-related deaths remain a public health problem.

The Peruvian Ministry of Health (Ministerio de Salud, MINSA, in Spanish) has established that skilled health care professionals perform adequate ANC with at least six ANC visits, the first of which is made during the first trimester of pregnancy.[9] ANC includes guidance and counseling for pregnant women, tests to support diagnosis and prophylaxis, prenatal stimulation, individual psychological consultation, dental consultation, nutrition consultation, as well as social services and legal consultations.[9] To date, adequate compliance with ANC according to the MINSA and WHO recommendations has not been studied among pregnant women in Peru. Therefore, the objective of the present study was to determine the adequacy of compliance with ANC and to identify factors associated with compliance in Peru.

Methods

Study Design and Population

A cross-sectional and analytical study of the data of women and their last delivery within the five years preceding the completion of the 2019 ENDES was conduct. The present manuscript was written following the guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.[10]

According to its geographic characteristics, Peru is divided into three regions differentiated by geographic, climatic, and sociodemographic aspects. The Coast region borders the Pacific Ocean, and it is where Lima, the capital of the country, is located. The Andean region has the highest levels of altitude and the population with the lowest level of wealth in the country. Lastly, the Amazon is the region with the greatest biodiversity; however, its population does not have adequate access to health services due to geographic limitations regarding access.[11]

The ENDES is a population-based survey conducted by the National Institute of Statistics and Informatics (Instituto Nacional de Estadística e Informática, INEI, in Spanish), which provides information on the sociodemographic and health characteristics of the population. It uses complex sampling in two stages: the first is the selection of clusters, and the second, of households. It is representative of urban and rural areas throughout Peru according to the geographic (Coast, Andean, and Amazon) and administrative regions. The ENDES uses direct interviews to collect data, and these are performed by trained personnel who visit the selected homes to fill out three questionnaires (a household questionnaire, for households and their members; an individual questionnaire, for all women of childbearing age; and a health questionnaire applied to the head of the household, which collects information on household characteristics, for persons 15 years of age or older). Detailed information on the sampling, collection, and processing of ENDES data are available on the INEI web site.[12]

Variables and Measurements

The dependent variable was adequate ANC compliance in the last pregnancy by women aged 15 to 49 years within the 5 years preceding the date of the survey. The ANC was considered adequate when fulfilling the following aspects: performed by a skilled healthcare personnel (doctors, nurses, or midwives, as reported in the ENDES data);[2] first visit before the end of the first trimester of pregnancy; six or more visits during pregnancy; and visits including all of the required services (with appropriate content). Non-completion of these aspects was considered as inadequate compliance. These components of adequate compliance with ANC have been previously used by other studies in the literature.[5–7] The content of the ANC visits evaluated was based on the WHO recommendations,[3] considering only the data of pregnant women participating in the survey (the WHO recommends including the measurement of blood pressure; urine and blood analysis, HIV and syphilis testing, administration of iron tablets, protection against tetanus, and information on pregnancy complications and where to go if they occur). The absence of any of these features in ANC visits was considered as non-compliance. For the present study, the minimum number of ANC visits required was six, as established by the MINSA in 2013 for the care of pregnant women in Peru.[9]

According to the literature, the independent variables considered to be associated with adequate compliance with ANC[5–7,13] are: maternal age ([V012]: 15 to 19 years; 20 to 34 years; 35 to 49 years); level of schooling ([V106]: no education or primary education; secondary education; higher education); wealth quintile ([V190]: very poor; poor; intermediate; rich; richest); geographic region (SHREGION: Metropolitan Lima; rest of the Coast; Andean; Amazon); area of residence ([V025]: urban; rural); having public health insurance ([S413]: yes; no); ethnic self-identification ([V131]: non-native; native); birth order ([BORD]: first birth; second or third births; $\geq$ fourth birth); desired pregnancy ([M10]: yes; no); and type of pregnancy (B0: multiple; single).

Statistical Analysis

The 2019 ENDES databases were imported, combined, and analyzed using the Stata (StataCorp., LLC, College Station, TX, US) software, version 16. In every analysis, the weighting factors and specifications of the complex sample design of the ENDES were considered, using the *svy* command in the Stata software. Likewise, values of $p < 0.05$ were considered statistically significant for all statistical tests.

Sociodemographic characteristics and those related to the last pregnancy of the study population were reported using absolute frequencies and weighted proportions for the categorical variables, and averages with standard deviations for the numerical variables. Likewise, the spatial distribution of adequate compliance with ANC was represented according to the administrative regions of Peru (HV023: [24 departments and the constitutional province of Callao]).

To evaluate the association of the sociodemographic variables and those related to the last pregnancy with adequate compliance with ANC, prevalence ratios (PRs) and their 95% confidence intervals (95%CIs) were calculated using a log-binomial regression model. Finally, a multivariate analysis was performed to estimate the adjusted PR (aPR) for all independent variables with statistically significant values in the crude analysis.

Results

The data of 18,386 women who had delivered a child in the 5 years preceding the study were analyzed (►**Fig. 1**).

Regarding the sociodemographic characteristics of the women analyzed (►**Table 1**), 45.7% had a secondary education as their level of schooling, and 18.7% had no education or had only reached the primary level. Most of the women lived in the Coast region (56.2%). In addition, 74.8% lived in urban areas. Regarding health insurance, 67.2% had comprehensive health insurance (seguro integral de salud, SIS, in Spanish). In relation to ethnic self-identification, 6.3% reported belonging to a native ethnic group. Regarding pregnancy, 33.6% declared that they had had their first delivery; half of the women reported having had 2 or 3 deliveries, and 46.2% said that their last birth was an unwanted pregnancy. With respect to the type of pregnancy, less than 1% (0.9%) had a twin pregnancy.

The ANC visits were adequate in 35.0% of the women analyzed (►**Table 2**). Based on the administrative regions of

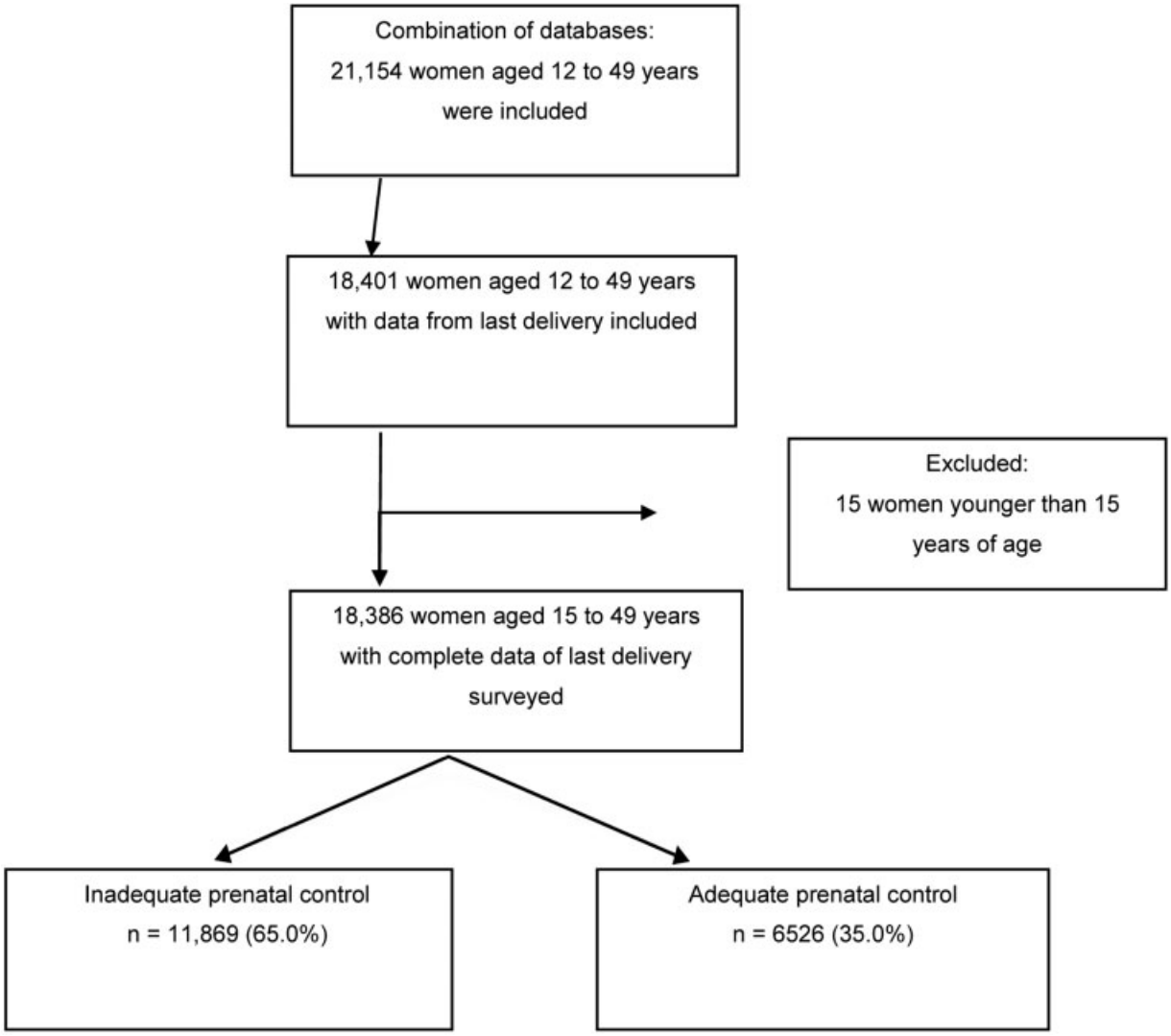

Fig. 1 Flowchart of the selection of the study sample.

the Peruvian territory, in general, the lowest proportions of ANC visits were found in the Andean region (►**Fig. 2**). According to the services included in adequate ANC, almost all women (98.3%) were cared for by skilled health care personnel. The first ANC visit was in the first trimester of pregnancy for 81.10%, and 89.70% underwent six or more visits. Regarding compliance with the content of ANC as stipulated by the MINSA, 42.6% of the women underwent ANC visits with appropriate content.

In relation to the analysis of the association between sociodemographic variables and adequate compliance with ANC (►**Table 3**), we found that women in the age groups of 20 to 34 years (aPR: 1.38; 95%CI: 1.19–1.60) and from 35 to 49 years (aPR: 1.36; 95%CI: 1.16–1.61) had a higher probability of presenting adequate compliance with ANC compared with adolescent pregnant women. Regarding the level of schooling, women with secondary (aPR: 1.19; 95%CI: 1.10–1.29) or higher education (aPR: 1.17; 95%CI: 1.06–1.30) had a higher probability of having adequate ANC compared with those with no education or only primary education. According to the wealth quintile, poorer women (quintile 1) were generally less likely to have had appropriate ANC compared with those in quintile 2 (aPR: 1.12; 95%CI: 1.02–1.23) and quintile 3 (aPR: 1.18; 95%CI: 1.06–1.31). As for the geographic domain, in the Andean region, women were less likely to receive proper ANC compared with those from the Coast region (aPR: 0.73; 95%CI: 0.67–0.79), while women from the Amazon region were more likely to have adequate ANC compared with those from the Coast (aPR: 1.26; 95%CI: 1.17–1.35). No significant differences were found between women in rural and urban areas concerning the probability of presenting adequate compliance with ANC ($p = 0.188$). Regarding ethnic self-identification, women of native ethnicity were less likely to have adequate ANC (aPR: 0.83; 95%CI: 0.71–0.96).

Regarding the variables related to the last pregnancy and their association with adequate compliance with ANC (►**Table 3**), women in older age groups had a higher probability of presenting adequate compliance with the ANC visits. A significant difference was observed between women with

Table 1 Characteristics of Peruvian women between the ages of 15 and 49 included in the 2019 ENDES survey

Characteristics	Absolute frequency ($n = 18,386$)	Weighted proportion* (95%CI)	Inadequate ANC (95%CI)	Adequate ANC (95%CI)	*p*-value**
Age group (years)					
15–19	921	4.8 (4.4–5.3)	73.7 (69.8–77.2)	26.3 (22.8–30.2)	< 0.001
20–34	12,090	65.0 (64.0–65.9)	63.7 (62.5–64.9)	36.3 (35.1–37.5)	
35–49	5,375	30.2 (29.3–31.1)	66.6 (64.8–68.4)	33.4 (31.6–35.2)	
Level of schooling					
No education / Primary education	3,533	18.7 (17.9–19.5)	71.7 (69.6–73.7)	28.3 (26.3–30.4)	< 0.001
Secondary education	8,576	45.7 (44.6–46.7)	62.8 (61.3–64.2)	37.2 (35.8–38.7)	
Higher education	6,277	35.6 (34.6–36.7)	64.4 (62.8–66.0)	35.6 (34.0–37.2)	
Wealth quintile					
Quintile 1 (lowest)	4,845	23.2 (22.3–24.1)	71.2 (69.4–73.0)	28.8 (27.0–30.6)	< 0.001
Quintile 2	5,062	24.7 (23.7–25.7)	62.7 (60.7–64.7)	37.3 (35.3–39.3)	
Quintile 3	3,735	19.9 (19.1–20.7)	60.4 (58.3–62.5)	39.6 (37.5–41.7)	
Quintile 4	2,787	17.5 (16.6–18.4)	62.7 (60.0–65.3)	37.3 (34.7–40.0)	
Quintile 5 (highest)	1,957	14.8 (14.0–15.6)	68.3 (65.3–71.1)	31.7 (28.9–34.7)	
Geographic region					
Coast	7,908	56.2 (55.1–57.3)	63.0 (61.4–64.5)	37.0 (35.5–38.6)	< 0.001
Andean	5,988	26.9 (25.7–28.1)	74.9 (73.2–76.4)	25.1 (23.6–26.8)	
Amazon	4,490	16.9 (16.0–17.9)	56.3 (54.1–58.4)	43.7 (41.6–45.9)	
Residence area					
Urban	13,230	74.8 (74.0–75.6)	63.3 (62.0–64.5)	36.7 (35.5–38.0)	< 0.001
Rural	5,156	25.2 (24.4–26.0)	70.3 (68.3–72.2)	29.7 (27.8–31.7)	
Pulic health insurance					
Not	5,191	32.8 (31.8–33.8)	68.1 (66.2–70.0)	31.9 (30.0–33.8)	< 0.001
Yes	13,195	67.2 (66.2–68.2)	63.5 (62.3–64.7)	36.5 (35.3–37.7)	
Ethnicity					
Non-native	16,808	93.7 (93.1–94.3)	64.2 (63.1–65.3)	35.8 (34.7–36.9)	<0.001
Native	1,578	6.3 (5.7–6.9)	77.0 (73.8–79.9)	23.0 (20.1–26.2)	
Birth order					
1	6,033	33.6 (32.7–34.4)	63.1 (61.4–64.8)	36.9 (35.2–38.6)	0.001
2 to 3	9,146	50.0 (49.1–50.9)	65.2 (63.8–66.5)	34.8 (33.5–36.2)	
≥ 4	3,207	16.4 (15.8–17.1)	68.5 (66.3–70.6)	31.5 (29.4–33.7)	
Desired pregnancy					
Yes	8,470	46.2 (45.2–47.1)	61.2 (59.7–62.7)	38.8 (37.3–40.3)	< 0.001
No	9,916	53.8 (52.9–54.8)	68.3 (67.0–69.6)	31.7 (30.4–33.0)	
Type of pregnancy					
Multiple	168	0.9 (0.7–1.0)	63.9 (54.6–72.3)	36.1 (27.7–45.4)	0.801
Single	18,218	99.1 (99.0–99.3)	65.0 (64.0–66.1)	35.0 (33.9–36.0)	

Abbreviation: CI, confidence interval; ENDES, Encuesta Demográfica y de Salud Familiar (Demographic and Family Health Survey).
Notes: *The weighting factor and sample specifications of the 2019 ENDES were included; **the p-value was calculated using the Chi-squared test.

a second or third birth compared with those delivering for first time (aPR: 0.92; 95%CI: 0.87–0.98). There was no difference in adequate compliance with ANC between women who had a first birth or those with four or more births ($p = 0.070$). Women with an unwanted pregnancy had a lower probability of adequate compliance with ANC (aPR: 0.82; 95%CI: 0.78–0.86). On the other hand, there were no differences between women who had a single birth compared with those with multiple births in relation to adequate compliance with ANC ($p = 0.800$).

Table 2 Proportion of compliance with the antenatal care (ANC) components among Peruvian women according to the 2019 ENDES

Characteristic	Absolute frequency ($n = 18,386$)	Weighted proportion* (95% confidence interval)
Care for by skilled health care personnel	18,042	98.3 (97.9–98.6)
Six antenatal care visits or more	16,499	89.7 (89.1–90.3)
First antenatal care visit within first trimester	14,768	81.1 (80.3–81.9)
Antenatal care with appropriate content	7,921	42.6 (41.5–43.7)
All four characteristics	6,526	35.0 (33.9–36.0)

Abbreviation: ENDES, Encuesta Demográfica y de Salud Familiar (Demographic and Family Health Survey).
Note: *The weighting factor and sample specifications of the 2019 ENDES were included.

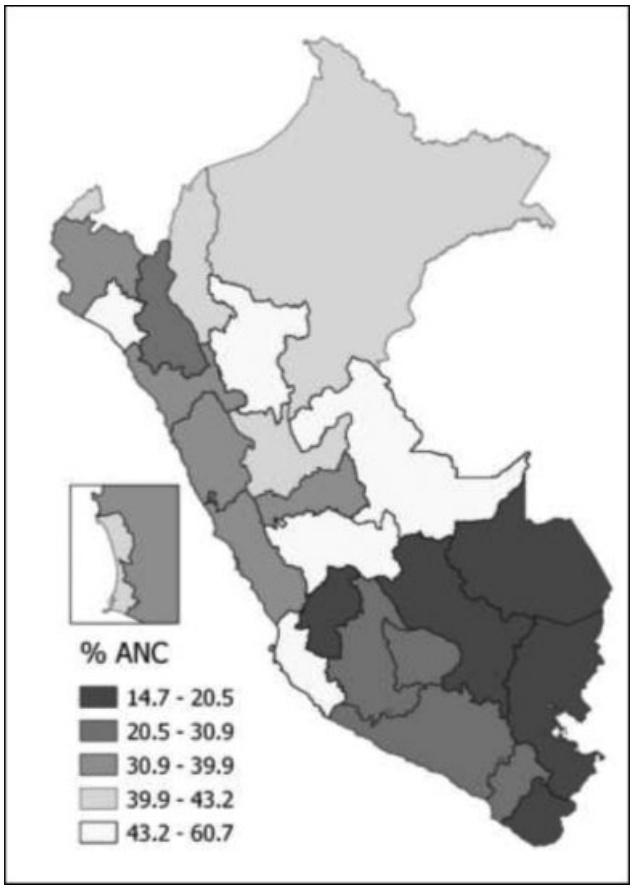

Fig. 2 Proportion of compliance with the antenatal care (ANC) components among Peruvian women by region, according to the 2019 Peruvian Demographic and Family Health Survey (Encuesta Demográfica y de Salud Familiar, ENDES, in Spanish).

Discussion

Antenatal care is a strategy that is promoted throughout the world to improve maternal health, and it is included in the Peruvian regulations for the adequate care of pregnant women. However, the results of the present study indicate that less than half of pregnant women in Peru received adequate ANC during the five years prior to the 2019 ENDES survey. The component with the lowest ANC compliance was the provision of the complete ANC content (care by skilled health care personnel, the first visit made before the end of the first trimester of pregnancy, and six or more ANC visits during pregnancy). Likewise, the sociodemographic factors, such as age, level of schooling, well-being index, ethnicity, and region of origin, as well as factors related to pregnancy, such as the order of the number of deliveries and desired pregnancy, were associated with non-compliance with ANC.

The 2019 ENDES showed that only 3 out of 10 pregnant Peruvian women presented adequate compliance with ANC, and the lowest proportion of compliance was regarding the fulfillment of all the content required to receive an adequate ANC (4 out of 10). In a study performed in 2019,[14] 1 in 2 pregnant women received ANC with adequate compliance to the content, which is similar to the findings of the present study. Similarly, in low and middle-income countries, low proportions of compliance with the contents of ANC have been reported.[5–7] In relation to maternal health, in recent decades, there has been an increase in the number of ANC visits per pregnant women, as well as in ANC provided by skilled health care personnel.[15] Likewise, in Peru, ANC provided by skilled health care personnel has increased from 54.6% (1986) to 98.2% (2019).[14] These increases are consistent with the global panorama, which reveals an increase in the number of ANC visits, as well as in the proportion of women who start ANC early, and in the care provided by skilled health care personnel.[15] Given the widely-studied relationship between inadequate ANC and the presence of negative maternal and perinatal outcomes, such as maternal and fetal death or low birth weight,[16,17] it is necessary to strengthen ANC in Peru. Improvements in ANC should include not only sustaining the rise in the number of pregnant women cared for by skilled health care personnel with an adequate initiation and number of ANC visits, but also strategies to enable the recruitment of pregnant women who are not yet receiving this care, as well as to improve compliance with ANC content during pregnancy.

In relation to sociodemographic factors, we found that the higher the level of education, the more likely the mother was to present adequate compliance with ANC, and that women with a higher level of schooling were more likely to adequately comply with ANC visits, which is similar to what has been described worldwide.[5–7,18–20] It has been observed that women with a higher level of schooling tend to value more the ANC, which would explain the greater adequate compliance with ANC in this group. Similarly, several studies[5–7,21] have shown that women with a higher socioeconomic status are more likely to receive adequate ANC and even a larger number of services. In Peru, the SIS provides free public insurance that covers ANC visits and childbirth for every woman in the Peruvian territory who does not have another

Table 3 Factors associated with adequate compliance with antenatal care among women who received prenatal care for the last birth according to the 2019 ENDES

Characteristic	Bivariate model		Adjusted model*	
	PR (95%CI)	*p*-value	aPR (95%CI)	*p*-value
Age group (years)				
15–19	Ref.		Ref.	
20–34	1.38 (1.20–1.59)	< 0.001	1.38 (1.19–1.60)	< 0.001
35–49	1.27 (1.09–1.47)	0.002	1.36 (1.16–1.61)	< 0.001
Level of schooling				
No education / Primary education	Ref.		Ref.	
Secondary education	1.31 (1.21–1.42)	< 0.001	1.19 (1.10–1.29)	< 0.001
Higher education	1.26 (1.15–1.37)	< 0.001	1.17 (1.06–1.30)	0.002
Wealth quintile				
Quintile 1 (lowest)	Ref.		Ref.	
Quintile 2	1.30 (1.19–1.41)	< 0.001	1.12 (1.02–1.23)	0.021
Quintile 3	1.38 (1.27–1.49)	< 0.001	1.18 (1.06–1.31)	0.002
Quintile 4	1.30 (1.18–1.43)	< 0.001	1.13 (1.00–1.28)	0.058
Quintile 5 (highest)	1.10 (0.99–1.23)	0.085	1.00 (0.87–1.16)	0.948
Geographic region				
Coast	Ref.		Ref.	
Andean	0.68 (0.63–0.73)	< 0.001	0.73 (0.67–0.79)	< 0.001
Amazon	1.18 (1.11–1.26)	< 0.001	1.26 (1.17–1.35)	< 0.001
Residence area				
Urban	Ref.		Ref.	
Rural	0.81 (0.75–0.87)	< 0.001	0.94 (0.85–1.03)	0.188
Public health insurance				
No	Ref.		Ref.	
Yes	1.14 (1.07–1.22)	< 0.001	1.25 (1.16–1.34)	< 0.001
Ethnicity				
Non-native	Ref.		Ref.	
Native	0.64 (0.56–0.74)	< 0.001	0.83 (0.71–0.96)	0.010
Birth order				
1	Ref.		Ref.	
2 to 3	0.94 (0.89–1.00)	0.052	0.92 (0.87–0.98)	0.006
≥ 4	0.85 (0.79–0.93)	<0.001	0.92 (0.84–1.01)	0.070
Desired pregnancy				
Yes	Ref.		Ref.	
No	0.82 (0.78–0.86)	<0.001	0.82 (0.78–0.86)	< 0.001
Type of pregnancy				
Multiple	Ref.		Not included	
Single	0.97 (0.76–1.24)	0.800		

Abbreviations: aPR, adjusted prevalence ratio; CI, confidence interval; PR, prevalence ratio.
Note: *A generalized linear model of the binomial family was performed for complex samples. Variables with a *p*value < 0.05 in the bivariate analysis were included.

type of health insurance. One of the findings of the present study was that women with SIS showed a higher proportion of adequate compliance with ANC. The results of the present study describe a scenario in which, despite ANC visits and ANC content being covered and regulated for all women in the Peruvian territory, adequate ANC differs according to the level of schooling and the socioeconomic level, as well as having health insurance.

Regarding the geographic domain, women from the Andean region were less likely to adequately comply with ANC compared with those from the Coast. In the five years preceding the study, the proportion of women seen in the first trimester increased; however, in 2018, a lower proportion of women from the Andean region had their first ANC visit during this period compared with those from the Coast region (74.8% versus 84.6%, respectively).[22] On the other hand, women from the Amazon region were more likely to have ANC with adequate compliance compared with those from the Coast region. Similarly to the Andean region, there has been an increase in the proportion of women in the Amazon region undergoing ANC visits in recent years, as well as in the number of those who started ANC during the first trimester of pregnancy. Nonetheless, Peruvian women from the Andean and Amazon regions have the highest proportion of maternal mortality in the country;[8,23] therefore, increased efforts are needed to develop strategies to reduce this health indicator.

There were no differences between women living in rural areas and those living in urban areas in relation to less adequate compliance with ANC. In recent years, the proportion of rural women who have undergone an ANC visit during the first trimester has increased, in addition to the number of visits received and other useful strategies to reduce maternal mortality, such as institutionalized delivery.[12] However, there is still a gap between women in urban and rural areas regarding ANC.[12] A similar situation has been observed throughout the world,[15] and it has been reported that rurality is associated with an increase in maternal mortality.[24] Taking into account that the area of residence is related to ANC onset and delay,[25] joint efforts are needed to sustain the increase in the number of ANC visits received by women in rural areas and to achieve adequate compliance with ANC, to contribute to reduce morbidity and mortality in both the mother and the fetus.

Regarding the age of the pregnant women, the probability of having adequate ANC increased with age. Previous studies[6,7] performed in other countries report that the older the pregnant woman, the more likely she is to receive adequate ANC. A previous study[11] have reported that there was no difference between the age groups of women of childbearing age in terms of receiving the required ANC content. A possible explanation for why older women have greater adequate compliance with ANC could be that they may be considered as being at a greater risk by health care personnel, or that the experience of previous pregnancies sensitizes these women about the importance of undergoing ANC visits, thereby increasing the likelihood of adequate compliance. Regarding ethnic identification, self-identified native women were less likely to adequately comply with the ANC visits. In Latin America and the Caribbean, a lower proportion of women of native ethnic groups have access to ANC and delivery care provided by skilled health care personnel compared to women of non-native ethnic groups,[26] with reports of difficulties in the implementation of programs to increase the number of women of native ethnic groups receiving ANC.[27]

In relation to the characteristics of the pregnancy, women with four or more previous deliveries had a lower probability of presenting adequate compliance with ANC. Some studies[13,28,29] performed in other countries describe that the greater the number of births a woman has had, the less likely she will attend ANC visits, possibly due to the feeling of security that the experiences of the previous births bring her. On the other hand, studies have observed that women with an unwanted pregnancy are less likely to adequately comply with ANC or to start ANC controls early, because of they have less interest in this care. These results could explain the differences found in the present study. In Peru, there is an unmet need for family planning, and women have difficulty preventing unwanted pregnancies, in addition to the social stigma of continuing with a pregnancy when they are in a socially-undesirable personal situation or undergoing an abortion[30] (which occurs at a high rate, despite being illegal in the country, except for medical reasons). Unwanted pregnancies are described in the literature[31–33] as associated with an increased risk of maternal and neonatal health complications, among other adverse outcomes, including a higher risk of cesarean sections, inappropriate weight gain, and mental health disorders. These characteristics associated with non-compliance with ANC indicate which population subgroups of women of childbearing age would not receive adequate ANC in accordance with the WHO recommendations and Peruvian standards, and a major concern of decision-makers is the achievement of adequate compliance with ANC in these women.

In regard to the limitations of the present study, since it is a study of secondary data, it is likely that some of the data may not be adequate. Another limitation of the study related to the ENDES is the possibility of introducing a recall bias and the lack of understanding of some questions by the respondents, a situation that is typical of any study based on surveys that collect data on past events. In addition, the ENDES does not record data on diseases or risk factors for women that could be of great use to health services, including ANC visits. However, the ENDES has protocols that ensure the quality of the recorded data, and it is widely used by public institutions and researchers as a source of information for the development of research and decision-making regarding health care in Peru. Moreover, it is a survey of national and regional representation, which records data that are used to evaluate adequate compliance with ANC based on the recommendations of the WHO and regulated by the MINSA.

Conclusion

In conclusion, in 2019, we observed that 3 out of 10 women in Peru presented adequate compliance with ANC, as recommended by the WHO and the MINSA standards. In relation to the components of adequate compliance with ANC, only 4 out of 10 women had undergone ANC that included the required care content. In addition, population subgroups, such as women from the Andean region and rural areas, native ethnic groups, and those with lower level of schooling

and socioeconomic status were less likely to present adequate compliance with ANC. These findings reveal the need to strengthen ANC among the Peruvian population, with an emphasis on providing the required content during care and developing strategies for population subgroups with a lower likelihood of having adequate compliance with ANC.

Ethical Considerations

The Institutional Research Ethics Committee of Universidad Científica del Sur approved the present study (under registration code: 349–2019-PRE15).

Contributors

All of the authors contributed with the project and data interpretation, the writing of the article, the critical review of the intellectual content, and with the final approval of the version to be published.

Conflict of Interests

The authors have no conflict of interests to declare.

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References

1 Trends in maternal mortality: 1990 to 2015: estimates by WHO, UNICEF, UNFPA, World Bank Group and the United Nations Population Division [Internet]. Geneva: World Health Organization; 2015 [cited 2020 Sep 30]. Available from: http://www.who.int/reproductivehealth/publications/monitoring/maternal-mortality-2015/en/
2 World Health Organization. Definition of skilled health personnel providing care during childbirth: the 2018 joint statement by WHO, UNFPA, UNICEF, ICM, ICN, FIGO and IPA [Internet]. 2018 [cited 2020 Sep 30]. Available from: https://apps.who.int/iris/bitstream/handle/10665/272818/WHO-RHR-18.14-eng.pdf?ua=1
3 World Health Organization. WHO recommendations on antenatal care for a positive pregnancy experience [Internet]. Geneva: World Health Organization; 2016 [cited 2020 Sep 28]. Available from: http://www.who.int/reproductivehealth/publications/maternal_perinatal_-health/anc-positive-pregnancy-experience/en/
4 Kerber KJ, de Graft-Johnson JE, Bhutta ZA, Okong P, Starrs A, Lawn JE. Continuum of care for maternal, newborn, and child health: from slogan to service delivery. Lancet. 2007;370(9595):1358–1369. Doi: 10.1016/S0140-6736(07)61578-5
5 Heredia-Pi I, Servan-Mori E, Darney BG, Reyes-Morales H, Lozano R. Measuring the adequacy of antenatal health care: a national cross-sectional study in Mexico. Bull World Health Organ. 2016;94(06):452–461. Doi: 10.2471/BLT.15.168302
6 Siddique AB, Perkins J, Mazumder T, et al. Antenatal care in rural Bangladesh: Gaps in adequate coverage and content. PLoS One. 2018;13(11):e0205149. Doi: 10.1371/journal.pone.0205149
7 Singh L, Dubey R, Singh S, Goel R, Nair S, Singh PK. Measuring quality of antenatal care: a secondary analysis of national survey data from India. BJOG. 2019;126(Suppl 4):7–13. Doi: 10.1111/1471-0528.15825
8 Gil CF. Situación epidemiológica de la mortalidad materna en el Perú, SE 52. Bol Epidemiol Perú [Internet]. 2019 [cited 2020 Sep 29];28(52):1334–40. Available from: http://www.dge.gob.pe/portal/docs/vigilancia/boletines/2019/52.pdf
9 Ministerio de Salud. NTS No. 105: Norma Técnica de Salud para la Atención Integral de Salud Materna [Internet]. 2013 [cited 2020 Sep 30]. Available from: http://www.diresacusco.gob.pe/salud_-individual/dais/materno/NORMAS%20RTN/03/RM827-2013%20-%20NTS%20DE%20SALUD%20MATERNA.pdf
10 von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JPSTROBE Initiative. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ. 2007;335(7624):806–808. Doi: 10.1136/bmj.39335.541782.AD
11 You H, Yu T, Gu H, et al. Factors Associated With Prescribed Antenatal Care Utilization: A Cross-Sectional Study in Eastern Rural China. Inquiry. 2019;56:46958019865435. Doi: 10.1177/0046958019865435
12 Instituto Nacional de Estadística e Informática. Perú: Encuesta Demográfica y de Salud Familiar - ENDES 2019 [Internet] 2020 [cited 2020 Sep 20]. Available from: https://www.inei.gob.pe/media/MenuRecursivo/publicaciones_digitales/Est/Endes2019/
13 Abame DE, Abera M, Tesfay A, et al. Relationship between unintended pregnancy and antenatal care use during pregnancy in Hadiya Zone, Southern Ethiopia. J Reprod Infertil. 2019;20(01):42–51
14 Hernández-Vásquez A, Vargas-Fernández R, Bendezu-Quispe G. Factores asociados a la calidad de la atención prenatal en Perú. Rev Peru Med Exp Salud Publica. 2019;36(02):178–187. Doi: 10.17843/rpmesp.2019.362.4482
15 UNICEF. Antenal Care [Internet] 2019 [cited 2020 Sep 10]. Available from: https://data.unicef.org/topic/maternal-health/antenatal-care/
16 Chen XK, Wen SW, Yang Q, Walker MC. Adequacy of prenatal care and neonatal mortality in infants born to mothers with and without antenatal high-risk conditions. Aust N Z J Obstet Gynaecol. 2007;47(02):122–127. Doi: 10.1111/j.1479-828X.2007.00697.x
17 da Fonseca CR, Strufaldi MW, de Carvalho LR, Puccini RF. Adequacy of antenatal care and its relationship with low birth weight in Botucatu, São Paulo, Brazil: a case-control study. BMC Pregnancy Childbirth. 2014;14:255. Doi: 10.1186/1471-2393-14-255
18 Muyunda B, Makasa M, Jacobs C, Musonda P, Michelo C. Higher educational attainment associated with optimal antenatal care visits among childbearing women in Zambia. Front Public Health. 2016;4:127. Doi: 10.3389/fpubh.2016.00127
19 Babalola S. Women's education level, antenatal visits and the quality of skilled antenatal care: a study of three African countries. J Health Care Poor Underserved. 2014;25(01):161–179. Doi: 10.1353/hpu.2014.0049
20 Afaya A, Azongo TB, Dzomeku VM, et al. Women's knowledge and its associated factors regarding optimum utilisation of antenatal care in rural Ghana: A cross-sectional study. PLoS One. 2020;15(07):e0234575. Doi: 10.1371/journal.pone.0234575
21 Pandey S, Karki S. Socio-economic and demographic determinants of antenatal care services utilization in Central Nepal. Int J MCH AIDS. 2014;2(02):212–219
22 Instituto Nacional de Estadística e Informática. Informe Perú: indicadores de resultados de los programas presupuestales, primer semestre 2019 elaborado con los resultados de la Encuesta Demográfica y de Salud Familiar (ENDES) [Internet]. 2019 [cited 2020 Sep 25]. Available from: https://proyectos.inei.gob.pe/endes/2019/ppr/Indicadores_de_Resultados_de_los_Programas_Presupuestales_ENDES_Primer_Semestre_2019.pdf
23 dl Carpio Ancaya L. [Situation of maternal mortality in Peru, 2000 - 2012]. Rev Peru Med Exp Salud Publica. 2013;30(03):461–464
24 World health Organization. Maternal health [Internet] 2020 [cited 2020 Oct 3]. Available from: https://www.who.int/health-topics/maternal-health#tab=tab_1

25 Ewunetie AA, Munea AM, Meselu BT, Simeneh MM, Meteku BT. DELAY on first antenatal care visit and its associated factors among pregnant women in public health facilities of Debre Markos town, North West Ethiopia. BMC Pregnancy Childbirth. 2018;18(01):173. Doi: 10.1186/s12884-018-1748-7
26 Mesenburg MA, Restrepo-Mendez MC, Amigo H, et al. Ethnic group inequalities in coverage with reproductive, maternal and child health interventions: cross-sectional analyses of national surveys in 16 Latin American and Caribbean countries. Lancet Glob Health. 2018;6(08):e902–e913. Doi: 10.1016/S2214-109X(18)30300-0
27 Comisión Económica para América Latina y el Caribe. Salud materno-infantil de pueblos indígenas y afrodescendientes de América Latina: aportes para una relectura desde el derecho a la integridad cultural [Internet]. 2010 [cited 2020 Sep 30]. Available from: https://www.cepal.org/es/publicaciones/3797-salud-materno-infantil-pueblos-indigenas-afrodescendientes-america-latina-aportes
28 Erol N, Durusoy R, Ergin I, Döner B, Ciçeklioğlu M. Unintended pregnancy and prenatal care: a study from a maternity hospital in Turkey. Eur J Contracept Reprod Health Care. 2010;15(04):290–300. Doi: 10.3109/13625187.2010.500424
29 Kassahun EA, Zeleke LB, Dessie AA, et al. Factors associated with unintended pregnancy among women attending antenatal care in Maichew Town, Northern Ethiopia, 2017. BMC Res Notes. 2019;12(01):381. Doi: 10.1186/s13104-019-4419-5
30 Palomino N, Padilla MR, Talledo BD, Mazuelos CG, Carda J, Bayer AM. The social constructions of unwanted pregnancy and abortion in Lima, Peru. Glob Public Health. 2011;6(Suppl 1):S73–S89
31 Bahk J, Yun SC, Kim YM, Khang YH. Impact of unintended pregnancy on maternal mental health: a causal analysis using follow up data of the Panel Study on Korean Children (PSKC). BMC Pregnancy Childbirth. 2015;15:85
32 Dehingia N, Dixit A, Atmavilas Y, et al. Unintended pregnancy and maternal health complications: cross-sectional analysis of data from rural Uttar Pradesh, India. BMC Pregnancy Childbirth. 2020;20(01):188
33 Omani-Samani R, Ranjbaran M, Mohammadi M, et al. Impact of unintended pregnancy on maternal and neonatal outcomes. J Obstet Gynaecol India. 2019;69(02):136–141. Doi: 10.1007/s13224-018-1125-5

Prenatal Diagnosis of Aberrant Right Subclavian Artery: Association with Genetic Abnormalities

Diagnóstico pré-natal de artéria subclávia direita anômala: associação com anormalidades genéticas

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Abstract

Objective The objective of the present study was to determine the frequency of malformations and chromosomal abnormalities in a population of fetuses with an aberrant right subclavian artery (ARSA).

Methods This is a 6-year retrospective study of fetuses with a prenatal diagnosis of ARSA conducted during the period between September 2013 and June 2019 at a fetal medicine unit. Data were collected from ultrasound, fetal echocardiograms, genetic studies, and neonatal records.

Results An ARSA was diagnosed in 22 fetuses. An ARSA was an isolated finding in 18 out of 22 cases (82%). Associated abnormal sonographic findings were found in 4 cases. All cases underwent invasive testing. In 1 of the cases, a chromosomal abnormality was detected (mos 45,X [13]/46,X,e(X) (p22.1q22.1)). No cases of congenital heart disease were found in any of these fetuses. There were two cases in which the postnatal evaluation revealed a malformation: one case of hypospadias and 1 case of cleft palate.

Conclusion The presence of an isolated ARSA is benign and is not associated with chromosomal abnormalities. The finding of ARSA, however, warrants a detailed fetal ultrasound in order to exclude major fetal abnormalities and other soft markers.

Keywords

- aberrant right subclavian artery
- prenatal diagnosis
- screening
- genetic abnormalities

Resumo

Objetivo O objetivo do presente estudo foi determinar a frequência de malformações e anomalias cromossômicas em uma população de fetos com artéria subclávia direita aberrante (ARSA).

Métodos Este é um estudo retrospectivo de 6 anos de fetos com diagnóstico pré-natal de ARSA realizado durante o período de setembro de 2013 a junho de 2019 em uma unidade de medicina fetal. Os dados foram coletados de ultrassom, ecocardiograma fetal, estudos genéticos e registros neonatais.

Resultados Um ARSA foi diagnosticado em 22 fetos. Um ARSA foi um achado isolado em 18 dos 22 casos (82%). Achados ultrassonográficos anormais associados foram encontrados em 4 casos. Todos os casos foram submetidos a testes invasivos. Em um

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Palavras-chave
- artéria subclávia direita aberrante
- diagnóstico pré-natal
- triagem
- anormalidades genéticas

dos casos, foi detectada uma anormalidade cromossômica (mos 45, X [13] / 46, X, e (X) (p22.1q22.1)). Nenhum caso de doença cardíaca congênita foi encontrado em qualquer um desses fetos. Houve dois casos em que a avaliação pós-natal revelou a malformação: um caso de hipospádia e 1 caso de fenda palatina.

Conclusão A presença de ARSA isolado é benigna e não está associada a anormalidades cromossômicas. O achado de ARSA, no entanto, justifica uma ultrassonografia fetal detalhada para excluir anormalidades fetais importantes e outros marcadores leves.

Introduction

An abnormal origin of the right subclavian artery (RSA) is the most common aortic branching abnormality, and it has been reported postnatally in ~ 1 to 2% in the general population in autopsy series.[1–6] In contrast to the normal aortic arch branching pattern, in which the right subclavian artery branches off the brachiocephalic trunk, an aberrant right subclavian artery (ARSA) arises as a 4th aortic arch vessel and passes behind the trachea and the esophagus and courses to the right arm.[7–11]

Being usually asymptomatic and considered as a normal variant, an ARSA can sometimes cause clinical symptoms due to its trajectory behind the trachea and the esophagus (dysphagia, cough, and dyspnea).[8–11] In the postnatal period, and, most recently, also in the prenatal period, ARSA was found significantly more often in subjects with congenital heart disease[12] or with chromosomal abnormalities, particularly trisomy 21,[2,13] with the relative risk multiplied by 3.94.[14] However, most fetuses with trisomy 21 have additional anatomic features in addition to the ARSA.[3,5,8,13] An ARSA has also been reported in fetuses with other, less common, genetic anomalies.[5,6,15–21] Some authors recommend invasive testing even if an ARSA is isolated.[7] However, more recent studies did not find an association between isolated ARSA and chromosomal abnormalities and, therefore, do not recommend invasive testing in these cases.[19–22]

The aim of our study was to determine the frequency and the nature of associated anomalies, such as malformations and chromosomal abnormalities, in a population of fetuses diagnosed with an ARSA through screening or diagnostic ultrasound, and to assess the postnatal outcome.

Methods

An ARSA was prospectively sought in all patients who underwent obstetric ultrasound during the 2nd trimester of gestation. The examinations were performed using Voluson E8 Expert ultrasound devices (GE Healthcare, Chicago, IL, USA) by a transabdominal approach between August 2013 and June 2019 by 6 sonographers experts in obstetrics ultrasonography. They included all patients referred for 2nd trimester ultrasound in our department, including high- and low-risk pregnancies. During fetal heart assessment, the course of the RSA was observed after the assessment of 4-chamber view, outflow tracts and the 3 vessel and trachea view according to the technique described by Chaoui et al.[2] In addition to the B-mode segmental view approach, color Doppler ultrasonography was used for visualizing the transverse 3-vessel and tracheal view. The normal RSA in the axial plane was visualized as an S-shaped vessel passing anterior to the trachea at the clavicle level. An ARSA was detected as a vessel arising separately from the junction of the aortic arch and ductus arteriosus and having a retrotracheal course toward the right arm. The course of the ARSA was straight, without an S-shape proximal concavity surrounding the trachea anteriorly. All cases were referred for fetal echocardiogram performed by a pediatric cardiologist. The cases of ARSA were categorized as isolated if ARSA was the only sonographic finding, and as nonisolated in cases of associated ultrasound abnormalities or 2nd trimester soft markers. Soft markers included the sonographic findings associated with an increased risk of chromosomal abnormality – increased nuchal fold, nasal bone hypoplasia, echogenic bowel, echogenic intracardiac focus, choroid plexus cyst, pyelectasis and femur or humerus length $< 5^{th}$ centile. After the diagnosis, patients were offered invasive testing by amniocentesis. Until 2018, karyotype and fluorescence in situ hybridization (FISH) for 22q11.2 microdeletion were offered, and after 2018, QF-PCR and cGH-array were offered. Follow-up scans were performed in all cases in which the pregnancy continued. An ARSA was not evaluated postnatally by imaging in liveborn infants, while it was systematically investigated in cases of termination of pregnancy. Outcomes were collected from all ARSA fetuses from hospital records.

Results

Between August 2013 and June 2019, an ARSA was diagnosed in 22 fetuses in the 2nd trimester (between 20 and 22 weeks). A total of 8,699 second trimester ultrasounds were done, resulting in a prevalence of 0.25%. The mean maternal age was 29 years old (range 18–38 years old). The fetal gender was mainly female. Only 3 out of 22 ARSA cases were male. An ARSA was an isolated finding in 18 out of 22 cases (82%). An ARSA was associated with other sonographic findings in the remaining 4 out of 22 cases (18%). A total of 21 cases underwent 1st trimester screening ultrasound, 19 cases underwent 1st trimester combined screening, and 1 case underwent 2nd trimester screening. Of these, there were 18 cases of 1st trimester low-risk screening and 1 case of

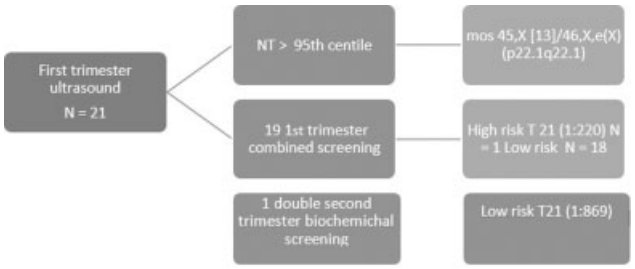

Fig. 1 Results of first trimester screening in fetus with ARSA. NT = nuchal translucency; 1st = first; T21 = trisomy 21.

increased risk for trisomy 21 (1:220). There was 1 case with nuchal translucency > 95th centile, and the patient opted for invasive testing (chorionic villous sampling) (►**Fig. 1**).

There were no cases of associated congenital heart disease. There were 3 cases of associated ultrasound signs in the 2nd trimester: 1 case of choroid plexus cysts, 1 case of a megacisterna magna, and 1 case with measurements of long bones < 5th centile and head circumference < 5th centile. There was 1 case of head circumference < 5th centile in the 3rd trimester. The ARSA was apparently isolated in 18 cases.

Fetal genetic testing was systematically proposed and analyzed in all fetuses (karyotype and FISH for 22q11.2 microdeletion or QF-PCR and cGH array) during the prenatal period.

Genetic testing proved to be normal for 21 out of 22 (95%) and abnormal for 1 out of 22 fetuses (5%). This case had nuchal translucency > 95th centile in the 1st trimester and measurements of long bones < 5th centile and head circumference < 5th centile in the second trimester, having an unusual abnormality, such as mos 45,X [13]/46,X,e(X) (p22.1q22.1). No chromosomal abnormalities were detected in fetuses with an isolated ARSA. There were no cases in this population of trisomy 21 or 22q11.2 microdeletion.

In what concerns obstetrics outcomes, there was 1 case of termination of pregnancy, 1 case of intrauterine fetal demise at 32 weeks, and 2 cases of head circumference below the 5th centile in the 3rd trimester whose fetal cerebral magnetic resonance imaging (MRI) were normal.

On ongoing pregnancies, there were two cases of late preterm birth. All the remaining cases reached full term. There were 2 cases of birthweight < 2500 g, corresponding to the 2 preterm births.

All neonates were examined postnatally, and two congenital anomalies with no prenatal diagnosis were seen: one case of hypospadias and one case of cleft palate. All born children had normal development (►**Table 1**)

Discussion

The presence of an ARSA in fetuses with Down syndrome was described for the first time by Chaoui et al.[2] In their preliminary study, they identified an ARSA in 35.7% of fetuses with Down syndrome in the 2nd and 3rd trimester. Since then, several studies reported that an ARSA was one of the most powerful independent markers for Down syndrome.

Chaoui et al.[2] found a fetus with trisomy 21 in whom the only ultrasonographic abnormality was an ARSA. In this case, the maternal age was 42 years old, the nuchal translucency thickness was < 95th percentile, and the result of serum markers was not mentioned. It is very likely that fetal karyotyping was performed because of the initially high risk of aneuploidy.

Table 1 Outcomes in fetuses with ARSA

Case	First trimester screening	Method of diagnosis	Fetal Karyotype	Associated abnormalities/ soft markers	Outcome
1	NT > 95th centile	CVS and amniocentesis	mos 45,X [13]/46, X,e(X) (p22.1q22.1)	long bones < 5th centile and head circumference < 5th centile	TOP at 23 weeks
2	Low risk	amniocentesis	Normal	Choroid plexus cyst	Balanic Hypospadias Normal development
3	Low risk	amniocentesis	Normal	Megacysterna magna	Normal fetal cerebral MRI Normal development
4	High risk (1:220 T21)	Amniocentesis	Normal	None	Normal development
5	Low risk	Amniocentesis	Normal	Head circumference < 5th centile in the 3rd trimester	Normal fetal cerebral MRI Normal development
6	Low risk	Amniocentesis	Normal	none	Intrauterine fetal demise at 32 weeks Autopsy: ARSA, subendocardic elastosis; fetal distress
7	Low risk	Amniocentesis	Normal	None	Postnatal diagnosis of cleft palate. Normal development

Abbreviations: CVS, chorionic villous sampling; MRI, magnetic resonance imaging; NT, nuchal translucency; T21, trisomy 21; TOP, termination of pregnancy.

In the study by Borenstein et al.,[5] an ARSA was also isolated in one fetus with Down syndrome. However, the population included in their study was at a high risk of chromosomal abnormality.

Rembouskos et al.[6] revealed 2 chromosomal abnormalities in fetuses with isolated ARSAs: in a fetus with trisomy 21, the 1st trimester combined risk was 1/39, and in a fetus with a trisomy 21 mosaicism, the combined risk was 1/402. Cardiovascular defects were the most frequently associated abnormality in euploid fetuses. Therefore, the authors concluded that fetal echocardiography should be offered in all cases of ARSA.

Paladini et al.[13] found 8 fetuses carrying trisomy 21 with an isolated ARSA, and ARSA revealed as the one of the most important 2nd trimester marker for Down syndrome together with hypoplasic nasal bone and increased nuchal fold. However, at that time, the standard combined 1st-trimester screening test was not applied routinely, so there is no information regarding 1st trimester risk in the apparently isolated cases of ARSA.

In the study by Esmer et al.,[7] 6 fetuses with trisomy 21 were classified as having isolated ARSA. A review of these cases showed that in 4 out 6 of these patients the combined 1st trimester screening was high risk for trisomy 21, and in 2 out of 6 patients the 1st trimester risk was not evaluated; these 2 patients were 37 and 38 years old.

Gursoy Erzincan et al.[9] found a weak association with Down syndrome in a low-risk population. An ARSA is more commonly detected in fetuses with Down syndrome than in euploid fetuses, and, in most cases, it is associated with other pathologic sonographic findings. The authors conclude that ARSA by itself does not create a sufficient indication for invasive testing.

Fehmi Yazicioğlu et al.[19] studied the prevalence of an ARSA in a mixed population and found a prevalence rate of 1.1%. However, the study was composed of high-risk pregnancies, owing to the high incidence of Down syndrome in their study population.

In a meta-analysis, Agathokleous et al.[14] reported that an ARSA increased the Down syndrome risk by 3.94, and emphasized that most of the studies included in the meta-analysis were performed in high-risk pregnancies.

Considering the meta-analysis by De León-Luis et al.,[16] we must differentiate isolated from non-isolated ARSAs. The ARSAs detected among cases of Down syndrome were all associated with other markers of trisomy 21. They did not find any correlation between an isolated ARSA and Down syndrome.

Scala et al.,[4] in a systematic review and meta-analysis evaluating an ARSA in fetuses with Down syndrome, showed that an ARSA is a clinically important marker of trisomy 21, but not sufficient to recommend fetal karyotyping in isolated cases.

In recent studies, no cases of Down syndrome or pathogenic copy number variants were reported in fetuses with an isolated ARSA[4,16,19–22] Svirsky et al.[20] report the findings of chromosomal microarray analysis in 62 fetuses referred for genetic counseling due to the finding of ARSA. In the 41 patients with isolated ARSA, no cases of trisomy 21 or any other chromosomal aberration were found. Maya et al.[21] showed that in 36 fetuses with isolated ARSA, pathogenic copy number variants were not found. These studies reported that all Down syndrome cases with an ARSA were associated with other markers. Sagi-Dain et al.[22] report the results of chromosomal microarray analysis in 246 fetuses with isolated ARSA. In 1 case, a trisomy 21 was detected, but this frequency did not significantly differ from the control population. In this report, there is no reference to the results of 1st trimester screening in these cases. Aberrant right subclavian artery has a female predominance,[6,11] and our results also reflected a higher incidence of ARSA in females than in males. Some studies do not support this female predominance.

The present study has some limitations. Aberrant right subclavian artery was routinely searched only in 2nd trimester ultrasound. This might have resulted in a lower incidence than that reported by previous studies.

Moreover, there was no systematic postnatal verification of the presence of an ARSA. The course of the RSA is not readily detectable by postnatal echocardiography, while other postnatal tests, such as MRI, are nonjustifiable in the absence of specific indications. Therefore, the only way of confirming the course of the RSA in asymptomatic cases remains fetal echocardiography. As is well known, the accuracy of fetal echocardiography at 20 to 22 weeks of gestation is well documented, so that we took the midtrimester scan as the gold standard.[6] In our series, fetal echocardiography has confirmed the initial diagnosis of ARSA, which underlines that, once the specific landmarks described are observed, a straightforward diagnosis of ARSA can be achieved.[6]

Conclusion

The conflicting evidence in the literature regarding the association of ARSA and chromosomal abnormalities is probably because the earlier studies did not differentiate between isolated ARSA and ARSA with additional ultrasound findings. The analysis of the literature combined with the results of our study suggest that in patients in whom the combined risk of chromosomal abnormalities in the 1st trimester was evaluated, the presence of an isolated ARSA is a condition rarely associated with a chromosomal abnormality. In the case of an isolated ARSA, an ultrasound scan must be performed in a reference center, and especially an echocardiography, to confirm that there is no associated anomaly.

Contributors
All authors were involved in the design and interpretation of the analyses, contributed to the writing of the manuscript, read and approved the final manuscript.

Conflict of Interests
The authors have no conflict of interests to declare.

References

1 Zapata H, Edwards JE, Titus JL. Aberrant right subclavian artery with left aortic arch: associated cardiac anomalies. Pediatr Cardiol. 1993;14(03):159–161. Doi: 10.1007/BF00795645

2 Chaoui R, Heling KS, Sarioglu N, Schwabe M, Dankof A, Bollmann R. Aberrant right subclavian artery as a new cardiac sign in second- and third-trimester fetuses with Down syndrome. Am J Obstet Gynecol. 2005;192(01):257–263. Doi: 10.1016/j.ajog.2004.06.080

3 Chaoui R, Rake A, Heling KS. Aortic arch with four vessels: aberrant right subclavian artery. Ultrasound Obstet Gynecol. 2008;31(01):115–117. Doi: 10.1002/uog.5240

4 Scala C, Leone Roberti Maggiore U, Candiani M, Venturini PL, Ferrero S, Greco T, et al. Aberrant right subclavian artery in fetuses with Down syndrome: a systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2015;46(03):266–276. Doi: 10.1002/uog.14774

5 Borenstein M, Minekawa R, Zidere V, Nicolaides KH, Allan LD. Aberrant right subclavian artery at 16 to 23 + 6 weeks of gestation: a marker for chromosomal abnormality. Ultrasound Obstet Gynecol. 2010;36(05):548–552. Doi: 10.1002/uog.7683

6 Rembouskos G, Passamonti U, De Robertis V, Tempesta A, Campobasso G, Volpe G, et al. Aberrant right subclavian artery (ARSA) in unselected population at first and second trimester ultrasonography. Prenat Diagn. 2012;32(10):968–975. Doi: 10.1002/pd.3942

7 Esmer AC, Gul A, Nehir A, Yuksel A, Dural O, Kalelioglu I, et al. Detection rate of trisomy 21 in fetuses with isolated and non-isolated aberrant right subclavian artery. Fetal Diagn Ther. 2013;34(03):140–145. Doi: 10.1159/000354650

8 Pico H, Mancini J, Lafouge A, Bault JP, Gorincour G, Quarello E. Prenatal associated features in fetuses diagnosed with an aberrant right subclavian artery. Fetal Diagn Ther. 2016;40(03):187–194. Doi: 10.1159/000443524

9 Gursoy Erzincan S, Karamustafaoglu Balci B, Tokgoz C, Kalelioglu IH. Incidence of an aberrant right subclavian artery on second-trimester sonography in an unselected population. J Ultrasound Med. 2017;36(05):1015–1019. Doi: 10.7863/ultra.16.05075

10 Ranzini AC, Hyman F, Jamaer E, van Mieghem T. Aberrant right subclavian artery: correlation between fetal and neonatal abnormalities and abnormal genetic screening or testing. J Ultrasound Med. 2017;36(04):785–790. Doi: 10.7863/ultra.16.05028

11 Song MJ, Han BH, Kim YH, Yoon SY, Lee YM, Jeon HS, et al. Prenatal diagnosis of aberrant right subclavian artery in an unselected population. Ultrasonography. 2017;36(03):278–283. Doi: 10.14366/usg.16046

12 Ramaswamy P, Lytrivi ID, Thanjan MT, Nguyen T, Srivastava S, Sharma S, Ko HH, et al. Frequency of aberrant subclavian artery, arch laterality, and associated intracardiac anomalies detected by echocardiography. Am J Cardiol. 2008;101(05):677–682. Doi: 10.1016/j.amjcard.2007.10.036

13 Paladini D, Sglavo G, Pastore G, Masucci A, D'Armiento MR, Nappi C. Aberrant right subclavian artery: incidence and correlation with other markers of Down syndrome in second-trimester fetuses. Ultrasound Obstet Gynecol. 2012;39(02):191–195. Doi: 10.1002/uog.10053

14 Agathokleous M, Chaveeva P, Poon LC, Kosinski P, Nicolaides KH. Meta-analysis of second-trimester markers for trisomy 21. Ultrasound Obstet Gynecol. 2013;41(03):247–261. Doi: 10.1002/uog.12364

15 Willruth AM, Dwinger N, Ritgen J, Stressig R, Geipel A, Gembruch U, et al. Fetal aberrant right subclavian artery (ARSA) - a potential new soft marker in the genetic scan? Ultraschall Med. 2012;33(07):E114–E118. Doi: 10.1055/s-0029-1245935

16 De León-Luis J, Gámez F, Bravo C, Tenías JM, Arias Á, Pérez R, et al. Second-trimester fetal aberrant right subclavian artery: original study, systematic review and meta-analysis of performance in detection of Down syndrome. Ultrasound Obstet Gynecol. 2014;44(02):147–153. Doi: 10.1002/uog.13336

17 Rauch R, Rauch A, Koch A, Zink S, Kaulitz R, Girisch M, et al. Laterality of the aortic arch and anomalies of the subclavian artery-reliable indicators for 22q11.2 deletion syndromes? Eur J Pediatr. 2004;163(11):642–645. Doi: 10.1007/s00431-004-1518-6

18 McElhinney DB, Clark BJ III, Weinberg PM, Kenton ML, McDonald-McGinn D, Driscoll DA, et al. Association of chromosome 22q11 deletion with isolated anomalies of aortic arch laterality and branching. J Am Coll Cardiol. 2001;37(08):2114–2119. Doi: 10.1016/s0735-1097(01)01286-4

19 Fehmi Yazıcıoğlu H, Sevket O, Akın H, Aygün M, Özyurt ON, Karahasanoğlu A. Aberrant right subclavian artery in Down syndrome fetuses. Prenat Diagn. 2013;33(03):209–213. Doi: 10.1002/pd.4042

20 Svirsky R, Reches A, Brabbing-Goldstein D, Bar-Shira A, Yaron Y. Association of aberrant right subclavian artery with abnormal karyotype and microarray results. Prenat Diagn. 2017;37(08):808–811. Doi: 10.1002/pd.5092

21 Maya I, Kahana S, Yeshaya J, Tenne T, Yacobson S, Agmon-Fishman I, et al. Chromosomal microarray analysis in fetuses with aberrant right subclavian artery. Ultrasound Obstet Gynecol. 2017;49(03):337–341. Doi: 10.1002/uog.15935

22 Sagi-Dain L, Singer A, Josefsberg S, Peleg A, Lev D, Nasser SamraN, et al. Microarray analysis has no additional value in fetal aberrant right subclavian artery: description of 268 pregnancies and systematic literature review. Ultrasound Obstet Gynecol. 2019;53(06):810–815. Doi: 10.1002/uog.20208

Screening of Variants in the Transcript Profile of Eutopic Endometrium from Infertile Women with Endometriosis during the Implantation Window

Rastreio de variantes no perfil de tanscritos do endométrio eutópico de mulheres inférteis com endometriose durante a janela de implantação

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Abstract

Objective Abnormalities in the eutopic endometrium of women with endometriosis may be related to disease-associated infertility. Although previous RNA-sequencing analysis did not show differential expression in endometrial transcripts of endometriosis patients, other molecular alterations could impact protein synthesis and endometrial receptivity. Our aim was to screen for functional mutations in the transcripts of eutopic endometria of infertile women with endometriosis and controls during the implantation window.

Methods Data from RNA-Sequencing of endometrial biopsies collected during the implantation window from 17 patients (6 infertile women with endometriosis, 6 infertile controls, 5 fertile controls) were analyzed for variant discovery and identification of functional mutations. A targeted study of the alterations found was performed to understand the data into disease's context.

Results None of the variants identified was common to other samples within the same group, and no mutation was repeated among patients with endometriosis, infertile and fertile controls. In the endometriosis group, nine predicted deleterious mutations were identified, but only one was previously associated to a clinical condition with no endometrial impact. When crossing the mutated genes with the descriptors *endometriosis* and/or *endometrium*, the gene *CMKLR1* was associated either with inflammatory response in endometriosis or with endometrial processes for pregnancy establishment.

Conclusion Despite no pattern of mutation having been found, we ponder the small sample size and the analysis on RNA-sequencing data. Considering the purpose of the study of screening and the importance of the *CMKLR1* gene on endometrial

Keywords
- endometriosis
- infertility
- eutopic endometrium
- RNA-sequencing
- mutation

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modulation, it could be a candidate gene for powered further studies evaluating mutations in eutopic endometria from endometriosis patients.

Resumo

Objetivo Anormalidades no endométrio eutópico de mulheres com endometriose podem estar relacionadas à infertilidade associada à doença. Embora a análise prévia de sequenciamento de RNA não tenha evidenciado expressão diferencial em transcritos endometriais de pacientes com endometriose, outras alterações moleculares poderiam afetar a síntese de proteínas e a receptividade endometrial. Nosso objetivo foi rastrear mutações funcionais em transcritos de endométrios eutópicos de mulheres inférteis com endometriose e de controles durante a janela de implantação.

Métodos Os dados do sequenciamento de RNA de biópsias endometriais coletados durante a janela de implantação de 17 pacientes (6 mulheres inférteis com endometriose, 6 controles inférteis, 5 controles férteis) foram analisados para a descoberta de variantes e a identificação de mutações funcionais. Um estudo direcionado das alterações encontradas foi realizado para compreender os dados no contexto da doença.

Resultados Nenhuma das variantes identificadas foi comum a outras amostras dentro do mesmo grupo, assim como nenhuma mutação se repetiu entre pacientes com endometriose, controles inférteis e férteis. No grupo de endometriose, foram identificadas nove mutações deletérias preditas, mas apenas uma foi previamente associada a uma condição clínica sem impacto endometrial. Ao cruzar os genes mutados com os descritores *endometriose* e/ou *endométrio*, o gene *CMKLR1* foi associado a resposta inflamatória na endometriose e a processos endometriais para estabelecimento da gravidez.

Conclusão Apesar de nenhum padrão de mutação ter sido encontrado, ponderamos o pequeno tamanho da amostra e a análise dos dados de sequenciamento de RNA. Considerando o objetivo do estudo de triagem e a importância do gene *CMKLR1* na modulação endometrial, este poderia ser um gene candidato para estudos adicionais que avaliem mutações no endométrio eutópico de pacientes com endometriose.

Palavras-chave
- endometriose
- infertilidade
- endométrio eutópico
- sequenciamento de RNA
- mutação

Introduction

Endometriosis, a disease characterized by implantation and growth of endometrial tissue outside the uterine cavity,[1,2] has a high prevalence, affecting between 6 and 10% of women in reproductive age.[1] It is also frequently associated with infertility, being present in between 25 and 50% of infertile women,[3] with 30 to 50% of endometriosis patients being infertile.[3–6] However, the mechanisms underlying disease-related infertility are still poorly understood.

Evidence have suggested that changes in the endometrial receptivity, due to molecular and functional disorders in the eutopic endometrium, may be related to impaired fertility in women with endometriosis.[5,7–9] The success of embryonic implantation depends on an adequate embryonic development, on the arrival of a competent embryo to a receptive endometrium, and on an efficient communication between the embryo and the endometrium.[10–12] It is known that the human endometrium becomes receptive only during the implantation window,[10,13–16] a certain period that results from the synchronized interaction of a variety of molecules (ovarian hormones, growth factors, transcription factors, cytokines, adhesion molecules), with an important role in establishing uterine receptivity.[16–22] Thus, molecular changes in the eutopic endometrium of these patients could impair their endometrial receptivity, contributing to the infertility observed in women with the disease.

However, a recent comprehensive and integrated evaluation of eutopic endometria of infertile women with endometriosis, infertile and fertile controls during the implantation window through a transcriptome analysis (RNA-Seq), did not identify differentially expressed transcripts among the groups.[23] Likewise, the miRNA sequencing in the eutopic endometrium of the same patients did not find changes in those post-transcriptional regulatory molecules.[23] Together, the findings suggest that the eutopic endometrium of infertile women with the disease is molecularly similar to that of fertile women. However, the absence of alterations in mRNA and miRNA expression does not exclude the possibility of other molecular changes, with consequences for protein synthesis, which could impact the endometrial receptivity of these women. Single nucleotide variants (SNVs) are changes on a DNA sequence basis

and comprise both polymorphisms (single-nucleotide polymorfisms [SNPs]) and point mutations, which may result in the wrong translation of transcripts into truncated, inactive and/or altered proteins.[24,25] Since no study to date has evaluated SNVs in the eutopic endometrium of infertile women with endometriosis, we question whether the occurrence of functional mutations in the eutopic endometrium of those patients could impact the endometrial receptivity and contribute to disease-related infertility.

Total genome and/or exome sequencing are methodologies that allow the identification of point mutations in the DNA strands; however, with the disadvantage of having a high cost.[26] RNA sequencing can be a less costly alternative for the indirect study of mutations in transcripts, with the possibility of analyzing new variations that have occurred as a result of post-transcriptional changes.[27] In this sense, the use of data generated by RNA-Seq has been proposed by the literature for the indirect analysis of SNVs and mutations.[28–32]

Thus, the objectives of the present study were to screen for functional mutations in the transcripts of eutopic endometria of infertile women with endometriosis, and of infertile and fertile controls during the implantation window, through the analysis of data previously generated by RNA-Seq, as well as to conduct a targeted study of the changes found in the context of endometriosis.

Methods

Study Design

A prospective case-control study was performed at the Human Reproduction Division of the Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (HCFMRP-USP). The study was approved by the Research Ethics Committee of the Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (HCFMRP-USP) (grant number 6383/2011). Patients who met the inclusion criteria and expressed their desire to participate in the study signed the informed consent form prior to inclusion.

From November 2011 to November 2014, patients previously submitted to diagnostic videolaparoscopy or tubal ligation procedures in the Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (HCFMRP-USP) were evaluated according to the eligibility criteria, and those considered eligible were interviewed. Patients who agreed to participate had an endometrial sample collected during the implantation window.

Patients – Eligibility Criteria

We considered eligible those patients who presented regular cycles (every 24 to 38 days, 4.5 to 8 days of duration and flow up to 80 ml per cycle)[33] for at least 3 months prior to the study, aged between 18 and 45 years old, body mass index (BMI) $\leq 30\ kg/m^2$, absence of polycystic ovary syndrome and of other etiologies of chronic anovulation, hydrosalpinx and chronic diseases such as diabetes mellitus or other endocrinopathies, cardiovascular disease, dyslipidemia, systemic lupus erythematosus and other rheumatologic diseases, HIV infection, any active infection, alcohol, drugs or smoking habit, and use of hormonal medication or of anti-inflammatory drugs during the 3 months preceding the beginning of the study were included.

In the END group, 6 patients with infertility exclusively associated to pelvic endometriosis diagnosed and classified by videolaparoscopy according to the criteria of the American Society for Reproductive Medicine[34] were included. Among them, 2 patients were diagnosed with stage I endometriosis, 1 with stage II endometriosis, 1 with stage III endometriosis and 2 with stage IV endometriosis.

In the IC group, 6 patients with infertility attributable to male and/or tubal factors who had ruled out endometriosis and other pelvic diseases by videolaparoscopy were included. The FC group was composed by 5 patients undergoing tubal ligation who were proven fertile (at least one living child) without possible associated endometrial factors.

Sample Collection and RNA-sequencing

The patients had endometrial samples collected during the implantation window[35] (between the 20^{th} and 24^{th} days of the cycle). For data standardization, the ovulation day was considered as the 14^{th} day of a 28-day menstrual cycle.

Eutopic endometrial biopsies were collected during the implantation window from 17 patients (3 infertile women with endometriosis I/II, 3 infertile women with endometriosis III/IV, 6 infertile controls, and 5 fertile controls).

Total RNA was extracted with the RiboPure kit (Ambion, Life Technologies, Carlsbad, California, USA), treated with DNase (DNA KIT Free, Ambion - Life Technologies). Total RNA concentration was determined by spectrophotometry (NanoDrop 2000c; Thermo Scientific, Wilmington, DE, USA) at 260 nm, while total RNA integrity was evaluated with Agilent Technologies 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) according to the instructions of the manufacturer. Samples with RNA Integrity Number (RIN) ≥ 7.0 were considered appropriate. mRNA libraries were prepared using TruSeq RNA Sample Preparation v2 kit (Illumina, San Diego, CA, USA) according to the instructions of the manufacturer. RNA sequencing was performed using the commercial TruSeq SBS kit v5 kit (Illumina Inc.), as instructed by the manufacturer. In total, 17 libraries were distributed in 3 lanes and sequenced paired end (PE 2×101pb) in the HISEq. 2500 Illumina Platform, through High Output run. Data regarding the differential expression of transcripts were previously presented.[23]

Mutation Screening and Annotation

Mutation screening was performed on RNA-Seq data generated previously.[23] The mapping of the generated fragments (reads) was performed with STAR (Spliced Transcripts Alignment to a Reference),[36] and variant calling was performed using the Genome Analysis Toolkit (GATK; https://gatk.broadinstitute.org/hc/en-us/articles/360035531192?id=3891), following the best practices for variant discovery in RNA-Seq data,[37] filtered using the hard filtering method (-window 35 -cluster 3 -FS > 30.0 -QD (Quality By Depth.) < 2.0 -DP (Coverage) > 10.0). The annotation of SNPs and Indels was performed with the VarAFT tool (https://varaft.eu/).

Table 1 Number and type of variants identified in the transcripts of eutopic endometrium of infertile women with endometriosis, women with tubal and/or male infertility factor (infertile control) and fertile women (fertile control) during the implantation window, from RNA-Seq data before and after application of filters

Group	Pacient ID	Variants		*Indel*		SNV		Total after filtering/ prediction
		Before filtering	After filtering/ prediction	Before filtering	After filtering/ prediction	Before filtering	After filtering/ prediction	
Endometriosis	1	72239	5	1286	0	70953	5	9
	2	16482	0	975	0	15507	0	
	3	14955	0	210	0	14745	0	
	4	84156	1	4743	0	79413	1	
	5	69363	2	1111	0	68252	2	
	6	146610	1	8595	0	138015	1	
Fertile control	1	79967	4	4694	0	75273	4	14
	2	66279	5	1505	0	64774	5	
	3	98901	2	5775	0	93126	2	
	4	157215	1	9525	0	147690	1	
	5	84380	2	4940	0	79440	2	
Infertile control	1	149952	2	9262	0	140690	2	19
	2	118616	4	7285	0	111331	4	
	3	97232	2	5600	0	91632	2	
	4	89246	1	5148	0	84098	1	
	5	88790	7	1906	0	86884	7	
	6	84869	3	4976	0	79893	3	

Abbreviation: SNV, single nucleotide variant.

In Silico Analysis to Identify Functional Mutations

Functional mutations were selected based on quality and selection criteria (such as: depth >10, genome region, variant function and register in the NCBI database dbSNP) and on the pathogenicity scores of the following *in silico* prediction tools: CADD (Combined Annotation Dependent Depletion); PROVEAN (Protein Variation Effect Analyzer); SIFT (Sort Intolerant From Tolerant) and Polyphen2. Only those classified as damaging, deleterious or possibly damaging in the 4 predictors were considered functional.

With the identification of possibly deleterious mutations, in order to interpret the data in the context of the disease, we performed a targeted study of the selected variants in NCBI databases such as Single Nucleotide Polymorphism Database (dbSNP) of Nucleotide Sequence Variation (https://www.ncbi.nlm.nih.gov/snp/), which brings described polymorphisms, and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), which brings disease-associated mutations.

Specifically, regarding the endometriosis group, in order to target the changes found in the context of the disease, we conducted a search in PubMed crossing the genes related to each mutation with the descriptors *endometriosis* and/or *endometrium*.

Statistical Analysis

An exploratory data analysis was performed by measurements of central position and dispersion and box-plot graphs. The

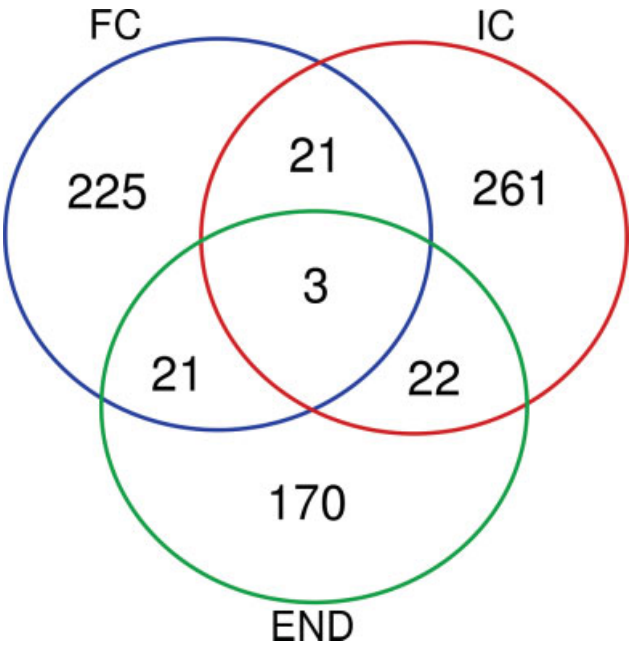

Fig. 1 Venn diagram: number of single nucleotide variants (SNV) with depth ≥ 10, located in exonic and splicing regions, not synonymous, found in eutopic endometrial RNA-Seq data from infertile women with endometriosis (END), infertile controls (IC) and fertile controls (FC) during the implantation window.

Table 2 Variants identified after filtering and predicting data obtained from eutopic endometrium RNA-Seq of infertile women with endometriosis, women with tubal and/or male infertility factor (infertile control), and fertile women (fertile control) during the implantation window

Group	Patient ID	Chromosome	Reference allele	Mutant allele	Genotype	Depth	SNV score	Gene	1000 g	dbSNP NCBI	CADD
CF	1	2	C	T	het	10	62.77	*TTN*	0.076877	rs4894028	24.0
		3	A	G	het	10	52.77	*ZNF502*	0.10603	rs56084453	17.61
		17	G	A	het	10	109.77	*EVPL*	0.0081869	rs150149800	33.0
		19	G	A	het	10	106.77	*DOCK6*	0.519569	rs12978266	22.9
	5	1	G	A	het	10	103.77	*ATAD3B*	0.00239617	rs141377718	23.5
		3	C	T	het	10	32.77	*DNAH1*	0.0299521	rs419752	34.0
		6	T	C	het	10	66.77	*GSTA3*	0.000199681	rs139422505	21.8
		8	C	A	het	10	58.77	*MAPK15*	0.095647	rs60732298	28.2
		12	A	C	het	10	71.77	*CLEC7A*	0.00858626	rs16910527	25.2
	8	1	C	T	het	10	124.77	*OXCT2*	–	rs150795467	22.6
		19	T	C	het	10	81.77	*ZNF836*	0.0129792	rs61739527	18.91
	9	1	A	C	het	10	24.78	*PLEKHN1*	–	rs181207265	20.5
	32	1	G	C	het	10	224.77	*ANKRD45*	0.00199681	rs191985325	24.7
		10	A	G	het	10	30.77	*PPP1R3C*	0.00199681	rs143318107	24.6
CI	2	1	C	T	het	10	127.77	*KMO*	0.000798722	rs200044625	28.8
		11	A	T	het	10	166.77	*CCDC88B*	0.000399361	rs572682028	29.4
	6	5	G	A	het	10	93.77	*PCDHB5*	0.0297524	rs17844422	18.71
		11	G	A	het	10	54.77	*SLC25A45*	0.0101837	rs34400381	26.0
		16	C	A	het	10	204.77	*MT1A*	0.470647	rs11640851	18.37
		18	G	A	het	10	69.77	*ALPK2*	0.0203674	rs79863383	24.1
	7	1	C	G	het	10	56.77	*TRAF3IP3*	0.00139776	rs147791408	22.8
		10	G	A	het	10	31.77	*CFAP58*	–	rs143080879	29.2
	17	1	G	A	het	10	67.77	*C1orf87*	–	rs772501233	26.5
	19	3	G	A	het	10	234.77	*CCDC13*	0.167732	rs17238798	24.8
			C	G	het	10	59.77	*IQCG*	0.281749	rs67877771	26.2
		5	C	T	het	10	91.77	*C5orf51*	0.00159744	rs151191974	33.0
		6	T	C	het	10	190.77	*CRYBG1*	0.0201677	rs61741114	27.0
			G	A	het	10	113.77	*LAMA4*	0.0309505	rs11757455	34.0
		11	C	T	het	10	152.77	*RIN1*	0.0183706	rs140145986	24.7
		17	G	A	het	10	94.77	*ITGAE*	0.265375	rs1716	25.0
	22	8	C	T	het	10	184.77	*MICU3*	0.000399361	rs201776772	26.8
		9	G	A	het	10	140.77	*FAM166B*	0.0333466	rs75679360	33.0
		12	G	C	het	10	49.77	*CAPRIN2*	0.0111821	rs73079976	28.0
END	3	4	C	T	het	10	136.77	*NSG1*	0.00139776	rs142822048	32.0
		12	G	A	het	10	111.77	*CMKLR1*	0.000199681	rs201809939	29.0
		14	G	A	hom	10	241.41	*AHNAK2*	0.538538	rs10438247	24.7
		17	A	T	het	10	108.77	*EFCAB13*	0.0892572	rs72825679	24.7
		20	T	C	het	10	97.77	*DHX35*	0.014976	rs36053162	23.0
	27	4	C	T	het	10	227.77	*SLC2A9*	0.294129	rs3733591	22.8
	28	17	G	A	het	10	44.77	*ASB16*	0.0141773	rs74491716	24.2
		19	A	T	het	10	131.77	*IZUMO4*	0.0107827	rs45506200	25.6
	31	5	C	T	het	10	224.77	*JMY*	0.0141773	rs116121324	24.5

Abbreviations: Hom, Homozygous; het, heterozygous; 1000 g, frequency described in the 1000 Genomes bank.

Table 3 Data from the dbSNP and ClinVar databases for the predicted pathogenic variants identified in eutopic endometrial RNA-Seq data from fertile women (fertile control; FC), women with tubal and/or male infertility factor (infertile control; IC), and infertile women with endometriosis (END) during the implantation window

Group	ID	Chr	Ref	Mut	NCBI register	Gene Symbol	Official name	Codon impact	Molecular consequence (dbSNP)	Interpretation (ClinVar)	Associated condition (ClinVar)
CF	1	2	C	T	rs4894028	*TTN*	titin	R (Arg) > H (His)	Missense variant	Benign / Likely benign	Dilated Cardiomyopathy, Myopathy, Hypertrophic cardiomyopathy, Limb-Girdle Muscular Dystrophy, Distal myopathy Markesbery-Griggs type
		3	A	G	rs56084453	*ZNF502*	zinc finger protein 502	Q (Gln) > R (Arg)	Missense variant	NR	–
		17	G	A	rs150149800	*EVPL*	envoplakin	R (Arg) > C (Cys)	Missense variant	NR	–
		19	G	A	rs12978266	*DOCK6*	dedicator of cytokinesis 6	P (Pro) > L (Leu)	Missense variant	Benign	Adams-Oliver syndrome 2
	2	1	G	A	rs141377718	*ATAD3B*	ATPase family AAA domain containing 3B	V (Val) > M (Met)	Missense variant	NR	–
		3	C	T	rs419752	*DNAH1*	dynein axonemal heavy chain 1	R (Arg) > C (Cys)	Missense variant	Benign	• Ciliary dyskinesia, Spermatogenic failure
		6	T	C	rs139422505	*GSTA3*	glutathione S-transferase α 3	N (Asn) > S (Ser)	Missense variant	NR	–
		8	C	A	rs60732298	*MAPK15*	Mitogen-activated protein kinase 15	T (Thr) > K (Lys)	Missense variant	NR	–
		12	A	C	rs16910527	*CLEC7A*	C-type lectin domain containing 7A	I (Ile) > S (Ser)	Missense variant	NR	–
	3	1	C	T	rs150795467	*OXCT2*	3-oxoacid CoA-transferase 2	D (Asp) > N (Asn)	Missense variant	NR	–
		19	T	C	rs61739527	*ZNF836*	zinc finger protein 836	E (Glu) > G (Gly)	Missense variant	NR	–
	4	1	A	C	rs181207265	*PLEKHN1*	pleckstrin homology domain containing N1	T (Thr) > P (Pro)	Missense variant	NR	–
	5	1	G	C	rs191985325	*ANKRD45*	ankyrin repeat domain 45	L (Leu) > V (Val)	Missense variant	NR	–
		10	A	G	rs143318107	*PPP1R3C*	protein phosphatase 1 regulatory subunit 3C	F (Phe) > S (Ser)	Missense variant	NR	–
CI	1	1	C	T	rs200044625	*KMO*	kynurenine 3-monooxygenase	T (Thr) > I (Ile)	Missense variant	NR	–
		11	A	T	rs572682028	*CCDC88B*	coiled-coil domain containing 88B	E (Glu) > V (Val)	Missense variant	NR	–
	2	5	G	A	rs17844422	*PCDHB5*	protocadherin β 5	S (Ser) > N (Asn)	Missense variant	NR	–
		11	G	A	rs34400381	*SLC25A45*	solute carrier family 25 member 45	R (Arg) > C (Cys)	Missense variant	NR	–
		16	C	A	rs11640851	*MT1A*	metallothionein 1A	T (Thr) > N (Asn)	Missense variant	NR	–
		18	G	A	rs79863383	*ALPK2*	α kinase 2	T (Thr) > I (Ile)	Missense variant	NR	–
	3	1	C	G	rs147791408	*TRAF3IP3*	TRAF3 interacting protein 3	D (Asp) > E (Glu)	Missense variant	NR	–
		10	G	A	rs143080879	*CFAP58*	cilia and flagella associated protein 58	R (Arg) > H (His)	Missense variant	NR	–
	4	1	G	A	rs772501233	*C1orf87*	chromosome 1 open reading frame 87	A (Ala) > V (Val)	Missense variant	NR	–
	5	3	G	A	rs17238798	*CCDC13*	coiled-coil domain containing 13	R (Arg) > W (Trp)	Missense variant	NR	–
		3	C	G	rs67877771	*IQCG*	IQ motif containing G	D (Asp) > H (His)	Missense variant	NR	–

Table 3 (*Continued*)

Group	ID	Chr	Ref	Mut	NCBI register	Gene Symbol	Official name	Codon impact	Molecular consequence (dbSNP)	Interpretation (ClinVar)	Associated condition (ClinVar)
		5	C	T	rs151191974	*C5orf51*	chromosome 5 open reading frame 51	P (Pro) > L (Leu)	Missense variant	NR	–
		6	T	C	rs61741114	*CRYBG1*	crystallin β-gamma domain containing 1	L (Leu) > P (Pro)	Missense variant	NR	–
		6	G	A	rs11757455	*LAMA4*	laminin subunit α 4	R (Arg) > W (Trp)	Missense variant	Benign	–
		11	C	T	rs140145986	*RIN1*	Ras and Rab interactor 1	A (Ala) > T (Thr)	Missense variant	NR	–
		17	G	A	rs1716	*ITGAE*	integrin subunit α E	R (Arg) > W (Trp)	Missense variant	NR	–
END	1	4	C	T	rs142822048	*NSG1*	neuronal vesicle trafficking associated 1	P (Pro) > S (Ser)	Missense variant	NR	–
		12	G	A	rs201809939	*CMKLR1*	chemerin chemokine-like receptor 1	R (Arg) > C (Cys)	Missense variant	NR	–
		14	G	A	rs10438247	*AHNAK2*	AHNAK nucleoprotein 2	P (Pro) > L (Leu)	Missense variant	NR	–
		17	A	T	rs72825679	*EFCAB13*	EF-hand calcium-binding domain-containing protein 13	D (Asp) > V (Val)	Missense variant	NR	–
		20	T	C	rs36053162	*DHX35*	DEAH-box helicase 35	I (Ile) > T (Thr)	Missense variant	NR	–
	4	4	C	T	rs3733591	*SLC2A9*	solute carrier family 2 member 9	R (Arg) > H (His)	Missense variant	Benign	Familial renal hypouricemia
	5	17	G	A	rs74491716	*ASB16*	ankyrin repeat and SOCS box containing 16	A (Ala) > T (Thr)	Missense variant	NR	–
		19	A	T	rs45506200	*IZUMO4*	IZUMO family member 4	Y (Tyr) > F (Phe)	Missense variant	NR	–
	6	5	C	T	rs116121324	*JMY*	junction mediating and regulatory protein, p53 cofactor	P (Pro) > L (Leu)	Missense variant	NR	–

Abbreviations: Chr, chromosome; ID, patient identification; Mut, mutated allele; NR, not reported; Ref, reference allele.

Kruskal-Wallis test was used for the comparison of clinical characteristics (age, height, weight, and BMI) among the groups.

Results

Clinical Characteristics of the Patients

The patients from the endometriosis, infertile control and fertile control groups were similar in relation to age, weight, height and BMI (►**Supplemental Table S1** (online only).

RNA sequencing

All samples that proceeded to RNA-Seq were evaluated for total RNA integrity in the 2100 BioanalyzerTM (Agilent Technologies) and were considered suitable for the technique (RIN $\geq$ 7). Paired-end libraries from the 17 RNA samples were sequenced: 6 women with endometriosis (3 with initial endometriosis and 3 with advanced endometriosis), 6 infertile controls and 5 fertile controls, distributed in 3 lanes, yielding $\sim$ 73 million reads each. Approximately 90% of the reads were mapped, with a phred-score > 30. Of the mapped reads, 1.5% were singleton, and 1% had multiple alignments, which have been removed from the analysis. The uniformity of reads mapped across all samples was considered good.

Variant Discovery

The analyzes performed in the GATK, following the best practices recommended for discovering variants in RNA-Seq data identified 885,515 variants. The detailed data by sample and group are shown in ►**Table 1**.

After filtering for quality, 793 variants were identified, 225 of which were exclusive to samples from the fertile control group, 261 from the infertile control group, and 170 from the endometriosis group, in addition to the 21 common to the fertile and infertile control groups, 21 to the fertile control and endometriosis groups, 22 common to the infertile control and endometriosis groups, and 3 common to the three groups (►**Fig. 1**). According to the predictors of pathogenicity, 42 variants were selected, 14 in the fertile control group, 19 in the infertile control group, and 9 in the endometriosis group. ►**Table 2** shows the data for the variants in each group after applying the filters. Within the endometriosis group, two samples did not present any mutation

predicted as deleterious. In the other groups, all samples showed at least one mutation.

Targeted Study of Variants Found

The search of functional mutations was, then, performed in the dbSNP and ClinVar databases. The general data for each variant are presented in ►**Table 3**. All the mutations found were classified as missense.

According to the findings (►**Table 3**), in the fertile control group, two patients had mutations corresponding to clinical conditions. Among them, patient 1 presented two mutations with associated pathological conditions, being one related to cardiomyopathy and the other to Adams-Oliver syndrome 2, both with benign significance. Patient 2 presented one mutation related to spermatogenic failure and ciliary dyskinesia, also with benign significance. The infertile control group did not have any mutations with an associated clinical condition. In the endometriosis group, only patient 4 presented a mutation associated to a clinical condition (familial renal hypouricemia), with a benign significance.

Specifically, regarding the endometriosis group, when we performed a search in the PubMed database, by crossing the mutated genes identified with the descriptors *endometriosis* and/or *endometrium*, only the *CMKLR1* gene was associated with those descriptors. Accordingly, the protein encoded by *CMKLR1* is increased in the peritoneal fluid of women with endometriosis when compared with controls. In addition, its mRNA protein and receptor appear to be increased in ovarian endometrioma compared with the eutopic endometrium of control women.

Discussion

Endometriosis is a disease related to infertility whose underlying mechanisms that impair the fertility of women are still under investigation.[1] An endometrial factor has been considered, since molecular and functional alterations of the eutopic endometrium could affect embryo implantation.[3,5,7–9] Despite a recent study that evidenced no differential expression in the mRNA and miRNA profile in the endometrium of those patients,[23] other molecular aberrations could impair protein synthesis and, consequently, endometrial receptivity. However, there is no study to date that evaluated eutopic endometrial mutations in endometriosis patients during the implantation window, which could bring important information regarding functional alterations in their endometrium. Because RNA-Seq data may be useful to identify variants in the transcriptome,[26–32] the aim of the present study was to screen for functional mutations in the transcripts (mRNA) of eutopic endometria of infertile women with endometriosis and of controls during the implantation window, through the analysis of data previously generated by RNA-Seq.[38]

According to the findings, none of the variants found were common to other samples within the same group, suggesting no pattern of mutations in those patients. Also, no variant was repeated among women with endometriosis, infertile controls, and fertile controls. Interestingly, the endometriosis group had the lower number of variants, followed by the fertile control group, with the infertile control group having the highest number of mutations. However, it is important to highlight the small sample size of the groups, which may represent a bias and precludes groups comparison. Powered studies are necessary to confirm those results.

All the filtered mutations were classified as missense, which means that the substitution of a single base pair alters the genetic code and produces an aminoacid which is different from the usual, which is able to affect the protein function.[39] It is known that the phenotypic effects of a mutation can be more severe the greater the difference in the chemical nature of the side chains of the aminoacid residues, and that they also depend on the role that this residue plays in the structure and function of the protein.[39] Nevertheless, in the endometriosis group, only one patient presented a mutation associated with a clinical condition (familial renal hypouricemia). Renal hypouricemia is characterized by impaired reabsorption of uric acid in the apical membrane of proximal renal tubule cells caused by dysfunction of renal urate reabsorption transporters.[40] Patients are usually asymptomatic, but, in some cases, they may present exercise-induced acute renal failure and nephrolithiasis.[41,42] However, the disease has no relation with the endometrium or with infertility.

Regarding the endometriosis group, there are evidence relating one of the mutated genes (*CMKLR1*) with endometriosis and/or the endometrium. The *CMKLR1* gene encodes a protein called chemerin, which is an adipokine expressed in several human organs.[43–45] This protein has been associated with several systemic and focal inflammatory processes.[43–47] It modulates chemotaxis and activates inflammatory macrophages and cytokines.[48] The *CMKLR1* gene is also associated with important endometrial events for pregnancy, such as accumulation of deciduous natural killer (NK) cells and vascular remodeling. In this sense, chemerin levels seems to be higher in stromal endometrial cells of pregnant women compared with nonpregnant or menopausal fertile women, being regulated positively during decidualization.[49]

Interestingly, chemerin plays a role in pelvic inflammation related to endometriosis, and its concentration is increased in the peritoneal fluid of women with the disease when compared with controls. In addition, its mRNA, protein and receptor appear to be increased in ovarian endometrioma compared with the eutopic endometrium of control women.[38] However, there is no data about the expression of *CMKLR1* in the eutopic endometrium of women with endometriosis comparing them to fertile controls. In this sense, given its role in the inflammatory process, chemerin could have a role in the impairment of fertility of those patients. The endometrial *CMKLR1* gene mutation could be involved in reduced chemotaxis, less activation of macrophages and decreased release of inflammatory cytokines. Considering that the inflammatory process is important for endometrial receptivity and embryo implantation[50–52] and that chemerin plays a direct role in the establishment of pregnancy,[49] it is questioned whether the mutation of the *CMKLR1* gene could be related to the impairment of those important events in

women with endometriosis, being able to participate in the etiopathogenesis of disease-related infertility. However, this should be clarified in future studies with appropriate methodologies.

The present study has limitations, such as the small sample size, which does not allow us to state whether there are differential mutations among women with endometriosis compared with fertile and infertile controls, nor the identification of a pattern of mutations in the endometriosis group. Moreover, the search for variants was performed on RNA-Seq data, which may add bias by evaluating only expressed transcripts. It is unknown whether other mutations, in regulatory regions, for example, may characterize those patients and impact the phenotype.

In summary, no pattern of functional mutations was identified in the transcripts of the eutopic endometria from infertile women with endometriosis during the implantation window. However, it is necessary to consider the small sample size and that the analyses were performed on RNA-Seq data. Interestingly, one of the mutations found in one endometriosis patient was related to a gene (*CMKLR1*) already associated with endometriosis, endometrial function, and initial gestational development.

Conclusion

Considering the aim of the present study of screening analysis and the importance of the *CMKLR1* gene in endometrial modulation, *CMKLR1* could be suggested as a candidate gene for further studies evaluating mutations in the eutopic endometrium from endometriosis patients. Thus, according to the present findings, future studies with appropriate casuistry, which investigate the *CMKLR1* mutation in DNA samples (and not in transcripts) and evaluate the respective protein (chemerin) in the eutopic endometria of infertile women with endometriosis may clarify this issue and contribute to the understanding of endometriosis-related infertility.

Contributors

Da Broi M. G. was responsible for the study design, acquisition of data, data analysis, results interpretation, and manuscript writing. Plaça J. R. was responsible for the bioinformatics analysis and contributed to the data interpretation. Silva Jr, W. A. contributed to data interpretation and manuscript review. Ferriani R. A. contributed to revising critically the manuscript for important intellectual content. Navarro P. A. contributed to the study design, interpretation of data, critic review of the manuscript, and was the coordinator of the project. All authors have approved the final version and the submission of the manuscript.

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Conflict to Interests

The authors have no conflict of interests to declare.

References

1 Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril. 2012;98(03):511–519. Doi: 10.1016/j.fertnstert.2012.06.029
2 Gupta S, Agarwal A, Krajcir N, Alvarez JG. Role of oxidative stress in endometriosis. Reprod Biomed Online. 2006;13(01):126–134. Doi: 10.1016/s1472-6483(10)62026-3
3 Practice Committee of the American Society for Reproductive Medicine. Endometriosis and infertility: a committee opinion. Fertil Steril. 2012;98(03):591–598. Doi: 10.1016/j.fertnstert.2012.05.031
4 Garrido N, Navarro J, Remohí J, Simón C, Pellicer A. Follicular hormonal environment and embryo quality in women with endometriosis. Hum Reprod Update. 2000;6(01):67–74. Doi: 10.1093/humupd/6.1.67
5 Giudice LC, Kao LC. Endometriosis. Lancet. 2004;364(9447):1789–1799. Doi: 10.1016/S0140-6736(04)17403-5
6 Gupta S, Goldberg JM, Aziz N, Goldberg E, Krajcir N, Agarwal A. Pathogenic mechanisms in endometriosis-associated infertility. Fertil Steril. 2008;90(02):247–257. Doi: 10.1016/j.fertnstert.2008.02.093
7 Practice Committee of the American Society for Reproductive Medicine. Endometriosis and infertility. Fertil Steril. 2006;86(05, Suppl 1):S156–S160. Doi: 10.1016/j.fertnstert.2006.08.014
8 Wei Q, St Clair JB, Fu T, Stratton P, Nieman LK. Reduced expression of biomarkers associated with the implantation window in women with endometriosis. Fertil Steril. 2009;91(05):1686–1691. Doi: 10.1016/j.fertnstert.2008.02.121
9 Bulletti C, Coccia ME, Battistoni S, Borini A. Endometriosis and infertility. J Assist Reprod Genet. 2010;27(08):441–447. Doi: 10.1007/s10815-010-9436-1
10 Giudice LC, Telles TL, Lobo S, Kao L. The molecular basis for implantation failure in endometriosis: on the road to discovery. Ann N Y Acad Sci. 2002;955:252–264, discussion 293–295, 396–406. Doi: 10.1111/j.1749-6632.2002.tb02786.x
11 Minici F, Tiberi F, Tropea A, Orlando M, Gangale MF, Romani FR, et al. Endometriosis and human infertility: a new investigation into the role of eutopic endometrium. Hum Reprod. 2008;23(03):530–537. Doi: 10.1093/humrep/dem399
12 Singh M, Chaudhry P, Asselin E. Bridging endometrial receptivity and implantation: network of hormones, cytokines, and growth factors. J Endocrinol. 2011;210(01):5–14. Doi: 10.1530/JOE-10-0461
13 Kresowik JD, Devor EJ, Van Voorhis BJ, Leslie KK. MicroRNA-31 is significantly elevated in both human endometrium and serum during the window of implantation: a potential biomarker for optimum receptivity. Biol Reprod. 2014;91(01):17. Doi: 10.1095/biolreprod.113.116590
14 Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update. 2006;12(06):731–746. Doi: 10.1093/humupd/dml004
15 Bourgain C, Devroey P. Histologic and functional aspects of the endometrium in the implantatory phase. Gynecol Obstet Invest. 2007;64(03):131–133. Doi: 10.1159/000101735
16 Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. N Engl J Med. 1999;340(23):1796–1799. Doi: 10.1056/NEJM199906103402304
17 Altmäe S, Esteban FJ, Stavreus-Evers A, Simón CS, Giudice L, Lessey BA, et al. Guidelines for the design, analysis and interpretation of 'omics' data: focus on human endometrium. Hum Reprod Update. 2014;20(01):12–28. Doi: 10.1093/humupd/dmt048
18 Díaz-Gimeno P, Ruíz-Alonso M, Blesa D, Simón C. Transcriptomics of the human endometrium. Int J Dev Biol. 2014;58(2-4):127–137. Doi: 10.1387/ijdb.130340pd

19 Hu S, Yao G, Wang Y, Xu H, Ji X, He Y, et al. Transcriptomic changes during the pre-receptive to receptive transition in human endometrium detected by RNA-Seq. J Clin Endocrinol Metab. 2014;99 (12):E2744–E2753. Doi: 10.1210/jc.2014-2155

20 Paulson RJ. Hormonal induction of endometrial receptivity. Fertil Steril. 2011;96(03):530–535. Doi: 10.1016/j.fertnstert.2011.07. 1097

21 von Grothusen C, Lalitkumar S, Boggavarapu NR, Gemzell-Danielsson K, Lalitkumar PG. Recent advances in understanding endometrial receptivity: molecular basis and clinical applications. Am J Reprod Immunol. 2014;72(02):148–157. Doi: 10.1111/aji.12226

22 Aghajanova L, Hamilton AE, Giudice LC. Uterine receptivity to human embryonic implantation: histology, biomarkers, and transcriptomics. Semin Cell Dev Biol. 2008;19(02):204–211. Doi: 10.1016/j.semcdb.2007.10.008

23 Da Broi MG, Meola J, Plaça JR, Peronni KC, Rocha CV, Silva WA, et al. Is the profile of transcripts altered in the eutopic endometrium of infertile women with endometriosis during the implantation window? Hum Reprod. 2019;34(12):2381–2390. Doi: 10.1093/humrep/dez225

24 Katsonis P, Koire A, Wilson SJ, Hsu T-K, Lua RC, Wilkins AD, et al. Single nucleotide variations: biological impact and theoretical interpretation. Protein Sci. 2014;23(12):1650–1666. Doi: 10.1002/pro.2552

25 Mueller SC, Backes C, Kalinina OV, Meder B, Stöckel D, Lenhof H-S, et al. BALL-SNP: combining genetic and structural information to identify candidate non-synonymous single nucleotide polymorphisms. Genome Med. 2015;7(01):65. Doi: 10.1186/s13073-015-0190-y

26 Cirulli ET, Singh A, Shianna KV, Ge D, Smith JP, Maia JM, et al; Center for HIV/AIDS Vaccine Immunology (CHAVI) Screening the human exome: a comparison of whole genome and whole transcriptome sequencing. Genome Biol. 2010;11(05):R57. Doi: 10.1186/gb-2010-11-5-r57

27 Han L, Vickers KC, Samuels DC, Guo Y. Alternative applications for distinct RNA sequencing strategies. Brief Bioinform. 2015;16(04): 629–639. Doi: 10.1093/bib/bbu032

28 Sheng Q, Zhao S, Li CI, Shyr Y, Guo Y. Practicability of detecting somatic point mutation from RNA high throughput sequencing data. Genomics. 2016;107(05):163–169. Doi: 10.1016/j.ygeno.2016.03.006

29 Quinn EM, Cormican P, Kenny EM, Hill M, Anney R, Gill M, et al. Development of strategies for SNP detection in RNA-seq data: application to lymphoblastoid cell lines and evaluation using 1000 Genomes data. PLoS One. 2013;8(03):e58815. Doi: 10.1371/journal.pone.0058815

30 Chepelev I, Wei G, Tang Q, Zhao K. Detection of single nucleotide variations in expressed exons of the human genome using RNA-Seq. Nucleic Acids Res. 2009;37(16):e106. Doi: 10.1093/nar/gkp507

31 Cánovas A, Rincon G, Islas-Trejo A, Wickramasinghe S, Medrano JF. SNP discovery in the bovine milk transcriptome using RNA-Seq technology. Mamm Genome. 2010;21(11-12):592–598. Doi: 10.1007/s00335-010-9297-z

32 Peng Z, Cheng Y, Tan BC, Tian Z, Zhu Y, Zhang W, et al. Comprehensive analysis of RNA-Seq data reveals extensive RNA editing in a human transcriptome. Nat Biotechnol. 2012;30(03):253–260. Doi: 10.1038/nbt.2122

33 Fraser IS, Critchley HO, Munro MG, Broder MWriting Group for this Menstrual Agreement Process. A process designed to lead to international agreement on terminologies and definitions used to describe abnormalities of menstrual bleeding. Fertil Steril. 2007; 87(03):466–476. Doi: 10.1016/j.fertnstert.2007.01.023

34 Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril. 1997;67(05):817–821. Doi: 10.1016/s0015-0282(97)81391-x

35 Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Am J Obstet Gynecol. 1975;122(02):262–263. Doi: 10.1016/s0002-9378(16)33500-1

36 Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013; 29(01):15–21. Doi: 10.1093/bioinformatics/bts635

37 Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr Protoc Bioinformatics. 2013;43 (1110):1–33

38 Jin CH, Yi KW, Ha YR, Shin J-H, Park HT, Kim T, et al. Chemerin expression in the peritoneal fluid, serum, and ovarian endometrioma of women with endometriosis. Am J Reprod Immunol. 2015;74(04):379–386. Doi: 10.1111/aji.12405

39 Stefl S, Nishi H, Petukh M, Panchenko AR, Alexov E. Molecular mechanisms of disease-causing missense mutations. J Mol Biol. 2013;425(21):3919–3936. Doi: 10.1016/j.jmb.2013.07.014

40 Nakayama A, Matsuo H, Ohtahara A, Ogino K, Hakoda M, Hamada T, et al. Clinical practice guideline for renal hypouricemia (1st edition). Hum Cell. 2019;32(02):83–87

41 Dinour D, Gray NK, Campbell S, Shu X, Sawyer L, Richardson W, et al. Homozygous SLC2A9 mutations cause severe renal hypouricemia. J Am Soc Nephrol. 2010;21(01):64–72. Doi: 10.1681/ASN.2009040406

42 Windpessl M, Ritelli M, Wallner M, Colombi M. A novel homozygous SLC2A9 mutation associated with renal-induced hypouricemia. Am J Nephrol. 2016;43(04):245–250. Doi: 10.1159/000445845

43 Bozaoglu K, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, et al. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. Endocrinology. 2007;148(10):4687–4694. Doi: 10.1210/en.2007-0175

44 Bozaoglu K, Segal D, Shields KA, Cummings N, Curran JE, Comuzzie AG, et al. Chemerin is associated with metabolic syndrome phenotypes in a Mexican-American population. J Clin Endocrinol Metab. 2009;94(08):3085–3088. Doi: 10.1210/jc.2008-1833

45 Bozaoglu K, Curran JE, Stocker CJ, Zaibi MS, Segal D, Konstantopoulos N, et al. Chemerin, a novel adipokine in the regulation of angiogenesis. J Clin Endocrinol Metab. 2010;95(05):2476–2485. Doi: 10.1210/jc.2010-0042

46 Bondue B, Wittamer V, Parmentier M. Chemerin and its receptors in leukocyte trafficking, inflammation and metabolism. Cytokine Growth Factor Rev. 2011;22(5-6):331–338. Doi: 10.1016/j.cytogfr.2011.11.004

47 Roh SG, Song SH, Choi KC, Katoh K, Wittamer V, Parmentier M, et al. Chemerin–a new adipokine that modulates adipogenesis via its own receptor. Biochem Biophys Res Commun. 2007;362(04): 1013–1018. Doi: 10.1016/j.bbrc.2007.08.104

48 Weigert J, Neumeier M, Wanninger J, Filarski M, Bauer S, Wiest R, et al. Systemic chemerin is related to inflammation rather than obesity in type 2 diabetes. Clin Endocrinol (Oxf). 2010;72(03): 342–348. Doi: 10.1111/j.1365-2265.2009.03664.x

49 Carlino C, Trotta E, Stabile H, Morrone S, Bulla R, Soriani A, et al. Chemerin regulates NK cell accumulation and endothelial cell morphogenesis in the decidua during early pregnancy. J Clin Endocrinol Metab. 2012;97(10):3603–3612. Doi: 10.1210/jc.2012-1102

50 Maybin JA, Critchley HO, Jabbour HN. Inflammatory pathways in endometrial disorders. Mol Cell Endocrinol. 2011;335(01):42–51. Doi: 10.1016/j.mce.2010.08.006

51 King AE, Critchley HO. Oestrogen and progesterone regulation of inflammatory processes in the human endometrium. J Steroid Biochem Mol Biol. 2010;120(2-3):116–126. Doi: 10.1016/j.jsbmb.2010.01.003

52 Roberts M, Luo X, Chegini N. Differential regulation of interleukins IL-13 and IL-15 by ovarian steroids, TNF-alpha and TGF-beta in human endometrial epithelial and stromal cells. Mol Hum Reprod. 2005;11(10):751–760. Doi: 10.1093/molehr/gah233

Sexuality of Female Spina Bifida Patients: Predictors of a Satisfactory Sexual Function

Sexualidade feminina em pacientes com espinha bífida: preditores de uma função sexual satisfatória

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Abstract

Objective To assess the sexual function of women with spina bifida (SB), and to verify the factors that influence their sexual function.

Methods A cross-sectional study in which a validated female-specific questionnaire was applied to 140 SB female patients from four different cities (Porto Alegre, Brazil; and Barcelona, Madrid, and Málaga, Spain) between 2019 and 2020. The questionnaires collected data on the clinical characteristics of SB, and female sexual function was assessed using the 6-item version of the Female Sexual Function Index (FSFI-6) validated to Portuguese and Spanish.

Results Half of the patients had had sexual activity at least once in the life, but most (57.1%) did not use any contraception method. Sexual dysfunction was present in most (84.3%) patients, and all sexual function domains were impaired compared those of non-neurogenic women. The presence of urinary and fecal incontinence significantly affected the quality of their sexual activity based on the FSFI-6.

Conclusion The specific clinical aspects of the SB patients, such as urinary and fecal incontinence, should be properly addressed by their doctors, since they are associated with reduced sexual activity and lower FSFI-6 scores in the overall or specific domains. There is also a need to improve gynecological care among sexually-active SB patients, since most do not use any contraceptive methods and are at risk of inadvertent pregnancy.

Keywords
- sexuality
- spina bifida
- sexual dysfunction
- urinary incontinence
- myelomeningocele

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Resumo

Objetivo Analisar a função sexual de pacientes do sexo feminino com espinha bífida (EB), e avaliar quais fatores influenciam na função sexual.

Métodos Uma pesquisa transversal em que um questionário validado para mulheres foi aplicado em 140 pacientes com EB de quatro cidades diferentes (Porto Alegre, Brasil; e Barcelona, Madri e Málaga, Espanha) entre 2019 e 2020. Os questionários coletaram dados sobre características clínicas da espinha bífida, e a função sexual feminina foi avaliada com a versão de seis itens do Índice de Funcionamento Sexual Feminino (IFSF-6) nas versões validadas para português e espanhol.

Resultados Metade das pacientes havia praticado atividade sexual pelo menos uma vez na vida, mas a maioria (57.1%) não utilizava nenhum método contraceptivo. A disfunção sexual estava presente na maioria das pacientes (84.3%), sendo todos os domínios de função sexual prejudicados em comparação com os de mulheres não neurogênicas. A presença de incontinência urinária e fecal afetou significativamente a qualidade da atividade sexual das pacientes.

Conclusão Aspectos clínicos específicos da EB, como incontinência urinária e fecal, devem ser adequadamente abordados pelos médicos assistentes, visto que estão associados à redução na atividade sexual e piores resultados no IFSF-6. Também é necessário melhorar o atendimento ginecológico das pacientes sexualmente ativas, uma vez que a maioria não utiliza métodos contraceptivos e corre o risco de gravidez inadvertida.

Palavras-chave

- sexualidade
- espinha bífida
- disfunção sexual
- incontinência urinaria
- mielomeningocele

Introduction

Spina bifida (SB) is the main neurological birth defect that occurs due to an impaired closure of the neural tube, leading to multi-systemic dysfunctions such as neurogenic bladder.[1] The life expectancy of SB patients has increased as a result of improved medical care; therefore, adult-life issues, such as social life and sexuality, have become growing concerns among this population.[1,2] There is consistent data associating the complications of SB, such as urinary incontinence (UI) and fecal incontinence, with negative effects on socialization.[1,3] Sexuality among SB patients is considered an important topic of discussion, and it lead to many studies on the male population.[4,5] Studies on female SB patients, however, are limited, and most have small sample sizes, are single-institution surveys, or use non-validated questionnaires.[6–9] These studies revealed that women with SB present higher sexual dysfunction rates than the general female population, and they suggest that some clinical factors, such as spinal-cord level and UI, could predict their sexual outcomes. The aim of the present study was to assess the sexual function of women with SB and to verify the factors that influence their sexual function.

Methods

A cross-sectional study was implemented in four different SB centers (Spina Bifida associations inBarcelona, Madrid and Málaga, Spain; and the Urology Department at Hospital de Clínicas de Porto Alegre, in Porto Alegre, Brazil). Between 2019 and 2020, adult female SB patients who undergoing regular follow-up in the aforementioned centers were invited to participate in this study. Only women older than 18 years of age who could read and understand the questionnaire, after informed consent, were enrolled. The surveys were administered in person by trained interviewers who helped the patients to fulfill them. A non-probability purposive sampling of 210 patients was eligible and invited, with 140 accepting to participate after reading the informed consent (response rate of 66.6%).

The questionnaires collected data on demographics, socioeconomics, clinical and gynecological characteristics, and sexuality. Female sexual function was assessed using the 6-Item Version of the Female Sexual Function Index (FSFI-6) validated to Spanish and Portuguese.[10–12] It consists of a questionnaire that approaches the following sexual function domains: desire, arousal, lubrication, orgasm, satisfaction, and pain. Each item has a score varying from 0 to 5, whose sum provides the final score. A FSFI-6 total score $\leq$ 19 was considered a positive screening for female sexual dysfunction (FSD).[12] Sexual activity was defined as having a history of at least one sexual intercourse. The body mass index (BMI) was calculated using the patient's weight in kilograms divided by the square of height in meters, and obesity of was defined as a BMI score $\geq$ 30. Fecal incontinence or UI were defined as involuntary leakage of urine or feces. Psychological disorders were identified according to the patient's report, and they included the following conditions: depression, anxiety, or mood disorders.

The authors followed guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement during the preparation oif the present manuscript.[13] The following statistical tests utilized were used: Chi-squared, Fisher exact, and Mann-Whitney, and

they were analyzed using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, US) software, version 25.0. A Poisson logistic regression model was created to assess both sexual activity or dysfunction, and in each model we included the variables that presented significance of association ($p < 0.05$) or a trend to association ($p < 0.2$) in the bivariate analysis: BMI, UI and fecal incontinence. The present study was approved by each local institutional ethics committee under registration number (CAAE 96636518.3.0000.5327).

Results

In the present study, we analyzed 140 adult female SB patients, with a mean age of 27 (range: 18 to 42) years and a mean BMI of 26.2 (range: 18 to 43) Kg/m^2, who were interviewed in Spain (89.3%) and Brazil (10.7%). Most patients were single (85%) women living with their parents/family (82.1%) who economically-dependent on them (66.4%). Their level of schooling was most commonly Elementary School (61.4%). Myelomeningocele at lower levels (91.4%) associated with hydrocephalus (82.9%) was the most common SB presentation at birth. Approximately 77.1% presented mobility without the need of aids, while 22.9% were wheelchair-dependent. In total, UI occurred in 83.6% of the patients, fecal incontinence was present in 64.3%, and 16.4% claimed a history of psychological disorder.

Regular annual gynecological (GO) follow-up was a routine for 17.9% of the patients, irregular previous GO consultations occurred in 67.9%, and 14.3% had never had a single GO evaluation. Half of the patients had had sexual activity at least once in their life, and most (85%) were single women. Among those sexually active, most (57.1%) did not use any contraception method. Gestational history was present in 6 (4.3%) patients, all of them subitted to deliveries by cesarean section without complications (►**Table 1**). Sexual dysfunction was present in 84.3% of the sexually-active patients, with a median FSFI-6 total score of 14.5 (range: 4 to 26). The scores on specific domains of the FSFI-6 were also analyzed among the sexually-active women (►**Fig. 1**).

The clinical characteristics of the patients were compared with their sexual activity and the presence of sexual dysfunction (FSFI-6 overall score > 19). The type of SB, spinal cord level, hydrocephalus, use of wheelchair, psychological disorder, and fecal incontinence were not statistically associated with differences in the rates of sexual activity or dysfunction. Obesity (BMI $\geq$ 30) had a significant association with sexual dysfunction ($p = 0.004$; Fisher exact test), but no differences regarding sexual-activity rates ($p = 0.572$). The presence of UI was associated with significant lower rates of sexual activity (continent: 78.3% versus UI: 44%; $p = 0.003$; Chi-squared test) and higher rates of sexual dysfunction (continent: 50% versus UI: 96.2%; $p < 0.001$; Fisher exact test) (►**Table 2**).

A Poisson logistic regression model using BMI, UI and fecal incontinence was created to assess both sexual activity and dysfunction. The only clinical variable that demonstrated significance with lower sexual activity ($p = 0.006$) and more sexual dysfunction ($p = 0.004$) in the regression analysis was UI. Patients who suffered from UI presented a prevalence ratio of 1.46 (95% confidence interval [95%CI]: 1.21–1.76) of sexual dysfunction, and a prevalence ratio of 0.78 (95%CI: 0.67–0.9) of sexual activity.

The sexual-function domains were also analyzed quantitatively. Obesity, type of SB, and deambulation status did not influence the scores of any sexual function domain. Those without hydrocephalus had better scores only in the orgasm domain. Fecal incontinence and UI were significantly associated with lower scores in all domains, except for pain (►**Table 3**).

Table 1 Gynecological care and sexuality characteristics of spina bifida patients

Characteristic	n (%)
Gynecological examination	
Irregular visits	95(67.9)
Regular visits	25(17.9)
Has never undergone a gynecological examination	20(14.3)
Sexual activity	
No	70(50)
Yes	70(50)
Contraceptive method[a,b]	
No	40(57.1)
Yes	30(42.9)
Pregnancy	
No	134(95.7)
Yes	6(4.3)
Sexual dysfunction[a]	
No	11(15.7)
Yes	59(84.3)

Notes: [a]Assessed only among sexually-active women; [b]includes hormonal and non-hormonal contraceptives.

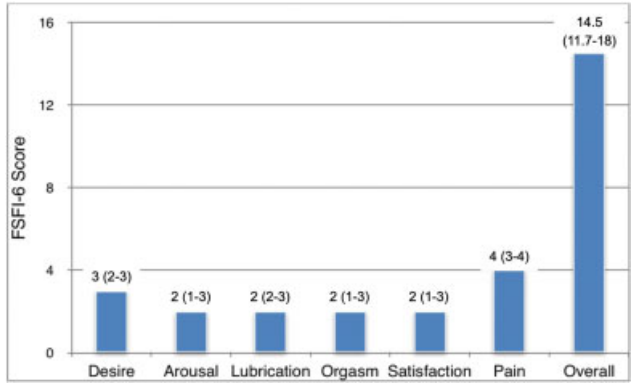

Fig. 1 Median total score and scores for the domains of the six-item version of the Female Sexual Function Index (FSFI-6) among sexually-active women with spina bifida. Data are presented as medians (25th percentile––75th percentile).

Table 2 Clinical characteristics and sexual outcomes

	Sexual activity n = 140		Sexual dysfunction* n = 70	
	No	Yes	No	Yes
Body mass index (Kg/m^2)				
$\leq$ 20	4(50)	4(50)	3(75)	1(25%)
20–25	18(41.9)	25(58.1)	1(4)	24(96%)
25–30	35(52.2)	32 (47.8)	6(18.8)	26(81.2%)
$\geq$ 30	13(59.1)	9(40.9)	1(11.1)	8(88.9)
p-value	0.572[a]		0.004[b]	
Myelomeningocele	64(50)	64 (50)	11(17.2)	53(82.8)
Meningocele/Others (includes spina bifida occulta)	6(50)	6(50)	0(0)	6(100)
p-value	1[a]		0.580[b]	
Spinal cord level				
Lumbar or lumbosacral	64(50)	64(50)	10(15.6)	54(84.4)
Thoracic or Thoracolumbar	6(50)	6(50)	1(16.7)	5(83.3)
p-value	1[a]		1[b]	
Hydrocephalus				
No	13(54.2)	11(45.8)	2(18.2)	9(81.8)
Yes	57(49.1)	59(50.9)	9(15.3)	50(84.7)
p-value	0.654[a]		1[b]	
Deambulation				
Deambulates	52(48.1)	56(51.9)	9(16.1)	47(83.9)
Wheelchair	18(56.3)	14(43.7)	2(14.3)	12(85.7)
p-value	0.421[a]		1[b]	
Urinary incontinence				
No	5(21.7)	18(78.3)	9(50)	9(50)
Yes	65(55.6)	52(44.4)	2(3.8)	50(96.2)
p-value	0.003[a]		$<$ 0.001[b]	
Fecal incontinence				
No	23(46)	27(54)	7(25.9)	20(74.1)
Yes	47(52.2)	43(47.8)	4(9.3)	39(90.7)
p-value	0.480[a]		0.092[b]	
Psychological disorder				
No	59(50.4)	58(49.6)	8(13.8)	50(86.2)
Yes	11(47.8)	12(52.%)	3(25)	9(75)
p-value	0.820[a]		0.386[b]	

Notes: [a]Chi-squared test; [b]Fisher exact test; female sexual dysfunction was assessed only among sexually-active women.

Discussion

Spina bifida is a complex group of anatomical changes characterized by impaired fusion of the vertebral arches in the first 28 days of the embryo, and it is considered the main neurological birth defect.[1] Traditionally considered a condition of the pediatric population, SB has undergone major changes due to better medical care, and, nowadays, most patients reach adulthood.[2] Thus, the increase in life expectancy leads to a rising importance of sexuality among this population.

A sexual activity rate of 50% among the SB patients in the present study is concordant with previous research[6–8,14] that reported rates of sexual activity among women with the same condition ranging from 32% to 68%. This demonstrates important differences compared with the general female population from the countries involved in the study – Brazil and Spain –,which presented sexual intercourse rates ranging from 83.6% to 85.3%.[15,16] Also, the prevalence of sexual dysfunction in the present study (84.3%), compared with a large Brazilian sample of non-SB women with dysfunction rates of 49%, highlights the need to improve sexual care in

Table 3 Clinical characteristics and specific domains of the 6-item version of the Female Sexual Function Index (FSFI-6) among sexually-active female spina bifida patients. Data are presented as medians (25th percentile–75th percentile)

	Desire	Arousal	Lubrication	Orgasm	Satisfaction	Pain	Overall
Type of lesion							
Myelomeningocele	3(2–3)	2(1–3)	2(2–3)	2(1–3)	2(1–3)	4(3–4)	14.5(11.25–18)
Meningocele/Others (includes spina bifida occulta)	2(2–3)	2(1.75–3.25)	2(1.75–3.25)	2(1.75–3)	1.5(1–2.25)	4.5(3–5)	15.5(11.75–17.5)
*p-value**	0.542	0.655	0.786	0.761	0.345	0.247	0.891
Spinal cord level							
Lumbar or lumbosacral	3(2–3)	2(1–3)	2(2–3)	2(1–3)	2(1–3)	4(3–4)	15(11.25–18)
Thoracic or thoracolumbar	1.5(1–4)	2(2–3.5)	2(1–4)	2(2–2.5)	1(1–3.5)	4(3.75–5)	14.3(11.75–20.75)
*p-value**	0.322	0.349	0.761	0.573	0.430	0.221	0.908
Hydrocephalus							
No	2(1–3)	2(1–3)	2(2–3)	3(2–4)	1(1–2)	4(4–5)	15(12–18)
Yes	3(2–3)	2(1–3)	2(2–3)	1(1–3)	2(1–3)	4(3–4)	14(11–18)
*p-value**	0.303	0.802	0.701	**0.015**	0.328	0.241	0.703
Deambulation							
Deambulates	2.5(2–3)	2(1–3)	2(2–3)	2(1.25–3)	2(1–3)	4(3–4)	15(11.25–18)
Wheelchair	3(2–4)	2(0.75–3)	2.5(1.5–3)	1(0.75–2.25)	2(1–3.25)	4(2.25–4)	13.5(11.5–16.25)
*p-value**	0.067	0.843	0.524	0.136	0.062	0.232	0.534
Urinary incontinence							
No	3.5(3–4)	3(3–3.25)	4(3–4)	3(2–3)	3.5(3–4)	4(3–4)	19.5(18–23)
Yes	2(1.25–3)	2(1–2)	2(1.25–3)	2(1–3)	1.5(1–2)	4(3–4)	13(11–15.75)
*p-value**	< **0.001**	< **0.001**	< **0.001**	**0.037**	< **0.001**	0.381	< **0.001**
Fecal incontinence							
No	3(2–4)	3(2–3)	3(2–4)	3(2–3)	3(2–4)	4(3–4)	18(13–21)
Yes	2(1–3)	2(1–2)	2(2–3)	2(1–2)	2(1–2)	4(3–5)	13(11–16)
*p-value**	**0.007**	**0.009**	**0.017**	**0.006**	**0.004**	0.065	**0.003**
Psychological disorder							
No	3(2–3)	2(1–3)	2(2–3)	2(1–3)	2(1–3)	4(3–4)	14.5(11.75–18)
Yes	3(2–3)	2(2–3)	2(2–3)	3(1.25–3)	2(1–3.75)	4(3–4.75)	15.5(11.5–21.75)
*p-value**	0.802	0.374	0.866	0.194	0.903	0.993	0.547

Note: *Mann-Whitney non-parametric test.

SB.[17] The weak median scores found in the overall and specific-domains of the FSFI-6 among our patients are similar to the scores found in previous studies that quantitatively assessed sexuality in a quantitative matter.[8,18] Lee et al.[18] found lower overall and specific-domain scores in the FSFI of SB patients when compared with non-SB women who also suffered from sexual dysfunction, showing that these neurologic patients demand more attention to their sexual life than regular patients.[18]

To comprehend the sexuality of female patients, it is important to assess their GO aspects. Our study demonstrated that only 17.9% of the SB patients had regular annual GO follow-up, meanwhile almost 15% had never undergone a single GO evaluation. Also, the prevalence of contraceptive methods used by sexually-active SB patients was much inferior compared with non-neurogenic female sexually-active patients who attended the gynecology outpatient clinic of the Brazilian institution in the present study (SB patients: 42.9%; regular patients: 91.9%), revealing the risk of inadvertent pregnancy among the population with SB.[19] Other studies[7,9] have already demonstrated a lack of contraception in these patients, which is believed to be related to inadequate sex education and unfamiliarity with the available options. Other factors that may contribute are the high rates of latex allergy and comorbidities associated with SB that restrict the use of contraceptives (such as epilepsy and the use of anticonvulsants; and reduced mobility and thromboembolic events).[20,21] The physiological process of pregnancy and the effects of fetal growth can exacerbate the manifestations of SB, such as bone abnormalities (mainly in

the spine and hips), which could restrict mobility, cause pain, make vaginal delivery difficult, and hinder epidural analgesia.[20,22] Spina bifida patients have been encouraged to perform vaginal deliveries and follow the obstetric indications for cesarean section respecting their orthopedic limitations (auch as narrow pelvis or severe scoliosis).[20,23] Despite these recommendations, SB patients are still most often submitted to cesarean sections when compared with the general population.[23] In the present study, in spite of the low rates of contraception, only 4.3% had a history of pregnancy, and there were no major complications during deliveries, which were all cesarean sections.

The clinical characteristics of SB and the sexual outcomes have been analyzed qualitatively and quantitatively. The UI status was the most relevant factor, since it impaired either sexual activity rates and worsened the overall and specific-domain scores on the FSFI-6. The only aspect that did not suffer significant influence from the UI was the pain domain. These findings are consonant with those of previous studies, including the specific data from Gamé et al.,[6] who also observed that the desire, arousal and lubrication domains suffered negative effects from UI among SB patients.[6–8] Other non-neurogenic conditions that caused UI also showed that it has major impact in female sexual life, mainly due to the fear of unpredictable incontinence during sex. Urinary incontinence impairs the self-esteem and promotes anxiety, which could contribute to these findings.[24,25] Although fecal incontinence did not promote significant differences in the rates of sexual activity, we found that it influenced negatively, in a similar manner to that of UI, in all sexual-function domains but pain. Few studies[26,27] assessed fecal incontinence and sexuality, with a limited inference that it could impair the social life and sexual perception of the SB patients.[26,27] Neurological characteristics (type of SB, spinal cord level, hydrocephalus, walking ability, and the presence of concomitant psychological disorders) showed little influence in the sexual outcomes. The only significant finding is that those without hydrocephalus had better scores on the orgasm domain of the FSFI-6. Two previous studies[9,28] have described that SB patients with hydrocephalus demonstrated inferior sexual activity, fewer sexual partners, and more sexual dysfunction. The fact that hydrocephalus is caused by Arnold-Chiari type-2 cerebellar malformation could explain the orgasm interference, since the cerebellum demonstrated increased activity during orgasm in functional magnetic resonance imaging studies.[29]

There are some limitations to the present study that should be considered potential bias. The instrument to evaluate sexual function (FSFI-6) was originally validated in women who attended outpatient clinics for reproductive medicine in Italy, and the Brazilian Portuguese version was assessed in middle-aged patients. There are no validated sexual questionaries specific for SB patients,in which is a limitation of the present study. Another limitation is that UI was simplified in yes or no groups, not taking into account the different types that could be present (sphincteric insufficiency, detrusor hyperactivity, or both), because it was not possible to access the videourodynamics exams from most of the sample.

Conclusion

The clinical aspects of SB patients, such as UI and fecal incontinence, should be properly addressed by their doctors, since they are associated with reduced sexual activity and lower FSFI-6 scores in the overall or specific domains. There is also a need to improve GO care among sexually-active SB patients, since most do not use any contraceptive methods, and are at risk of inadvertent pregnancy.

Contributors
All authors contributed to the writing of the article, relevant revision of the intellectual content, and approved the final version submitted for publication.

Conflict of Interests
The authors have no conflict of interests to declare.

References

1 Copp AJ, Adzick NS, Chitty LS, Fletcher JM, Holmbeck GN, Shaw GM. Spina bifida. Nat Rev Dis Primers. 2015;1:15007. Doi: 10.1038/nrdp.2015.7
2 Sawin KJ, Liu T, Ward E, et al; NSBPR Coordinating Committee. The National Spina Bifida Patient Registry: profile of a large cohort of participants from the first 10 clinics. J Pediatr. 2015;166(02): 444–50.e1. Doi: 10.1016/j.jpeds.2014.09.039
3 Vu Minh Arnell M, Abrahamsson K. Urinary continence appears to enhance social participation and intimate relations in adolescents with myelomeningocele. J Pediatr Urol. 2019;15(01):33.e1–33.e6. Doi: 10.1016/j.jpurol.2018.08.008
4 Wiener JS, Frimberger DC, Wood H. Spina bifida healthcare guidelines for men's health. Urology. 2018;116:218–226. Doi: 10.1016/j.urology.2018.01.005
5 Shiomi T, Hirayama A, Fujimoto K, Hirao Y. Sexuality and seeking medical help for erectile dysfunction in young adults with spina bifida. Int J Urol. 2006;13(10):1323–1326. Doi: 10.1111/j.1442-2042.2006.01559.x
6 Gamé X, Moscovici J, Guillotreau J, Roumiguié M, Rischmann P, Malavaud B. Sexual function of young women with myelomeningocele. J Pediatr Urol. 2014;10(03):418–423. Doi: 10.1016/j.jpurol.2013.07.016
7 Gatti C, Del Rossi C, Ferrari A, Casolari E, Casadio G, Scire G. Predictors of successful sexual partnering of adults with spina bifida. J Urol. 2009;182(4, Suppl):1911–1916. Doi: 10.1016/j.juro.2009.02.065
8 Choi EK, Kim SW, Ji Y, Lim SW, Han SW. Sexual function and qualify of life in women with spina bifida: Are the women with spina bifida satisfied with their sexual activity? Neurourol Urodyn. 2018;37(05):1785–1793. Doi: 10.1002/nau.23525
9 Verhoef M, Barf HA, Vroege JA, et al. Sex education, relationships, and sexuality in young adults with spina bifida. Arch Phys Med Rehabil. 2005;86(05):979–987. Doi: 10.1016/j.apmr.2004.10.042
10 Pérez-López FR, Fernández-Alonso AM, Trabalón-Pastor M, Vara C, Chedraui PMenopAuse RIsk Assessment (MARIA) Research Group. Assessment of sexual function and related factors in mid-aged sexually active Spanish women with the six-item Female Sex Function Index. Menopause. 2012;19(11):1224–1230. Doi: 10.1097/gme.0b013e3182546242
11 Dall'Agno ML, Ferreira CF, Ferreira FV, Pérez-López FR, Wender MCO. Validation of the six-item Female Sexual Function Index in middle-aged Brazilian women. Rev Bras Ginecol Obstet. 2019;41 (07):432–439. Doi: 10.1055/s-0039-1692694
12 Isidori AM, Pozza C, Esposito K, et al. Development and validation of a 6-item version of the female sexual function index (FSFI) as a

diagnostic tool for female sexual dysfunction. J Sex Med. 2010;7 (03):1139–1146. Doi: 10.1111/j.1743-6109.2009.01635.x
13 von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JPSTROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007;370 (9596):1453–1457. Doi: 10.1016/S0140-6736(07)61602-X
14 Streur CS, Schafer CL, Garcia VP, Quint EH, Sandberg DE, Wittmann DA. "If everyone else is having this talk with their doctor, why am I not having this talk with mine?": the experiences of sexuality and sexual health education of young women with spina bifida J Sex Med. 2019;16(06):853–859. Doi: 10.1016/j.jsxm.2019.03.012
15 Abdo CH, Oliveira WM Jr, Moreira ED Junior, Fittipaldi JA. [Sexual profile of brazilian population: results from Brazilian Study of Sexual Behavior (BSSB)]. RBM. Rev Bras Med. 2002;59(04): 250–257Portuguese.
16 Castroviejo Royo F, Conde Redondo C, Rodriguez Toves LA, Garcia-Tunon CM, Gonzalez Tejero C. Female sexual function in Castilla Y Leon (Spain): normal ranges. Gynecol Obstet (Sunnyvale). 2014; 4:1–6. Doi: 10.4172/2161-0932.1000240
17 Abdo CH, Oliveira WM Jr, Moreira ED Jr, Fittipaldi JA. Prevalence of sexual dysfunctions and correlated conditions in a sample of Brazilian women–results of the Brazilian study on sexual behavior (BSSB). Int J Impot Res. 2004;16(02):160–166. Doi: 10.1038/sj.ijir.3901198
18 Lee NG, Andrews E, Rosoklija I, et al. The effect of spinal cord level on sexual function in the spina bifida population. J Pediatr Urol. 2015;11(03):142.e1–142.e6. Doi: 10.1016/j.jpurol.2015.02.010
19 Lubianca JN, Cioba C, Martins DE, et al. Knowledge about emergency contraception among women referred for treatment at a university hospital in Brazil. Clin Biomed Res. 2014;34(01):60–66
20 Jackson AB, Mott PK. Reproductive health care for women with spina bifida. ScientificWorldJournal. 2007;7:1875–1883. Doi: 10.1100/tsw.2007.304
21 Nosek MA, Wuermser L, Walter LJ. Differences in contraceptive methods used by women with physical disabilities compared to women without disabilities. Prim Care Update Ob Gyns. 1998;5 (04):172–173. Doi: 10.1016/s1068-607x(98)00076-6
22 Sivarajah K, Relph S, Sabaratnam R, Bakalis S. Spina bifida in pregnancy: A review of the evidence for preconception, antenatal, intrapartum and postpartum care. Obstet Med. 2019;12(01): 14–21. Doi: 10.1177/1753495 × 18769221
23 Shepard CL, Yan PL, Hollingsworth JM, Kraft KH. Pregnancy among mothers with spina bifida. J Pediatr Urol. 2018;14(01):11.e1–11. e6. Doi: 10.1016/j.jpurol.2017.08.001
24 Pinheiro Sobreira Bezerra LR, Britto DF, Ribeiro Frota IP, et al. The Impact of Urinary incontinence on Sexual function: a systematic review. Sex Med Rev. 2020;8(03):393–402. Doi: 10.1016/j.sxmr.2019.06.009
25 Mota RL. Female urinary incontinence and sexuality. Int Braz J Urol. 2017;43(01):20–28. Doi: 10.1590/S1677-5538.IBJU.2016.0102
26 Verhoef M, Lurvink M, Barf HA, et al. High prevalence of incontinence among young adults with spina bifida: description, prediction and problem perception. Spinal Cord. 2005;43(06):331–340. Doi: 10.1038/sj.sc.3101705
27 von Linstow ME, Biering-Sørensen I, Liebach A, et al. Spina bifida and sexuality. J Rehabil Med. 2014;46(09):891–897. Doi: 10.2340/16501977-1863
28 Lassmann J, Garibay Gonzalez F, Melchionni JB, Pasquariello PS Jr, Snyder HM III. Sexual function in adult patients with spina bifida and its impact on quality of life. J Urol. 2007;178(4 Pt 2):1611–1614. Doi: 10.1016/j.juro.2007.03.162
29 Wise NJ, Frangos E, Komisaruk BR. Brain activity unique to orgasm in women: an fMRI analysis. J Sex Med. 2017;14(11):1380–1391. Doi: 10.1016/j.jsxm.2017.08.014

SARS-CoV-2 Infection and Placental Pathology

Infecção por SARS-CoV-2 e patologia placentária

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Abstract

Placental pathophysiology in SARS-CoV-2 infection can help researchers understand more about the infection and its impact on the maternal/neonatal outcomes. This brief review provides an overview about some aspects of the placental pathology in SARS-CoV-2 infection. In total, 11 papers were included. The current literature suggests that there are no specific histopathological characteristics in the placenta related to SARS-CoV-2 infection, but placentas from infected women are more likely to show findings of maternal and/or fetal malperfusion. The most common findings in placentas from infected women were fibrin deposition and intense recruitment of inflammatory infiltrates. The transplacental transmission of this virus is unlikely to occur, probably due to low expression of the receptor for SARS-CoV-2 in placental cell types. Further studies are needed to improve our knowledge about the interaction between the virus and the mother-fetus dyad and the impact on maternal and neonatal/fetal outcomes.

Keywords
- SARS-CoV-2
- Covid-19
- pregnancy
- placenta
- pathology

Resumo

A fisiopatologia da placenta na infecção por SARS-CoV-2 pode ajudar os pesquisadores a entender mais sobre a infecção e seu impacto nos resultados maternos/neonatais. Esta revisão breve fornece uma visão geral sobre alguns aspectos da patologia placentária na infecção por SARS-CoV-2. Ao todo, 11 artigos foram incluídos. A literatura atual sugere que não há características histopatológicas específicas nas placentas relacionadas à infecção por SARS-CoV-2, mas as placentas de mulheres infectadas têm maior probabilidade de apresentar achados de má perfusão materna e/ou fetal. Os achados mais comuns em placentas de mulheres infectadas foram deposição de fibrina e intenso recrutamento de infiltrado inflamatório. A transmissão transplacentária deste vírus é improvável, devido à baixa expressão do receptor para SARS-CoV-2 em tipos de células da placenta. Mais estudos são necessários para melhorar nosso conhecimento sobre a interação entre o vírus e a díade mãe-feto e o impacto nos resultados maternos e neonatais/fetais.

Palavras-chave
- SARS-CoV-2
- Covid-19
- gravidez
- placenta
- patologia

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Introduction

The most significant public health problem of the last decades is the coronavirus disease 2019 (Covid-19) pandemic, caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for more than 1 million deaths worldwide.[1] Some conditions have been associated with a higher risk of developing a severe illness, like advanced age, cardiovascular disease, diabetes mellitus, and hypertension.[2] However, data on the impact of the SARS-CoV-2 infection in pregnant women and in their fetuses or newborns are controversial. The available literature suggests that pregnant women have outcomes and clinical courses comparable to those of non-pregnant women of reproductive age,[3,4] and the newborns of infected mothers do not often show adverse clinical outcomes,[5] but there is few good-quality evidence to draw unbiased conclusions.[6] In any pregnancy infection, the placental pathophysiology can help researchers understand more about the disease and its impact on the maternal and neonatal outcomes.[7] The placenta is a transient pregnancy-related organ whose main function is to enable the maternal-fetal exchange of certain substances.[8] Some viruses can cross the placental barrier and infect the fetus, like the Zika virus, the cytomegalovirus, the rubella virus, and the herpesvirus.[9] So far, there is only one case report that showed unequivocal transplacental transmission of SARS-CoV-2.[10] There are many other papers about the vertical transmission of COVID-19, but no other convincing evidence has been found for the vertical transmission of this virus.[11] Besides being the possible key point for a fetal infection in pregnancy, the placenta itself can also be affected, morphologically and functionally, by the infection.[12] The aim of this brief review is to provide an overview about the data available in the literature about placental pathology in SARS-CoV-2 infection.

Methods

This brief and non-systematic review was based on a search carried out independently by two authors (CRVL and RAMM) on the PubMed, Scopus, SciELO and Cochrane databases. The following search terms were used: *placenta*; *placental pathology*; *SARS-CoV-2*; and *coronavirus*. Papers were selected after screening titles and articles. After data extraction and critical analysis, 11 case reports or series about placental alterations and pathophysiology in SARS-CoV-2 infection were included.[10,13–22]

Results

There are limited studies on SARS-CoV-2 infection and placental pathology. The most important aspects of each article found are shown in **table 1**.

So far, there is no evidence that SARS-CoV-2 can induce specific histopathological changes in placentas.[15,16,21,22] Some studies identified SARS-CoV-2 proteins in placental tissues or cells. Facchetti et al.[17] identified the virus in the villous syncytiotrophoblast, endothelial cells, fibroblasts, in maternal macrophages, in Hofbauer cells, and in fetal intravascular mononuclear cells. Hosier et al.[13] and Patanè et al.[20] found the virus in the syncytiotrophoblast, and Hsu et al.[14] identified virus proteins in chorionic villi endothelial cells and in trophoblasts.

Vivanti et al.[10] presented the first proven case of transplacental transmission of SARS-CoV-2. A 23-year-old pregnant woman infected by SARS-CoV-2 was submitted to a cesarean-section in full isolation. Amniotic fluid was collected before membrane rupture and tested positive for SARS-CoV-2 genes, as well as the placenta and other maternal and fetal tissues.

The most common findings in the placenta of pregnant women infected with SARS-CoV-2 are fibrin deposition and intense recruitment of inflammatory infiltrates. Fibrin depositions have been observed in three different patterns: subchorionic deposition,[18,22] deposition inside the villi,[21] and perivillous deposition,[10,13,17,21] and the last pattern was the most observed. The intense inflammatory infiltrates were composed mainly of macrophages,[17,20] neutrophils,[17] T lymphocytes[13,14] and histiocytes.[14]

In the study conducted by Smithgall et al.,[16] 51 third-trimester placentas from SARS-CoV-2-positive pregnant women (study group) and 25 third-trimester placentas from SARS-CoV-2-negative pregnant women (control group) were examined, and data were compared. As described before, no specific viral cytopathic modifications or evidence of vertical transmission were observed, but the study group showed evidence of maternal-fetal vascular malperfusion, with more villous agglutination ($p = 0.003$) and subchorionic thrombi ($p = 0.026$) than the control group. Ferraiolo et al.[18] also presented a case report of a third trimester SARS-CoV-2-positive placenta with villous agglutination.

Data also suggests that there is maternal and/or fetal malperfusion. Although the case reported by Hosier et al.[13] showed no decidual vasculopathy, the case report by Hsu et al.[14] demonstrated maternal vascular malperfusion (decidual hypertrophic arteriolopathy), with no fetal vascular malperfusion and, as aforementioned, Smithgall et al.[16] showed both maternal vascular malperfusion (decidual vasculopathy, intervillous thrombus, villus agglutination, and subchorionic thrombus) and fetal vascular malperfusion (avascular villi, fetal thrombotic vasculopathy, and chorangiosis). The study by Shanes et al.[19] indicated that placentas of SARS-CoV-2-positive pregnant women, compared to the control group (women with other medical conditions), were significantly more likely to exhibit intervillous thrombi ($p = 0.0002$) and at least one feature of maternal vascular malperfusion ($p = 0{,}046$), such as unusual or damaged maternal vessels.

The case series studied by Patanè et al.[20] presented 22 SARS-CoV-2-infected pregnant women. There were only two women whose newborns had SARS-CoV-2-positive nasopharyngeal swabs, and their placentas showed chronic intervillositis, accompanied by the existence of macrophages both in the intervillous and the villous spaces. Curiously, there were no significant alterations on the placenta of infected mothers whose newborns tested were negative.

Table 1 Summary of papers about placental pathology in SARS-CoV-2 infection

Authors	Study characteristics	Main findings
Vivanti et al.[10]	Case report of transplacental transmission of SARS-CoV-2 in a pregnant woman in the third trimester	The first case of proven transplacental transmission of SARS-CoV-2. The RT-PCR was positive for SARS-CoV-2 genes on the placenta, amniotic fluid and maternal, and fetal blood. Placental histological examination revealed diffuse perivillous fibrin deposition with infarction and acute and chronic intervillositis.
Hosier et al.[13]	Case report of second trimester SARS-CoV-2-infected pregnancy complicated by severe preeclampsia and placental abruption	Placental histological examination showed diffuse perivillous fibrin deposition and an inflammatory infiltrate consistent with histiocytic intervillositis. There were no features of decidual vasculopathy. Placenta and umbilical cord tested positive for SARS-COV-2 RNA. Virus proteins were localized predominantly in the syncytiotrophoblast cells.
Hsu et al.[14]	Case report of third trimester SARS-CoV-2-infected pregnant woman	Placental histological examination showed signs of maternal vascular malperfusion with hypertrophic arteriolopathy, but no fetal vascular malperfusion. There were signs of acute uterine hypoxia (subchorionic laminar necrosis) superimposed on chronic uterine hypoxia (extravillous trophoblasts and focal chronic villitis). Virus proteins were identified in chorionic villi endothelial cells and in trophoblasts.
Hecht et al.[15]	Case series and comparative study between 19 SARS-CoV-2 infected pregnant women and 3 sets of controls	There was no specific gross or characteristic histopathology present in the placentas, including the only two infected placentas.
Smithgall et al.[16]	Case series and comparative study between 51 SARS-CoV-2-infected pregnant women and 25 SARS-CoV-2-negative pregnant women	There were no specific histopathological characteristics in the placentas related to SARS-CoV-2 infection. None of the placentas tested positive for SARS-CoV-2. Maternal/fetal vascular malperfusion was identified in infected women, and their placentas showed more villous agglutination and subchorionic thrombi compared with non-infected women.
Facchetti et al.[17]	Case series of 15 SARS-CoV-2-infected pregnant women	Only 1 of the 15 placentas tested positive for SARS-CoV-2 genes. The comparison between this placenta and the other 14 showed no significant morphological differences, except for the prominent intervillous inflammation (showing variable changes compatible with fetal vascular malperfusion).
Ferraiolo et al.[18]	Case report of positive placental swabs for SARS-CoV-2 in an asymptomatic pregnant woman	Placental histological examination did not show substantial macroscopic alterations, except for mild to moderate subchorionic deposition of fibrin, for the presence of a single ischemic area in the thickness of the chorionic disc, for the appearance of villous agglutination, and for multiple organizing intervillous hemorrhages.
Shanes et al.[19]	Case series and comparative study between 16 SARS-CoV-2 infected pregnant women and 2 populations of controls	Third-trimester placentas were significantly more likely to show decidual arteriopathy or at least one characteristic of maternal vascular malperfusion (MVM), such as abnormal or injured maternal vessels and intervillous thrombi, when compared to controls. Placentas were not tested for SARS-CoV-2.
Patanè et al.[20]	Case series of 22 SARS-CoV-2-infected pregnant women in the third trimester	Only two newborns had SARS-CoV-2-positive nasopharyngeal swabs, whose placentas showed chronic intervillositis. On placental histological examination, no significant changes were observed in the other infected pregnant women.
Chen et al.[21]	Case series of three SARS-CoV-2-infected pregnant women in the third trimester	Placental histological examination showed various degrees of fibrin deposition inside and around the villi, but no pathological change of villitis or chorioamnionitis. There were no specific placental morphologic changes related to SARS-CoV-2 infection.
Taglauer et al.[22]	Case series and comparative study between 15 SARS-CoV-2-infected pregnant women and 10 SARS-CoV-2-negative pregnant women	There were no specific histopathological characteristics in the placentas related to SARS-CoV-2 infection. Placentas from infected women were notable for the presence of signs of inflammation and fibrin deposition, mostly intervillous and subchorionic deposition.

Abbreviations: RT-PCR, real-time polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Discussion

To date, little is known about placental pathology in SARS-CoV-2 infection, but the current literature suggests that there are no specific changes in the placenta of infected pregnant women. As aforementioned, the most common findings in the placenta of SARS-CoV-2-infected pregnant women are fibrin deposition and intense recruitment of inflammatory infiltrates. Compared to controls, the placentas of infected women showed a higher probability of exhibiting intervillous thrombi and at least one feature of maternal vascular malperfusion,[19] more villous agglutination, and subchorionic thrombi.[16]

Intervillous thrombi is the presence of a localized area of thrombosis in the chorionic villous stroma, while perivillous fibrinoid deposition is defined by the presence of fibrinoid material deposition in the intervillous space, and villous agglutination occurs when the distal villi are agglutinated by fibrin and bridging syncytial knots.[23] These patterns can be associated with processes of maternal malperfusion, such as placental insufficiency, fetal growth restriction, preeclampsia, thrombophilia, cardiovascular disease, renal abnormalities, or glucose intolerance.[23–26] Acute inflammatory lesions of the placenta are defined by diffuse infiltration of neutrophils and can involve every compartment of the placenta.[23,25] Chronic inflammatory lesions of the placenta are characterized by the infiltration of lymphocytes, plasma cells and macrophages, which may be a result of infections or may have an immune origin.[27] The main chronic inflammatory lesions of the placenta are villitis, chronic chorioamnionitis, and chronic deciduitis,[27] but chronic inflammation can also involve every compartment in the placenta, such as the intervillous space (intervillositis) or the umbilical cord (funisitis).[28] In our research, four articles showed two chronic inflammatory lesions in placentas from SARS-CoV-2-infected pregnant women: chronic intervillositis[10,13,20] and chronic villitis.[14] Both entities are usually reactions to infection, especially within the toxoplasmosis, other (syphilis, varicella-zoster, parvovirus B19), cytomegalovirus, and herpes simplex virus (TORCH) group, but when infectious causes are ruled out, they are called chronic intervillositis of unknown etiology (CIUE) and villitis of unknown etiology, both related to adverse obstetric outcomes, such as intrauterine growth restriction, preterm birth, and pregnancy loss.[28–30]

Specifically when talking about viral infections, some patterns are well studied, such as the correlation between maternal cytomegalovirus infection to the presence of chronic lymphoplasmacytic villitis and hemosiderin deposition,[23,26] as well as some reports of nonspecific intervillositis in the setting of the Zika and Dengue virus.[19] It seems that there is no association between the presence of chronic or even acute specific inflammatory patterns and placental findings of SARS-CoV-2-infected women,[19] only nonspecific inflammatory infiltrates composed of macrophages, neutrophils, T lymphocytes and histiocytes, as aforementioned.

Data on placental pathology in diseases caused by other coronaviruses, the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV), are scarce. Ng et al.[31] reported a case series of 7 placentas from pregnant women infected with SARS-CoV during the pandemic that occurred in Asia in 2003. Similar to SARS-CoV-2, there were no specific changes in those placentas. Two placentas of convalescent women who had the disease in the first trimester were normal, three placentas delivered in the acute stage of the disease showed increased subchorionic and intervillous fibrin deposition, and two placentas of convalescent women who had the disease in the third trimester showed extensive fetal thrombotic vasculopathy with sharply demarcated zones of avascular fibrotic villi (both had intrauterine growth restriction, oligohydramnios, and newborns small for gestational age). Data on MERS-CoV and placental pathology are even scarcer, but it seems that there is no relationship between this virus and specific placental disorders.[32]

In regard to the vertical transmission of SARS-CoV-2, most studies[11,33] show that this mode of transmission is unlikely to occur. Only Vivanti et al.[10] could prove the transplacental transmission of this virus; therefore, if vertical transmission exists, it happens at low rates and possibly in selected cases. One of the cornerstones in this issue is how the virus infects the cells: through the angiotensin-converting enzyme 2 (ACE-2) receptor and the transmembrane serine protease 2 (TMPRSS-2), widely expressed in many tissues.[34,35] It is well established that the more the cell expresses ACE-2, the greater the chances it will be infected by coronaviruses.[36] There is no consensus about how much placental tissue express ACE-2 and TMPRSS-2. Taglauer et al.[22] showed a predominance of ACE-2 expression in comparison with TMPRSS-2 in placenta from infected women, but there was a significant decrease in ACE-2 expression in those placentas compared to those of non-infected pregnant women. Pique-Regi et al.[37] reported that placental tissues poorly express ACE-2 and TMPRSS-2, but receptors for other viruses that cause congenital infections (such as cytomegalovirus and the Zika virus) are highly expressed by placental cell types, and that is why vertical transmission for SARS-CoV-2 is unlikely to occur. The expression of ACE-2 in the placenta can be increased in some diseases, such as preeclampsia,[38] so there would be a theoretical increased risk of vertical transmission in this setting, for example. Additional studies are needed to evaluate the expression of ACE-2 and TMPRSS-2 in placental cells in physiological and pathological conditions to investigate the infection and transmission of SARS-CoV-2.

Conclusion

In conclusion, in the present review, specific changes in the placentas of SARS-CoV-2-infected pregnant women were not found, but findings of maternal and/or fetal malperfusion were more likely to occur in infected than in non-infected women. The most common findings in the placentas from infected women were fibrin deposition and intense recruitment of inflammatory infiltrates. Little is known about placental pathology in SARS-CoV-2 infection, and further good evidence-based studies are needed in order to improve

our knowledge about the interaction between the virus and the mother-fetus dyad and the impact on maternal and neonatal/fetal outcomes.

Conflict of Interests
The authors have no conflict of interests to declare.

References

1 World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. 2020. [cited 2020 Oct 12]. Available from: https://covid19.who.int/
2 Petrilli CM, Jones SA, Yang J, Rajagopalan H, O'Donnell L, Chernyak Y, et al. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study. BMJ. 2020;369:m1966. Doi: 10.1136/bmj.m1966
3 Qiancheng X, Jian S, Lingling P, Lei H, Xiaogan J, Weihua, L, et al. sixth batch of Anhui medical team aiding Wuhan for COVID-19. Coronavirus disease 2019 in pregnancy. Int J Infect Dis. 2020; 95:376–383. Doi: 10.1016/j.ijid.2020.04.065
4 Della Gatta AN, Rizzo R, Pilu G, Simonazzi G. Coronavirus disease 2019 during pregnancy: a systematic review of reported cases. Am J Obstet Gynecol. 2020;223(01):36–41. Doi: 10.1016/j.ajog.2020.04.013
5 Kyle MH, Glassman ME, Khan A, Fernández CR, Hanft E, Emeruwa UN, et al. A review of newborn outcomes during the COVID-19 pandemic. Semin Perinatol. 2020;44(07):151286. Doi: 10.1016/j.semperi.2020.151286
6 Juan J, Gil MM, Rong Z, Zhang Y, Yang H, Poon LC. Effect of coronavirus disease 2019 (COVID-19) on maternal, perinatal and neonatal outcome: systematic review. Ultrasound Obstet Gynecol. 2020;56(01):15–27. Doi: 10.1002/uog.22088
7 Koi H, Zhang J, Parry S. The mechanisms of placental viral infection. Ann N Y Acad Sci. 2001;943:148–156. Doi: 10.1111/j.1749-6632.2001.tb03798.x
8 Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. Philos Trans R Soc Lond B Biol Sci. 2015;370 (1663):20140066. Doi: 10.1098/rstb.2014.0066
9 Arora N, Sadovsky Y, Dermody TS, Coyne CB. Microbial vertical transmission during human pregnancy. Cell Host Microbe. 2017; 21(05):561–567. Doi: 10.1016/j.chom.2017.04.007
10 Vivanti AJ, Vauloup-Fellous C, Prevot S, Zupan V, Suffee C, Cao JD, et al. Transplacental transmission of SARS-CoV-2 infection. Nat Commun. 2020;11(01):3572. Doi: 10.1038/s41467-020-17436-6
11 Simões E Silva AC, Leal CRV. Leal CRV. Is SARS-CoV-2 vertically transmitted? Front Pediatr. 2020;8:276. Doi: 10.3389/fped.2020.00276
12 Rosenberg AZ, Yu W, Hill DA, Reyes CA, Schwartz DA. Placental pathology of Zika virus: viral infection of the placenta induces villous stromal macrophage (Hofbauer cell) proliferation and hyperplasia. Arch Pathol Lab Med. 2017;141(01):43–48. Doi: 10.5858/arpa.2016-0401-OA
13 Hosier H, Farhadian SF, Morotti RA, Deshmukh U, Lu-Culligan A, Campbell KH, et al. SARS-CoV-2 infection of the placenta. J Clin Invest. 2020;130(09):4947–4953. Doi: 10.1172/JCI139569
14 Hsu AL, Guan M, Johannesen E, Stephens AJ, Khaleel N, Kagan N, et al. Placental SARS-CoV-2 in a pregnant woman with mild COVID-19 disease. J Med Virol. 2021;93(02):1038–1044. Doi: 10.1002/jmv.26386
15 Hecht JL, Quade B, Deshpande V, Mino-Kenudson M, Ting DT, Desai N, et al. SARS-CoV-2 can infect the placenta and is not associated with specific placental histopathology: a series of 19 placentas from COVID-19-positive mothers. Mod Pathol. 2020;33 (11):2092–2103. Doi: 10.1038/s41379-020-0639-4
16 Smithgall MC, Liu-Jarin X, Hamele-Bena D, Cimic A, Mourad M, Debelenko L, Chen X. Third-trimester placentas of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive women: histomorphology, including viral immunohistochemistry and in-situ hybridization. Histopathology. 2020;77(06): 994–999. Doi: 10.1111/his.14215
17 Facchetti F, Bugatti M, Drera E, Tripodo C, Sartori E, Cancila V, et al. SARS-CoV2 vertical transmission with adverse effects on the newborn revealed through integrated immunohistochemical, electron microscopy and molecular analyses of Placenta. EBioMedicine. 2020;59:102951. Doi: 10.1016/j.ebiom.2020.102951
18 Ferraiolo A, Barra F, Kratochwila C, Paudice M, Vellone VG, Godano E, et al. Report of positive placental swabs for SARS-CoV-2 in an asymptomatic pregnant woman with COVID-19. Medicina (Kaunas). 2020;56(06):306. Doi: 10.3390/medicina56060306
19 Shanes ED, Mithal LB, Otero S, Azad HA, Miller ES, Goldstein JA. Placental Pathology in COVID-19. Am J Clin Pathol. 2020;154(01): 23–32. Doi: 10.1093/ajcp/aqaa089
20 Patanè L, Morotti D, Giunta MR, Sigismondi C, Piccoli MG, Frigerio L, et al. Vertical transmission of coronavirus disease 2019: severe acute respiratory syndrome coronavirus 2 RNA on the fetal side of the placenta in pregnancies with coronavirus disease 2019-positive mothers and neonates at birth. Am J Obstet Gynecol MFM. 2020;2(03):100145. Doi: 10.1016/j.ajogmf.2020.100145
21 Chen S, Huang B, Luo DJ, Li X, Yang F, Zhao Y, Nie X, et al. [Pregnancy with new coronavirus infection: clinical characteristics and placental pathological analysis of three cases]. Zhonghua Bing Li Xue Za Zhi. 2020;49(05):418–423. Doi: 10.3760/cma.j.cn112151-20200225-00138Chinese.
22 Taglauer E, Benarroch Y, Rop K, Barnett E, Sabharwal V, Yarrington C, Wachman EM. Consistent localization of SARS-CoV-2 spike glycoprotein and ACE2 over TMPRSS2 predominance in placental villi of 15 COVID-19 positive maternal-fetal dyads. Placenta. 2020;100:69–74. Doi: 10.1016/j.placenta.2020.08.015
23 Jaiman S, Romero R, Pacora P, Jung E, Bhatti G, Yeo L, et al. Disorders of placental villous maturation in fetal death. J Perinat Med. 2020;•••:/j/jpme.ahead-of-print/jpm-2020-0030/jpm-2020-0030.xml. Doi: 10.1515/jpm-2020-0030 [ahead of print]
24 Derricott H, Jones RL, Heazell AE. Investigating the association of villitis of unknown etiology with stillbirth and fetal growth restriction - a systematic review. Placenta. 2013;34(10): 856–862. Doi: 10.1016/j.placenta.2013.07.003
25 Redline RW. The clinical implications of placental diagnoses. Semin Perinatol. 2015;39(01):2–8. Doi: 10.1053/j.semperi.2014.10.002
26 Khong TY, Mooney EE, Ariel I, Balmus NCM, Boyd TK, Brundler MA, et al. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. Arch Pathol Lab Med. 2016;140(07):698–713. Doi: 10.5858/arpa.2015-0225-CC
27 Kim CJ, Romero R, Chaemsaithong P, Kim JS. Chronic inflammation of the placenta: definition, classification, pathogenesis, and clinical significance. Am J Obstet Gynecol. 2015;213(4, Suppl): S53–S69. Doi: 10.1016/j.ajog.2015.08.041
28 Katzman PJ. Chronic inflammatory lesions of the placenta and preterm birth. NeoReviews. 2016;17(02):e80–86. Doi: 10 + 1542/neo.17–2-e80
29 Mattuizzi A, Sauvestre F, André G, Poingt M, Camberlein C, Carles D, et al. Adverse perinatal outcomes of chronic intervillositis of unknown etiology: an observational retrospective study of 122 cases. Sci Rep. 2020;10(01):12611. Doi: 10.1038/s41598-020-69191-9
30 Chen A, Roberts DJ. Placental pathologic lesions with a significant recurrence risk - what not to miss!. APMIS. 2018;126(07): 589–601. Doi: 10.1111/apm.12796
31 Ng WF, Wong SF, Lam A, Mak YF, Yao H, Lee KC, et al. The placentas of patients with severe acute respiratory syndrome: a pathophysiological evaluation. Pathology. 2006;38(03):210–218. Doi: 10.1080/00313020600696280
32 Jeong SY, Sung SI, Sung JH, Ahn YS, Kang ES, Chang YS, et al. MERS-CoV infection in a pregnant woman in Korea. J Korean Med Sci. 2017;32(10):1717–1720. Doi: 10.3346/jkms.2017.32.10.1717

33 Yang Z, Liu Y. Vertical transmission of severe acute respiratory syndrome coronavirus 2: a systematic review. Am J Perinatol. 2020;37(10):1055–1060. Doi: 10.1055/s-0040-1712161
34 Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181(02):271–280.e8. Doi: 10.1016/j.cell.2020.02.052
35 Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. Circ Res. 2020;126(10):1456–1474. Doi: 10.1161/CIRCRESAHA.120.317015
36 Jia HP, Look DC, Shi L, Hickey M, Pewe L, Netland J, et al. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. J Virol. 2005;79(23):14614–14621. Doi: 10.1128/JVI.79.23.14614-14621.2005
37 Pique-Regi R, Romero R, Tarca AL, Luca F, Xu Y, Alazizi A, Leng Y, et al. Does the human placenta express the canonical cell entry mediators for SARS-CoV-2? eLife. 2020;9:e58716. Doi: 10.7554/eLife.58716
38 Valdés G, Neves LA, Anton L, Corthorn J, Chacón C, Germain AM, et al. Distribution of angiotensin-(1-7) and ACE2 in human placentas of normal and pathological pregnancies. Placenta. 2006;27(2-3):200–207. Doi: 10.1016/j.placenta.2005.02.015

Morphology and Biochemistry of Ovulation

Morfologia e bioquímica da ovulação

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Abstract

The process of ovulation involves multiple and iterrelated genetic, biochemical, and morphological events: cessation of the proliferation of granulosa cells, resumption of oocyte meiosis, expansion of cumulus cell-oocyte complexes, digestion of the follicle wall, and extrusion of the metaphase-II oocyte. The present narrative review examines these interrelated steps in detail. The combined or isolated roles of the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are highlighted. Genes indiced by the FSH genes are relevant in the cumulus expansion, and LH-induced genes are critical for the resumption of meiosis and digestion of the follicle wall. A non-human model for follicle-wall digestion and oocyte release was provided.

Keywords
- granulosa cells
- follicle-stimulating hormone
- luteinizing hormone
- oocytes
- ovarian follicle

Resumo

O processo de ovulação envolve modificações genéticas, bioquímicas e morfológicas múltiplas e interrelacionadas: suspensão da proliferação das células da granulosa, reinício da meiose do oócito, expansão das células do complexo cumulus-oócito, digestão da parede folicular, e extrusão do oócito. Esta revisão narrativa examina em detalhes cada um desses eventos e os principais genes e proteínas envolvidos. Mais importante, a ação combinada ou isolada do hormônio folículo-estimulante (HFE) e do hormônio luteinizante (HL) é destacada. Detalha-se o papel do HFE na expansão do cumulus e do HL na digestão da parede folicular, permitindo a extrusão do oócito na superfície ovariana. Proveu-se um modelo não humano para explicar a digestão da parede folicular.

Palavras-chave
- células da granulosa
- hormônio folículo-estimulante
- hormônio luteinizante
- oócitos
- folículo ovariano

Introduction

Ovulation is the term used to define the ovarian release of the female mature gamete that is ready to be fertilized. The process of ovulation includes a series of morphological and biochemical events within the preovulatory follicle. Several genes are activated in the ovarian environment, leading to enzymatic and structural transformations under the influence of gonadotropins and sex steroids that are modulated by several growth factors. All of these events ensure that the oocyte becomes likely to be fertilized and extruded on the ovarian surface to form the corpus luteum.[1] The clinical marker of the

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beginning of the reproductive cycle responsible for the maturation and extrusion of the oocyte is menstruation. In regular cycles, at intervals of 24 to 38 days,[2] ovulation occurs mid-cycle, at around the 14th day. In this scenario, in an orchestrated way, the follicle-stimulating hormone (FSH), and the luteinizing hormone (LH) actively participate in the events that ensure ovulation, mostly through activation of multiple genes in theca and granulosa cells. The present review aims to examine the basic mechanisms of ovulation and describe the morphological and molecular events interconnected during the ovulatory process.

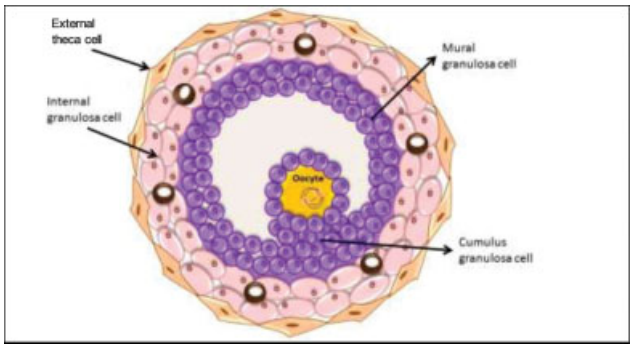

Fig. 1 Structure of the periovulatory follicle showing internal and external theca cell layers, granulosa cells, and the oocyte.

Methods

We searched for articles published in English in the PubMed and Google Scholar databases. The keywords were as follows: *menstrual cycle*, *menstrual cycle physiology*, *folliculogenesis*, *theca cells*, *granulosa cells*, *oocyte*, *oocyte-cumulus complex*, *follicular wall digestion*, *cumulus-oocyte-complex expansion*, *oocyte maturation*, *gene expression*, *FSH*, *LH*, and *progesterone receptor*. We expanded the search to the references of the retrieved articles.

Follicular Dynamics and Folliculogenesis

The more advanced stages of follicle development are characterized by the appearance of intercellular space filled by antral fluid. At this stage, the granulosa cells are differentiated into two distinct populations: cumulus cells, which are those closely linked to the oocyte, and wall or mural granulosa cells, which internally line the follicular wall. Although these two cell types share a common origin, there are differences in the production of transcribers and proteins.[3] At the end of follicular development, the FSH and estradiol promote the expression of the LH receptor (LHR) in granulosa cells. Most LH molecules bind to mural granulosa cells rather than to cumulus cells.[4] Cumulus cells provide energy input to the oocyte, controlling its growth and metabolism.[5] On the other hand, mural granulosa cells are responsible for steroid synthesis and differentiation in luteum cells after ovulation.[6] Cumulus granulosa cells play a smaller role regarding the function of the corpus luteum. Follicular architecture is provided by the inner and outer theca-cell layers (►**Fig. 1**). The theca cells, provided with LHR, are responsible for the capture of the substrate cholesterol and its enzymatic conversion into androgens, mainly testosterone (T) and androstenedione (A4). In turn, granulosa cells, which are adjacent to the theca cells, capture A4 and T and, by the action of the aromatase enzyme, convert them into estrone and estradiol respectively (►**Fig. 2**).[7]

Folliculogenesis begins with the formation of the primordial follicle, and ends with the preovulatory follicle.[8] The FSH, released by the anterior pituitary gland, promotes the recruitment of follicular waves that, in response, secrete estradiol and inhibin. When synthesized, these hormones modulate the release of pituitary FSH and LH in a pulsatile way. At the end of folliculogenesis, the preovulatory peaks of FSH and LH induce a complex sequence (or even a concurrence) of events: oocyte maturation, cumulus cell expansion, follicular wall digestion, and release of the cumulus-oocyte complex.[9]

Ovulation Process

Genetic Aspects Determining Ovulation

The ovulation process occurs in a coordinated and interrelated way in five complex steps: interruption of granulosa cell

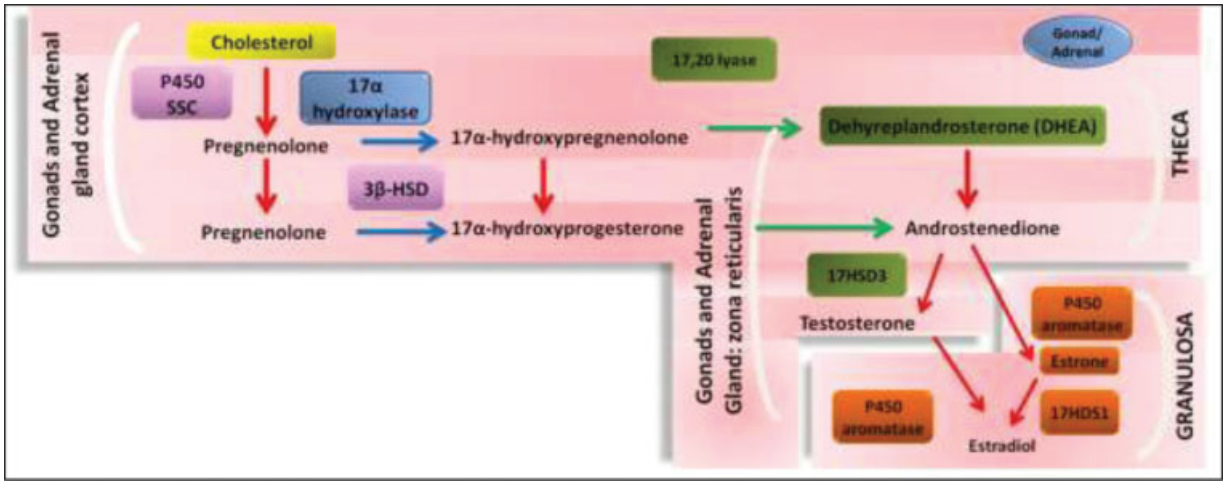

Fig. 2 Scheme showing the steroidogenesis of theca and granulosa ovarian cells. Abbreviations: P450ssc, cytochrome P450 for cleavage of cholesterol side chains; 3-βSHD, 3 β hydroxysteroid dehydrogenase; 17-HSD3, 17-hydroxysteroid dehydrogenase. **Source:** Medeiros et al.[7]

proliferation, resumption of meiosis, expansion of the cumulus with oocyte release inside the antrum, lysis of the follicular wall, and oocyte extrusion at the metaphase II (MII) stage. In mammals, oocytes are stationed in meiosis I at prophase I. The resumption of meiosis I occurs during puberty as a result of the gonadotropic stimulus in follicles in the preovulatory stage, culminating in the rupture of the germ vesicle.[10] The increase in the concentrations of LH and FSH in the mid-cycle in the presence of the preovulatory follicle, now provided with LHR in granulosa cells, promotes the activation of several genes that encode the synthesis of various proteins. This process is similar to inflammatory processes.[11] The LH activates cyclase, resulting in intracellular increases in cyclic adenosine monophosphate (cAMP) that activate cAMP-dependent kinases and the expression of the hyaluronic synthase 2 (HAS-2) and cyclooxygenase 2 (COX-2) enzymes, the tumor necrosis factor-inducible gene 6 protein (TSG-6), pentraxin 3 (PTX-3), and genes of the epidermal growth factor (EGF)-like family, such as amphiregulin (AREG), epiregulin (EREG), and betacellulin (BTC).[12–14] Tissue rearrangement occurs as a result of the activation of these genes participating in the cascade of ovulation events.

The Role of the Follicle-stimulating Hormone

Periovulatory gene expression induced by the FSH in cumulus cells plays a minor but necessary role in the mediation of ovulation (►**Fig. 3**). The occurrence of the FSH peak activates its own receptor (FSHR), stimulates the expression of steroidogenic factors, and induces LHR synthesis in granulosa cells. Such functions of the FSHR are related to the FSH activation of cAMP synthesis, and are triggered mainly through the expression of protein kinases A (PKA) and C (PKC) enzymes in granulosa cells.[15] The FSH activates the phosphatidylinositol 3-kinase/-protein kinase B (PI3K/Akt) pathway to mediate cell survival and granulosa proliferation, including the expression of the vascular endothelial growth factor (VEGF) gene, and it activates extracellular-regulated kinase (ERK) signaling in mural granulosa and cumulus cells, facilitating cumulus expansion.[16] The FSH may also induce COX-2 and other prostaglandin synthases through cAMP/PKA activation.[17] Activation of the COX-2 gene results mainly in prostaglandin F-α (PGF2α) that induces changes in the gene expression of the cumulus-oocyte-complex, which is critical for cumulus-oocyte-complex expansion.[18] Additionally, the FSH induces the expression of genes belonging to the family of disintegrin and metalloproteinases (A disintegrin and metalloproteinase with thrombospondin motifs, ADAMTS), molecules relevant in the process of cleavage of the extracellular matrix (►**Fig. 3**). It seems that these proteins are the main regulators of the release of EGF-like proteolytic factors in a soluble form (AREG, EREG, and BTC),[19] which activate the EGF receptor tyrosine kinase and the extracellular signal-regulated kinase (ERK) involved in cumulus expansion.[13] Metalloproteinases ADAMTS-1, ADAMTS-4, ADAMTS-5, and ADAMS-16, genes expressed in granulosa cells, are involved in the dissociation of the cumulus-oocyte complex and in the formation of the corpus luteum.[20,21] Then, the FSH, in the same way as in the mucification of the cumulus, plays a role with the LH in the synthesis of enzymes responsible for the digestion of the follicle wall.

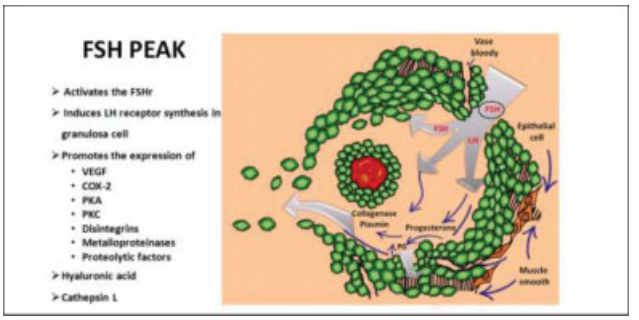

Fig. 3 Biochemical events initiated by the follicle stimulating hormone (FSH) in the preovulatory follicle. Abbreviations: VEGF, vascular endothelial growth factor; COX-2, cyclaoxygenase-2; PKA, protein kinase A; PKC, protein kinase C.

The Role of the Luteinizing Hormone

The role the LH in the ovulation process is complex and fundamental for the resumption of meiosis, loosening of the cumulus cells, and rupture of the follicle.[22] With the peak of the LH, the messenger ribonucleic acid (mRNA) for the progesterone receptor (PR) as well as other genes is now transcribed into the granulosa cells of preovulatory follicles (►**Fig. 4**).[23] The PR has an indirect influence on the synthesis of proteolytic enzymes cathepsin L and ADAMTS-1, which together play a role in tissue degradation and the remodeling of the extracellular matrix at the apex of the preovulatory follicle until ovulation occurs.[24] The LH peak, modulated by AMP, participates in the process of suppression of the proliferation of granulosa cells, and restarts meiosis, dissociation of the granulosa, digestion of the follicle wall, and luteinization.

Biochemical Aspects Determining Ovulation

Mucification and Cumulus Expansion

The genetic and biochemical events responsible for cumulus mucification are summarized in ►**Fig. 5**.[23] The matrix on which the cumulus cells move has three major components: hyaluronic acid (HA) and two HA binding proteins, TSG-6, and inter-α-trypsin inhibitor (ITI).[9,25] Induced by the peaks

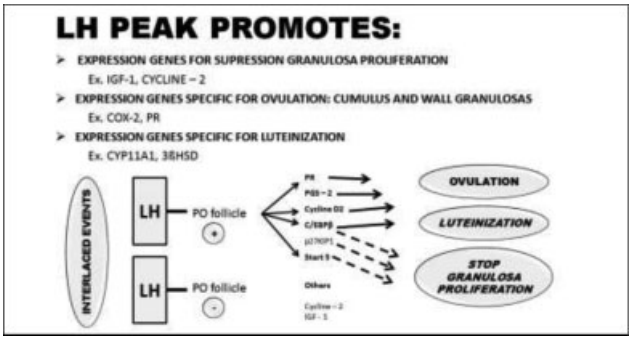

Fig. 4 Expression of several genes induced by the luteinizing hormone (LH) peak. **Source:** Richards et al.[23]Abbreviations: PO, preovulatory; PR, progesterone receptor; PGS, prostaglandins; C/EBPβ, *CAAT* enhancer-binding protein β; p27KIP1, cyclin-dependent kinase inhibitor 1B; Start 5, steroidogenic enzymes; IGF, insulin growth factor; COX, cyclooxygenase.

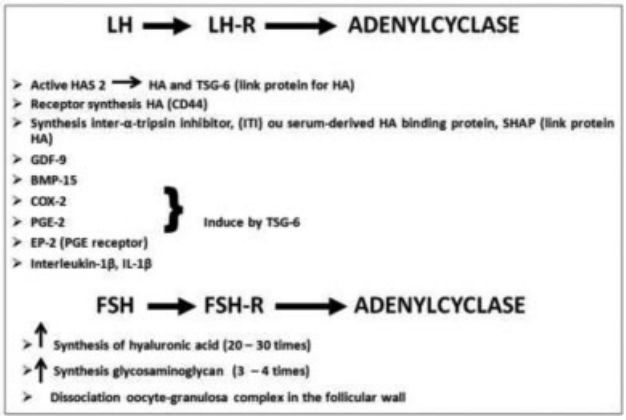

Fig. 5 Combined actions of the FSH and LH in the expansion of oocyte-cumulus cells. **Source:** Richards et al.[23] Abbreviations: HA, hyaluronic acid; GDF, growth defferentation factor; BMP, bone morphogenetic protein; PE, prostaglandin E receptor.

of FSH and LH, HAS-2 is the main enzyme responsible for the synthesis of arachidonic acids and HAs in the cumulus-oocyte complex, and, in synergy with COX-2, causes the synthesis of prostaglandins (PGs) from arachidonic acid in the granulosa cells of the cumulus. Thus, the expression of COX-2 in the cumulus cells promotes the synthesis of PGs, mainly prostaglandin E (PGE), and ensures the expansion of the cumulus.[18,25,26] However, cumulus expansion occurs only when the ITI enters the follicle. The TSG-6 and the proteoglycans brevican and versican, induced by high concentrations of LH and HA stabilization, are rapidly expressed in the cumulus granulosa cells of preovulatory follicles.[16] In the context of deficiency of the TSG-6 enzyme, the extracellular matrix is not structured, compromising cumulus expansion.[27] The PTX-3 protein, with an affinity for TSG6, is also responsible for the stability of the cumulus matrix. The interaction between these enzymes appears to be crucial for the structuring and expansion of the cumulus matrix, enabling the dispersion of the cumulus cells away from the oocyte.[20] Collectively, these observations indicate that HA, ITI, and COX-2, induced by the TSG-6 gene, are critical for cumulus matrix formation, cumulus cell differentiation, and, ultimately, cumulus expansion.

Oocyte Maturation

The oocyte maturation process aims to empower the female gamete and ensure its subsequent development until the activation of the embryonic genome occurs. Therefore, chromatin condensation is relevant in the continuity of meiosis, redistribution of organelles in the cytoplasm, and alterations in the cytoskeleton; all of these modifications are precisely regulated and coordinated (►**Fig. 6**).[28] For this to happen, there is paracrine cross-talk between the oocyte and cumulus cells. Cumulus cells penetrate the zona pellucida and limit the ooelema gap junction between the cumulus and the oocyte transfer of small molecules.[28] Biochemically, the oocyte regulates the metabolism of cumulus cells, which in turn provide ions, metabolites, amino acids, and small oocyte regulatory molecules (►**Fig. 7**).[29] Paracrine oocyte factors

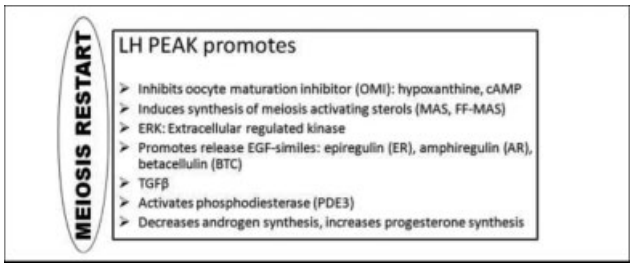

Fig. 6 The role of the LH in meiosis resumption. **Source:** Coticchio et al.[28] Abbreviations: TGFβ, transforming growth factor β; EGF, epidermal growth factor.

are soluble, and are generically referred to as oocyte-secreted factors (OSFs).[30] The growth differentiation factor 9 (GDF-9), the bone morphogenetic protein 15 (BMP15), and, to a lesser extent, the BMP6 are considered OSFs; all belong to the family of transforming growth factors β (TGFβ).[30,31] These factors coordinate the differentiation lineage and function of granulosa cells.

The functions of the OSFs include growth stimulation, prevention of apoptosis, inhibition of luteinization, regulation of energy metabolism, cholesterol biosynthesis, and regulation of cumulus expansion.[32–34] The factors that regulate the relationship between cumulus granulosa cells and the oocyte include ions, metabolites, amino acids, and small intracellular signaling molecules such as cAMP, cyclic guanosine monophosphate (cGMP), and inositol triphosphate-3 (IP3).[6,32] In the regulation of meiosis, cAMP synthesized by the oocyte itself and by cells of the mural granulosa and cumulus reaches the oocyte through the junctions of the hexameric lacunar canal composed of connectin proteins.[35,36]

In general, the properties of lacunar junctions enable the direct and bidirectional transport of small molecules between the oocyte and the granulosa cells. High intraoocyte levels of cAMP maintain the oocyte in the stage of germ vesicle, through suppression of the activity of the maturation-proimoting factor (MPF).[37–39] Follicle somatic cells also provide cGMP to the oocyte, inhibiting the phosphodiesterase enzyme type 3A (PDE3A), thereby preventing the

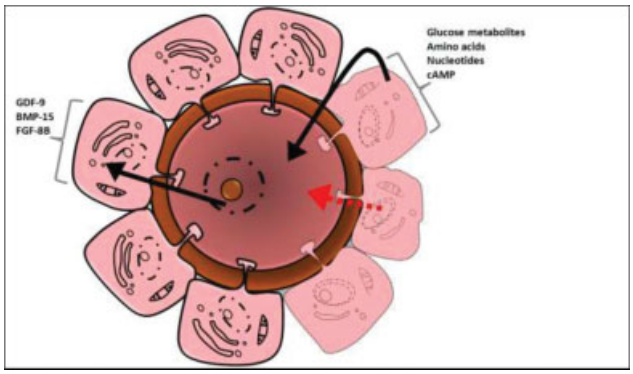

Fig. 7 Cell-cell signaling between the oocyte and granulosa cells in the final stage of follicle development. **Source:** Adapted from: Sutton et al.[29] Abbreviations: GDF-9, growth differentiation factor 9; BMP-15, bone morphogenetic protein 15; FGF-8B, fibroblast growth factor 8B.

degradation of cAMP with the accumulation of this factor and inhibition of the resumption of meiosis.[38,40] With the LH stimulus at high concentrations, the connectins close, decreasing the contribution of cAMP and cGMP from the cumulus cells to the oocyte. Therefore, the decrease in cAMP levels leads to the phosphorylation of PDE3A that degrades the cAMP. The degradation of cAMP enables the synthesis of the MPF, which promotes the resumption of meiosis I.[41]

In a recent study[42] in mice, the expression of natriuretic peptide type C (NPPC) was found in the mural granulosa cells, and natriuretic peptide receptor 2 (NPR2) was found in cumulus cells. With the communication between these two cell types the NPPC ligand and NPR2 stimulate the secretion of cGMP and cAMP. By adding NPPC to the culture media, an increase in the rates of oocytes that did not resume meiosis was observed, favoring the synchrony between nuclear maturation and cytoplasmic maturation.[42] During cytoplasmic maturation, there is a physical rearrangement of mitochondrial groups and endoplasmic reticulum, following the maturation time and energy dependence of the meiotic spindles so that chromatin is divided.

The meiotic spindles are responsible for the continuity of the meiotic division and extrusion of the two polar corpuscles. Initially, the mitochondrial groups are in a central position in the oocyte. As the maturation progresses, they migrate to the edges of the oocyte, close to the extruding regions of the polar body.[43,44] The MPF is the factor directly involved in cytoplasmic maturation, because, in addition to inducing the breakdown of the germ vesicle, it promotes the condensation of chromosomes, moving them from prophase I to metaphase I (MI), in which there is the formation of the meiotic spindle and the alignment of chromosomes in the center of the spindle. Then, anaphase I occurs, which consists of the separation of homologous chromosomes. Sequentially, telophase I begins with the extrusion of the first polar body, and the oocyte is in the metastasis II stage. At this stage, there is the formation of the second meiotic spindle and alignment of chromosomes, following anaphase II and telophase II and, finally, the extrusion of the second polar body.[45,46] The oocyte remains in this stage until ovulation occurs and there is the penetration of the sperm.

Follicular Wall Digestion

Morphological and biochemical changes that result in rupture of the follicular wall and oocyte extrusion occur basically by the action of the LH, because it induces the synthesis and secretion of various enzymes (►**Fig. 8**). The role of the FSH is smaller in this process, when the oocyte and cumulus cells are still fixed in the extracellular matrix (ECM). With the LH peak, LHR on the surface of the granulosa cells activates the digestion of the ECM within the theca layers and tunica albuginea at the ovarian surface via adenyl cyclase. The effectiveness of ECM digestion occurs through the balance between matrix components and proteases in the cumulus, oocyte, and endothelium cells that form the corpus luteum.[21]

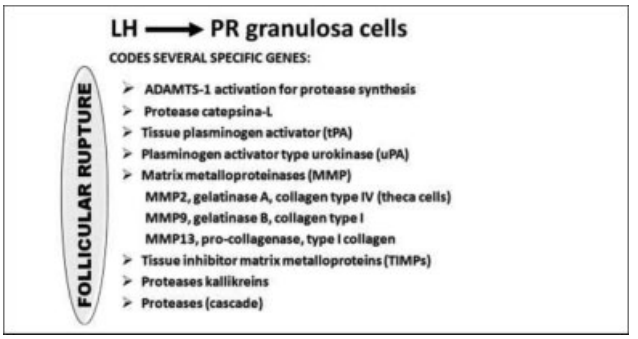

Fig. 8 Role of LH-induced genes in the digestion of the follicular wall.

Theca cells express a variety of matrix metalloproteinases (MMPs), including MMP2 (gelatinase A), MMP9 (gelatinase B), MMP13 (collagenase), MMP14, MMP16, MMP19, and tissue inhibitor of MMPs-1 (TIMP-1).[9]

The ADAMTS 16, present in luteinized granulosa cells, responds to FSH stimulation and actively participates in the process of structural follicle remodeling at the time of ovulation. The role of the LH on PR is mimicked by cAMP-inducing agonists (FSH, forskolin). Targets of PR appear to control the rupture of the follicle, mainly ADAMTS-1 (a disintegrin and metalloproteinase with thrombospondin) and cathepsin L. Among the proteases involved, thrombospondins 1 and 4 (ADAMTS1/4) promote the breakdown of the proteoglycan family structures, such as versican, through granulosa activation by PRs,[47] thereby contributing to the follicular rupture. Through its receptor in granulosa cells, the LH induces the transcription of early growth regulatory factor-1 (EGR-1), *CAAT* enhancer-binding protein β (C/EBPβ), PR, and other activator protein-1 family members (proto-oncogenes, c-Fos, c-Jun, Fra2, JunD), all involved in the functional activity of the granulosa cells of the ovulating follicle.

The proteoglycan (versican, brevican) components of the ECM induced by the LH peak, on either granulosa or theca cells, serve as substrates preferably for ADAMTS 1, culminating in follicular rupture.[47] Metalloproteinases such as plasminogen and collagenase are part of the follicular digestion process, and their control is mediated by metalloproteinase inhibitors, ensuring local homeostasis and completion of the ovulation process.[48] To illustrate, the model proposed by Ogiwara et al.[49] in the Japanese rice fish, also known as medaka, shows the involvement of proteinases in the lysis of the follicular wall (►**Fig. 9**).

After the rupture of the follicular wall, there is tissue reorganization by the activation of promatrix factors, which, in an organized and vascularized way, causes granulosa cell differentiation into luteal cells, thereby originating the corpus luteum. The corpus luteum is composed of functional cells for the synthesis of progesterone, the main regulator of the pituitary secretion of gonadotropins, the principal factors involved in the maintenance of the corpus luteum until initial gestation.[50] In the absence of maternal recognition of pregnancy, the corpus luteum regresses rapidly, and the ovarian cycle is resumed.[51]

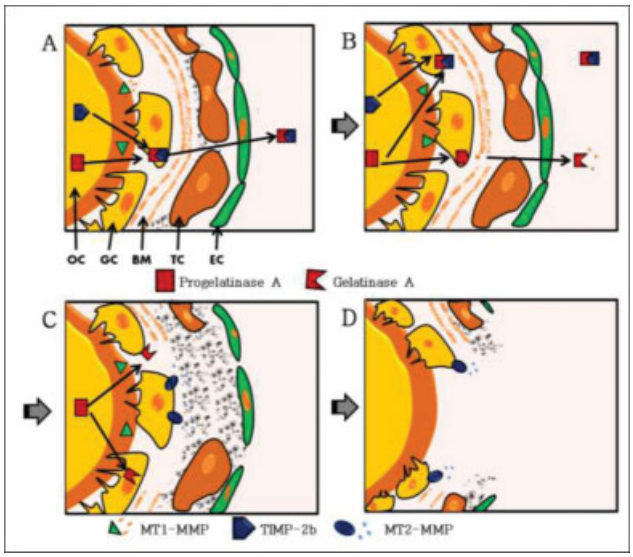

Fig. 9 A model of follicle rupture during ovulation in the Japanese rice fish, also known as medaka. (**A**) In the follicle, a few hours before ovulation, progelatinase A is activated by membrane type 1-matrix metalloproteinase (MT1-MMP) on the surface of the oocyte, whereas gelatinase A is immediately inactivated by the tissue inhibitor of matrix metalloproteinases-2b (TIMP-2b). (**B**) At the time of ovulation, the hydrolysis of basement membrane type-IV collagen is initiated by active gelatinase A at the follicle–ovarian surface contact site. (**C**) membrane type 2-matrix metalloproteinase (MT2-MMP), which is now expressed on the surface of the granulosa cells, can degrade the type-I collagen that is present in the theca cell layer.
(**D**) As a result, the oocyte is exposed at the contact site, leading to ovulation. Abbreviations: BM, basement membrane; EC, epithelial cell; GC, granulosa cell; OC, oocyte; TC, theca cell. Source: Ogiwara et al.[49] We would like to thank the National Academy of Sciences of the United States by permission

Conflict of Interests

The authors have no conflict of interests to declare.

References

1 Russell DL, Robker RL. Molecular mechanisms of ovulation: coordination through the cumulus complex. Hum Reprod Update. 2007;13(03):289–312. Doi: 10.1093/humupd/dml062
2 Fraser IS, Critchley HO, Munro MG, Broder M. Can we achieve international agreement on terminologies and definitions used to describe abnormalities of menstrual bleeding? Hum Reprod. 2007;22(03):635–643. Doi: 10.1093/humrep/del478
3 Latham KE, Bautista FD, Hirao Y, O'Brien MJ, Eppig JJ. Comparison of protein synthesis patterns in mouse cumulus cells and mural granulosa cells: effects of follicle-stimulating hormone and insulin on granulosa cell differentiation in vitro. Biol Reprod. 1999;61(02):482–492. Doi: 10.1095/biolreprod61.2.482
4 Peng XR, Hsueh AJ, LaPolt PS, Bjersing L, Ny T. Localization of luteinizing hormone receptor messenger ribonucleic acid expression in ovarian cell types during follicle development and ovulation. Endocrinology. 1991;129(06):3200–3207. Doi: 10.1210/endo-129-6-3200
5 Haghighat N, Van Winkle LJ. Developmental change in follicular cell-enhanced amino acid uptake into mouse oocytes that depends on intact gap junctions and transport system Gly. J Exp Zool. 1990;253(01):71–82. Doi: 10.1002/jez.1402530110
6 Albertini DF, Combelles CM, Benecchi E, Carabatsos MJ. Cellular basis for paracrine regulation of ovarian follicle development. Reproduction. 2001;121(05):647–653. Doi: 10.1530/rep.0.1210647
7 de Medeiros SF, Gil-Junior AB, Barbosa JS, Isaías ED, Yamamoto MM. New insights into steroidogenesis in normo- and hyperandrogenic polycystic ovary syndrome patients. Arq Bras Endocrinol Metabol. 2013;57(06):437–444. Doi: 10.1590/s0004-27302013000600005
8 van den Hurk R, Zhao J. Formation of mammalian oocytes and their growth, differentiation and maturation within ovarian follicles. Theriogenology. 2005;63(06):1717–1751. Doi: 10.1016/j.theriogenology.2004.08.005
9 Richards JS, Russell DL, Ochsner S, Espey LL. Ovulation: new dimensions and new regulators of the inflammatory-like response. Annu Rev Physiol. 2002;64:69–92. Doi: 10.1146/annurev.physiol.64.081501.131029
10 Schatten H, Sun QY. Centrosome dynamics during mammalian oocyte maturation with a focus on meiotic spindle formation. Mol Reprod Dev. 2011;78(10-11):757–768. Doi: 10.1002/mrd.21380
11 Espey LL. Ovulation as an inflammatory reaction–a hypothesis. Biol Reprod. 1980;22(01):73–106. Doi: 10.1095/biolreprod22.1.73
12 Park JY, Su YQ, Ariga M, Law E, Jin SL, Conti M. EGF-like growth factors as mediators of LH action in the ovulatory follicle. Science. 2004;303(5658):682–684. Doi: 10.1126/science.1092463
13 Ashkenazi H, Cao X, Motola S, Popliker M, Conti M, Tsafriri A. Epidermal growth factor family members: endogenous mediators of the ovulatory response. Endocrinology. 2005;146(01):77–84. Doi: 10.1210/en.2004-0588
14 Shimada M, Hernandez-Gonzalez I, Gonzalez-Robayna I, Richards JS. Paracrine and autocrine regulation of epidermal growth factor-like factors in cumulus oocyte complexes and granulosa cells: key roles for prostaglandin synthase 2 and progesterone receptor. Mol Endocrinol. 2006;20(06):1352–1365. Doi: 10.1210/me.2005-0504
15 Sirard MA, Desrosier S, Assidi M. In vivo and in vitro effects of FSH on oocyte maturation and developmental competence. Theriogenology. 2007;68(Suppl 1):S71–S76. Doi: 10.1016/j.theriogenology.2007.05.053
16 Ochsner SA, Day AJ, Rugg MS, Breyer RM, Gomer RH, Richards JS. Disrupted function of tumor necrosis factor-alpha-stimulated gene 6 blocks cumulus cell-oocyte complex expansion. Endocrinology. 2003;144(10):4376–4384. Doi: 10.1210/en.2003-0487
17 Joyce IM, Pendola FL, O'Brien M, Eppig JJ. Regulation of prostaglandin-endoperoxide synthase 2 messenger ribonucleic acid expression in mouse granulosa cells during ovulation. Endocrinology. 2001;142(07):3187–3197. Doi: 10.1210/endo.142.7.8268
18 Hizaki H, Segi E, Sugimoto Y, Hirose M, Saji T, Ushikubi F, et al. Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP(2). Proc Natl Acad Sci U S A. 1999;96(18):10501–10506. Doi: 10.1073/pnas.96.18.10501
19 Yamashita Y, Hishinuma M, Shimada M. Activation of PKA, p38 MAPK and ERK1/2 by gonadotropins in cumulus cells is critical for induction of EGF-like factor and TACE/ADAM17 gene expression during in vitro maturation of porcine COCs. J Ovarian Res. 2009;2:20. Doi: 10.1186/1757-2215-2-20
20 Richards JS, Hernandez-Gonzalez I, Gonzalez-Robayna I, Teuling E, Lo Y, Boerboom D, et al. Regulated expression of ADAMTS family members in follicles and cumulus oocyte complexes: evidence for specific and redundant patterns during ovulation. Biol Reprod. 2005;72(05):1241–1255. Doi: 10.1095/biolreprod.104.038083
21 Gao S, De Geyter C, Kossowska K, Zhang H. FSH stimulates the expression of the ADAMTS-16 protease in mature human ovarian follicles. Mol Hum Reprod. 2007;13(07):465–471. Doi: 10.1093/molehr/gam037
22 Dozortsev DI, Diamond MP. Luteinizing hormone-independent rise of progesterone as the physiological trigger of the ovulatory gonadotropins surge in the human. Fertil Steril. 2020;114(02):191–199. Doi: 10.1016/j.fertnstert.2020.06.016
23 Richards JS, Russell DL, Robker RL, Dajee M, Alliston TN. Molecular mechanisms of ovulation and luteinization. Mol Cell Endocrinol. 1998;145(1-2):47–54. Doi: 10.1016/s0303-7207(98)00168-3

24 Robker RL, Russell DL, Espey LL, Lydon JP, O'Malley BW, Richards JS. Progesterone-regulated genes in the ovulation process: ADAMTS-1 and cathepsin L proteases. Proc Natl Acad Sci U S A. 2000;97(09):4689–4694. Doi: 10.1073/pnas.080073497

25 Eppig JJ. Regulation by sulfated glycosaminoglycans of the expansion of cumuli oophori isolated from mice. Biol Reprod. 1981;25 (03):599–608. Doi: 10.1095/biolreprod25.3.599

26 Calder MD, Caveney AN, Westhusin ME, Watson AJ. Cyclooxygenase-2 and prostaglandin E(2)(PGE(2)) receptor messenger RNAs are affected by bovine oocyte maturation time and cumulus-oocyte complex quality, and PGE(2) induces moderate expansion of the bovine cumulus in vitro. Biol Reprod. 2001;65(01): 135–140. Doi: 10.1095/biolreprod65.1.135

27 Fülöp C, Szántó S, Mukhopadhyay D, Bárdos T, Kamath VR, Rugg MS, et al. Impaired cumulus mucification and female sterility in tumor necrosis factor-induced protein-6 deficient mice. Development. 2003;130(10):2253–2261. Doi: 10.1242/dev.00422

28 Coticchio G, Dal-Canto M, Guglielmo MC, Mignini-Renzini M, Fadini R. Human oocyte maturation in vitro. Int J Dev Biol. 2012;56(10-12):909–918. Doi: 10.1387/ijdb.120135gv

29 Sutton ML, Gilchrist RB, Thompson JG. Effects of in-vivo and in-vitro environments on the metabolism of the cumulus-oocyte complex and its influence on oocyte developmental capacity. Hum Reprod Update. 2003;9(01):35–48. Doi: 10.1093/humupd/dmg009

30 Gilchrist RB, Ritter LJ, Armstrong DT. Oocyte-somatic cell interactions during follicle development in mammals. Anim Reprod Sci. 2004;82-83:431–446. Doi: 10.1016/j.anireprosci.2004.05.017

31 Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Hum Reprod Update. 2008;14(02):159–177. Doi: 10.1093/humupd/dmm040

32 Buccione R, Schroeder AC, Eppig JJ. Interactions between somatic cells and germ cells throughout mammalian oogenesis. Biol Reprod. 1990;43(04):543–547. Doi: 10.1095/biolreprod43.4.543

33 Eppig JJ, Wigglesworth K, Pendola F, Hirao Y. Murine oocytes suppress expression of luteinizing hormone receptor messenger ribonucleic acid by granulosa cells. Biol Reprod. 1997;56(04): 976–984. Doi: 10.1095/biolreprod56.4.976

34 Hussein TS, Thompson JG, Gilchrist RB. Oocyte-secreted factors enhance oocyte developmental competence. Dev Biol. 2006;296 (02):514–521. Doi: 10.1016/j.ydbio.2006.06.026

35 Teilmann SC. Differential expression and localisation of connexin-37 and connexin-43 in follicles of different stages in the 4-week-old mouse ovary. Mol Cell Endocrinol. 2005;234(1-2):27–35. Doi: 10.1016/j.mce.2004.10.014

36 Gittens JE, Kidder GM. Differential contributions of connexin37 and connexin43 to oogenesis revealed in chimeric reaggregated mouse ovaries. J Cell Sci. 2005;118(Pt 21):5071–5078. Doi: 10.1242/jcs.02624

37 Aktas H, Wheeler MB, First NL, Leibfried-Rutledge ML. Maintenance of meiotic arrest by increasing [cAMP]i may have physiological relevance in bovine oocytes. J Reprod Fertil. 1995; 105(02):237–245. Doi: 10.1530/jrf.0.1050237

38 Tsafriri A, Chun SY, Zhang R, Hsueh AJ, Conti M. Oocyte maturation involves compartmentalization and opposing changes of cAMP levels in follicular somatic and germ cells: studies using selective phosphodiesterase inhibitors. Dev Biol. 1996;178(02):393–402. Doi: 10.1006/dbio.1996.0226

39 Bilodeau-Goeseels S. Cows are not mice: the role of cyclic AMP, phosphodiesterases, and adenosine monophosphate-activated protein kinase in the maintenance of meiotic arrest in bovine oocytes. Mol Reprod Dev. 2011;78(10-11):734–743. Doi: 10.1002/mrd.21337

40 Tsafriri A, Cao X, Ashkenazi H, Motola S, Popliker M, Pomerantz SH. Resumption of oocyte meiosis in mammals: on models, meiosis activating sterols, steroids and EGF-like factors. Mol Cell Endocrinol. 2005;234(1-2):37–45. Doi: 10.1016/j.mce.2004.09.009

41 Gilchrist RB, Smitz JEJ, Thompson JG. Current status and future trends of the clinical practice of human oocyte in vitro maturation. In: Gardner DK, Rizk BRMB, Falcone T, editors. Human assisted reproductive technology: future trends in laboratory & clinical practice. Cambridge: Cambridge University Press; 2011: 186–98

42 Zhang M, Su YQ, Sugiura K, Xia G, Eppig JJ. Granulosa cell ligand NPPC and its receptor NPR2 maintain meiotic arrest in mouse oocytes. Science. 2010;330(6002):366–369. Doi: 10.1126/science.1193573

43 Dalton CM, Carroll J. Biased inheritance of mitochondria during asymmetric cell division in the mouse oocyte. J Cell Sci. 2013;126 (Pt 13):2955–2964. Doi: 10.1242/jcs.128744

44 Coticchio G, Dal Canto M, Renzini MM, Guglielmo MC, Brambillasca F, Turchi D, et al. Oocyte maturation: gamete-somatic cells interactions, meiotic resumption, cytoskeletal dynamics and cytoplasmic reorganization. Hum Reprod Update. 2015;21(04): 427–454. Doi: 10.1093/humupd/dmv011

45 Kishimoto H, Hamada K, Saunders M, Backman S, Sasaki T, Nakano T, et al. Physiological functions of Pten in mouse tissues. Cell Struct Funct. 2003;28(01):11–21. Doi: 10.1247/csf.28.11

46 Sen A, Caiazza F. Oocyte maturation: a story of arrest and release. Front Biosci (Schol Ed). 2013;5:451–477. Doi: 10.2741/s383

47 Russell DL, Doyle KM, Ochsner SA, Sandy JD, Richards JS. Processing and localization of ADAMTS-1 and proteolytic cleavage of versican during cumulus matrix expansion and ovulation. J Biol Chem. 2003; 278(43):42330–42339. Doi: 10.1074/jbc.M300519200

48 Curry TE Jr, Dean DD, Sanders SL, Pedigo NG, Jones PB. The role of ovarian proteases and their inhibitors in ovulation. Steroids. 1989;54(05):501–521. Doi: 10.1016/0039-128x(89)90044-5

49 Ogiwara K, Takano N, Shinohara M, Murakami M, Takahashi T. Gelatinase A and membrane-type matrix metalloproteinases 1 and 2 are responsible for follicle rupture during ovulation in the medaka. Proc Natl Acad Sci U S A. 2005;102(24):8442–8447. Doi: 10.1073/pnas.0502423102

50 Kurita T, Wang YZ, Donjacour AA, Zhao C, Lydon JP, O'Malley BW, et al. Paracrine regulation of apoptosis by steroid hormones in the male and female reproductive system. Cell Death Differ. 2001;8 (02):192–200. Doi: 10.1038/sj.cdd.4400797

51 Smith RK, Carroll PM, Allard JD, Simon MA. MASK, a large ankyrin repeat and KH domain-containing protein involved in Drosophila receptor tyrosine kinase signaling. Development. 2002;129(01): 71–82

The Possible Contribution of being Born by Cesarean Section to Developing Childhood Overweight and Obesity in Later Life

A possível contribuição do nascimento por cesariana para o desenvolvimento do excesso de peso infantil e da obesidade na vida posterior

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Introduction

Obesity is now a major global epidemic. In 2016, 39% of adults worldwide ≥ 18 years old were overweight, and 13% were obese, as reported by the World Health Organization (WHO).[1] This current scenario is compounded in high-income countries such as the United States, where 31% of the population has a body mass index (BMI) > 30 kg/m^2 and 50% have a waist circumference ≥ 88 cm, in the case of women, or ≥ 102 cm in the case of men.[2] Overweight and obesity are rapidly becoming a major health issue, as these conditions are associated with severe chronic diseases such as diabetes, hypertension, and cardiovascular diseases in general.[3]

While it is largely agreed that obesity and overweight are the consequences of an unhealthy diet, mainly due to an increasing use of processed and preserved foods together with minimal physical activity, an association between cesarean delivery and obesity in later life has also been suggested. Cesarean section rates continue to increase well beyond what could be medically justified, and several studies conducted in different countries have found an association between being born by cesarean section and developing obesity in childhood or adulthood.[4]

Cesarean Section and the Development of Overweight and Obesity

The Growing Up Today Study (GUTS), a large prospective study of individuals followed-up from childhood through early adulthood in the United States, evaluated obesity as defined by the cutoff limit of BMI ≥ 30 kg/m^2, in accordance with the WHO criteria. The multivariate adjusted risk ratio (RR) for obesity was 1.15 for infants delivered by cesarean section compared with those born by vaginal delivery (95% confidence interval [CI]: 1.06–1.26; $p = 0.002$). The multivariate adjusted RR for obesity according to age group was: 1.23 (95%CI: 1.03–1.46) at ages between 9 and 12 years old, 1.16 (95%CI: 1.03–1.31) at ages between 13 and 18 years old, and 1.10 (95% CI: 0.98–1.24) at ages between 19 and 24 years old (*p-value* for heterogeneity = 0.13). The associations were similar both for females (1.12; 95%CI: 0.99–1.27) and for males (1.18; 95%CI: 1.04–1.34) (*p-value* for heterogeneity = 0.62).[5]

Three birth cohorts in the Brazilian city of Pelotas, state of Rio Grande do Sul, were followed-up into adulthood using a very similar methodology. All babies born in 1982, 1993 and 2004 whose mothers lived in the city were recruited to the studies. Fat mass index and BMI z-scores in the offspring were strongly and positively associated with cesarean birth in the crude analysis. However, following adjustment for socioeconomic characteristics and maternal factors such as BMI, parity, age, and smoking during pregnancy, the association disappeared, except for the group of 30-year-old women. In these women from the 1982 cohort, cesarean section remained associated with fat mass index ($\beta = 0.82$; 95%CI: 0.32–1.32) and BMI z-score ($\beta = 0.15$; 95%CI: 0.03–0.28).

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Following adjustment for potential confounders, the association persisted, indicating higher fat mass index and BMI at the age of 30 years old in those born by cesarean section compared with those delivered vaginally.[6]

The Project Viva enrolled participants between April 1999 and July 2002 in eastern Massachusetts, USA, forming a longitudinal prebirth cohort of mother-offspring pairs. In the children delivered by cesarean section, there was a 2-fold likelihood of developing obesity, with a higher BMI ($\sim$ 0.2 z-score units higher) and higher sum of triceps plus subscapular skinfold thicknesses ($\sim$ 1 mm) at the age of 3 years old compared with children delivered vaginally. These differences between the two modes of delivery persisted following adjustment for key potential confounding factors including maternal BMI and birthweight.[7]

In South Africa, an analysis of young adults aged between 21 and 24 years old from a longitudinal urban birth cohort showed that having been born by cesarean section was associated with obesity in early adulthood. In the crude analysis, birth by cesarean section was associated with an almost 2-fold increase in the risk of obesity among young people between 21 and 24 years old. Following adjustment for gender, birthweight, and the mother's parity and education level at delivery as possible confounders, the strength of the association was maintained.[8]

No such association was found, however, in a large, contemporary, prospective, longitudinal cohort study, the Millennium Cohort Study (MCS), conducted in the United Kingdom, which found no statistically significant difference in BMI at the ages of 3 and 14 years old in infants born by planned cesarean section compared with those born by vaginal delivery. Furthermore, there was no difference in body fat percentage at 7 and 14 years old between infants born by planned cesarean section and those born by vaginal delivery.[9]

A very large systematic review and meta-analysis conducted to investigate associations between mode of delivery and adult BMI, overweight, and obesity found that the mean BMI was 0.44 $kg.m^{-2}$ greater in subjects delivered by cesarean section compared with those delivered vaginally. The increased odds of overweight and obesity $>$ 20% applied to both genders.[10]

The Role of the Gut Microbiome as a Mechanism of Action in Overweight and Obesity

As accumulated global experience appears to confirm the association between being born by cesarean section and overweight and obesity in later life, a possible explanation for this association may lie in the limited microbial diversity reported in offspring delivered by cesarean section compared with that of those born by vaginal delivery.[11,12] Infants born by vaginal delivery are exposed to microorganisms mainly in the birth canal or in the vaginal environment, while those delivered by cesarean section are exposed to microflora on the mother's skin.[13] This is presumed to persist into adulthood.[5]

Experiments in rodents have shown the important role of microorganisms in the mechanism of obesity. Transferring fecal microbial content from normally raised mice to adult germ-free mice leads to a very rapid and voluminous increase in body fat within between 10 and 14 days, even when food consumption is reduced.[14] There are several mechanisms involved in these changes, including microbial fermentation of dietary polysaccharides that cannot be digested by mice, the contribution of monosaccharide and fatty acids to intestinal absorption, their metabolism in the liver, and the regulation of host genes that promote the deposition of lipids in adiposity.[14]

Infants delivered by cesarean section, particularly elective cesarean section, are generally not exposed to their mother's vaginal and fecal microbiota, which helps shape the initial composition of an infant's gut microbiota.[13] The diverseness and richness of their gut microbiome has been found to be poor.[15] According to some studies, the gut microbiota of infants born by cesarean section may have a tendency to harvest more dietary nutrients, thus predisposing them to being overweight or obese.[16,17]

Conclusion

Although the effect of the rise in the number of cesarean sections on the increasing rates of overweight and obesity may be minimal, it would nonetheless appear to constitute one more factor contributing to obesity, a worldwide health concern.

Contributions
Faúndes A. and Miranda L. had the original idea of revising the subject, based on isolated observation and in a revision of articles on microbioma. Faúndes A., Miranda L. and Bento S. F. reviewed the literature, selected the appropriate article, and summarized the results. Faúndes A., Miranda L. and Bento S. F. contributed to the writing of the final paper.

Conflict of Interests
The authors have no conflict of interests to declare.

References

1 World Health Organization. Obesity and overweight: Fact sheet [Internet]. 2020. [cited 2020 Nov 25]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
2 Miranda JJ, Herrera VM, Chirinos JA, Gómez LF, Perel P, Pichardo R, et al; The Latin American Consortium of Studies in Obesity (LASO) Major cardiovascular risk factors in Latin America: a comparison with the United States. PLoS One. 2013;8(01):e54056. Doi: 10.1371/journal.pone.0054056
3 Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, et al; Prospective Studies Collaboration. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet. 2009;373(9669):1083–1096. Doi: 10.1016/S0140-6736(09)60318-4
4 Betrán AP, Ye J, Moller AB, Zhang J, Gülmezoglu AM, Torloni MR. The increasing trend in caesarean section rates: global, regional

and national estimates: 1990–2014. PLoS One. 2016;11(02): e0148343. Doi: 10.1371/journal.pone.0148343

5 Yuan C, Gaskins AJ, Blaine AI, Zhang C, Gilman MW, Missmer SA, et al. Association between cesarean birth and risk of obesity in offspring in childhood, adolescence and early adulthood. JAMA Pediatr. 2016; 170(11):e162385. Doi: 10.1001/jamapediatrics.2016.2385

6 Barros AJ, Santos LP, Wehrmeister F, Motta JVS, Matijasevich A, Santos IS, et al. Caesarean section and adiposity at 6, 18 and 30 years of age: results from three Pelotas (Brazil) birth cohorts. BMC Public Health. 2017;17(01):256. Doi: 10.1186/s12889-017-4165-3

7 Huh SY, Rifas-Shiman SL, Zera CA, Edwards JWR, Oken E, Weiss ST, et al. Delivery by caesarean section and risk of obesity in preschool age children: a prospective cohort study. Arch Dis Child. 2012;97 (07):610–616. Doi: 10.1136/archdischild-2011-301141

8 Sogunle E, Masukume G, Nelson G. The association between caesarean section delivery and later life obesity in 21-24 year olds in an Urban South African birth cohort. PLoS One. 2019;14 (11):e0221379. Doi: 10.1371/journal.pone.0221379

9 Masukume G, Khashan AS, Morton SMB, Baker PN, Kenny LC, McCarthy FP. Caesarean section delivery and childhood obesity in a British longitudinal cohort study. PLoS One. 2019;14(10): e0223856. Doi: 10.1371/journal.pone.0223856

10 Darmasseelane K, Hyde MJ, Santhakumaran S, Gale C, Modi N. Mode of delivery and offspring body mass index, overweight and obesity in adult life: a systematic review and meta-analysis. PLoS One. 2014;9(02):e87896. Doi: 10.1371/journal.pone.0087896

11 Zhou X, Brown CJ, Abdo Z, Davis CC, Hansmann MA, Joyce P, et al. Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. ISME J. 2007;1(02): 121–133. Doi: 10.1038/ismej.2007.12

12 Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006;118(02):511–521. Doi: 10.1542/peds.2005-2824

13 Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010;107(26):11971–11975. Doi: 10.1073/pnas.1002601107

14 Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004;101(44):15718–15723. Doi: 10.1073/pnas.0407076101

15 Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Chari RS, et al; CHILD Study Investigators. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. CMAJ. 2013;185(05):385–394. Doi: 10.1503/cmaj.121189

16 Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027–1031. Doi: 10.1038/nature05414

17 Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, et al. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am J Clin Nutr. 2011;94(01):58–65. Doi: 10.3945/ajcn.110.010132

Vaccine for Covid-19 and Pregnant Women

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Dear Editor,

We would like to share ideas on "We have Vaccine for Covid-19! What to Recommend for Pregnant Women?"[1] Quintana[1] concluded that "*The final decision whether or not to receive the vaccine will be made by the woman after receiving the appropriate information. This same principle applies with even greater emphasis to puerperal and lactating women.*"[1] Indeed, the Covid-19 vaccine is considered risky for any subject. As a vaccine with an emergency-use authorization, the data on its safety and efficacy against COVID-19 is insufficient. According to a recent report on the joint International Federation of Fertility Societies (IFFS)/ European Society of Human Reproduction and Embryology (ESHRE) statement on COVID-19 vaccination for pregnant women, Ory et al.[2] concluded that "*individual risk, availability of the vaccine, and the potential recipients' concerns regarding unknown risks of the new vaccines*" should be important factors for considering and deciding to receive or not receive the vaccine. An important question is whether a pregnant woman has or not a higher risk of developing adverse effects than the general population. If a woman of childbearing age can bee vaccinated, there should be no increased risk for the mother. The remaining question to be researched is regarding the possibility that the immunity can cross the placenta and affect the fetus. Finally, while we wait for more data on the vaccine, there should be a careful reanalysis of the actual risk of pregnant subjects developing infections and a severe disease if infected. This is an interesting issue for further research in obstetrics.

Conflict of Interests

The authors have no conflict of interests to declare.

References

1 Quintana SM. We have vaccine for COVID-19! What to recommend for pregnant women? Rev Bras Ginecol Obstet. 2021;43(02):81–83. Doi: 10.1055/s-0041-1726090
2 Ory S, Veiga A, Horton M, Gianaroli L. Joint IFFS/ESHRE statement on COVID-19 vaccination for pregnant women and those considering pregnancy. Hum Reprod Open. 2021;2021(02):hoab016

Author's Reply

Reply to "Vaccine for COVID-19 and pregnant women"

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Dear Editor,

Thank you for the author's comments, and I am very pleased to share ideas on such an important topic as the prevention of Covid-19 infection in pregnant women through vaccines. The editorial reflects my opinion based on the studies available so far; however, I emphasize that the infection is recent, and we are learning on a daily basis. In Brazil, we have experienced a significant increase in maternal deaths associated with Covid-19, according to official data from the Brazilian Ministry of Healthl (https://observatorioobstetrico.shinyapps.io/covid_gesta_puerp_br/). This situation has brought great concern and the urgent need to reduce maternal deaths due to Covid-19. Although the studies on the Covid-19 vaccine did not include pregnant women and puerperal women in their designs, available data have shown that vaccinated pregnant women do not develop more serious or more frequent adverse events when receiving a vaccine compared with a non-pregnant population. Obviously, pregnant women and women who have recently given birth should be advised about the limited data on the immunogenicity and safety of the Covid-19 vaccines before deciding to get vaccinated or not. Given this context, the Brazilian Ministry of Health has released a vaccine calendar for all pregnant women in two stages. In the first phase, only pregnant women with comorbidities will receive the vaccine, and, in the second phase, all pregnant women will be vaccinated. I hope that pregnant women will have been vaccinated soon in order to reduce the maternal deaths from this infection in Brazil.

Conflict of Interests

The author has no conflict of interests to declare.

Impact of the Covid-19 Pandemic on Birth Rates in 2020: The Case of Colombia

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The global emergency arising from the rapid spread of the severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) virus, the etiological agent responsible for coronavirus disease 2019 (Covid-19), has resulted in a significant loss of more than 2.5 million human lives across the world until now. On March 12, 2020, Colombia's authorities declared a state of emergency due to Covid-19, and, by March 25, 2021, have reported 2,359,942 cases and 62,519 deaths attributed to the infection (https://www.ins.gov.co). Following the recommendation of the World Health Organization (WHO), just a year ago, Colombia's national government adopted mandatory preventive isolation to reduce new cases of Covid-19. However, despite implementing an early lockdown and having a 97,78% health coverage by the end of 2020, there is still great concern about the quality of care to respond effectively to the needs of infected patients, considering the 29% of accumulated excess of total mortality in 2020, compared with the national historical average.[1]

Besides the direct impact of the novel disease on individual health, the Covid-19 crisis has had significant consequences on the economic growth of the affected populations. Although the non-pharmaceutical interventions applied in most countries, including Colombia, to control the viral transmission and prevent the collapse of health care services, such as lockdowns, isolation, social distancing, and quarantine measures, have shown to be beneficial, they prompted a decline in productivity with a subsequent economic downturn, representing a significant challenge in some vulnerable countries with less developed health systems and lacking the financial ability to respond to the pandemic.[2]

Economic and social changes triggered by the widespread community transmission of the SARS-CoV-2 infection also negatively affect the population who wants to get pregnant, influencing fertility and birth rates.

The changes imposed by the pandemic not only affected the prenatal control program in Colombia, but also altered the mental state, intimacy, relationship dynamics, and reproductive desires in various ways worldwide. For instance, many Italian couples trying to conceive abandoned their intention to do so, precisely due to concerns of further economic difficulties and the repercussions on gestation.[3] Moreover, it has been reported that physiological changes during pregnancy may impact on the outcomes of mothers and newborns with Covid-19,[4] increasing the perception of pregnancy as a threat on the part of couples attempting conception. Collected statistics of existing research on sexual health during the pandemic closely relate to such remarks. Studies[5] report a significant decrease in sexual activity, probably due to the major changes at the beginning of the epidemic plus the perceived high risk of sexual practices. Altogether, current evidence thus raises awareness about the impairment in sexual and reproductive health and its forthcoming impact on the demographic composition of the affected nations.

An analysis using information from the Colombian National Administrative Department of Statistics (DANE, https://www.dane.gov.co/) regarding reports on births in Colombia from 2014 to 2020 was recorded for this letter. The data from 2014 to 2019 was consolidated and compared with the data reported for 2020. Birth records for

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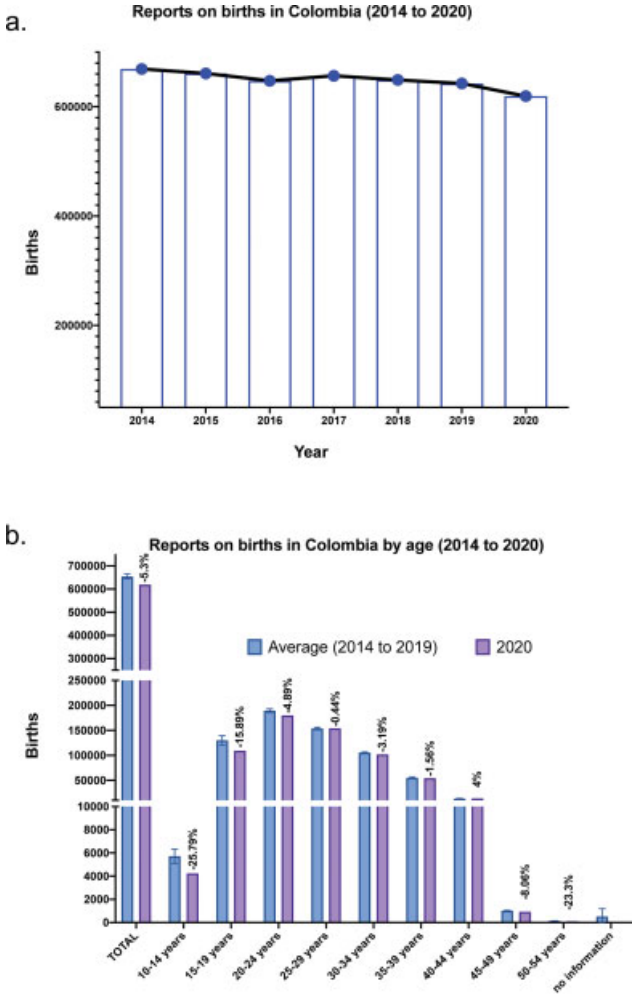

Fig. 1 The total (a) and range per age (b) of birth data for 2020 with respect to the data reported for the last 6 years (2014 to 2019).

2020 (preliminary report) were also compared with the average of the last 6 years (2014 to 2019) by yearly range (►**Fig. 1**).

The number of live births in Colombia during the last 6 years (2014 to 2019) is broadly stable (►**Fig. 1a**). However, there is a notable reduction of 5.32% in total births in 2020 compared with the mean national value for the past 6 years. When comparing the national average of the previous 6 years with 2020 ranges per age (►**Fig. 1b**), we also observed a decline in births in most age groups, with the highest decrease in women aged 10 to 14.

Although throughout the first 3 quarters of 2020 the number of births remained stable and comparable to that of previous years, it was in the last quarter of 2020 that births started to decline progressively, not redressing the rise in the mortality rate and the fatality rate of Covid-19. A similar situation occurs in France, Spain, and the United States, where there are important drops in births, contrary to the expected baby boom at this pandemic stage, based on prior epidemiological predictions.

The decrease in total births in Colombia in 2020, compared with average birth rates, has several implications. First, the social measures aimed to flatten the Covid-19 curve and lessen the economic impact are probably the main cause behind the negative effect on the fertility rate, both in high- and lower-to-middle-income economies. Notably, the economic crisis and employment uncertainty derived from policies against the spread of the virus might be the most significant circumstances accountable for such a decline, given their known influence in the reproductive decision-making as, in general, falling income has been associated to the postponement and abandonment of planned births, which may not be reversed regardless of improvements in economic indexes.[6,7] After that, the fall in fertility and birth rates has implications on the demographic structure, and ultimately, on the age distribution of the population, having an aggregate effect on economic affluence.[8]

The drop in the number of total births in 2020 in Colombia, will probably be followed by a gradual and modest increase in fertility rates in subsequent years, once the social and economic turbulence is over, in a pattern similar to how most epidemics affect demographyics.[9] As for now, even when vaccine efforts are being successful, the economic fallout will plausibly continue; hence, it is unlikely that the declining fertility trends alleviate in the short term, notwithstanding an apparent economic recovery. Furthermore, it is imperative to intervene on the main determinants regarding reproductive decision-making: are age, gender, schooling, and socioeconomic status. However, the large decline in newborns of women aged 10 to 14 years should be maintained through prioritizing investments in programs to educate men and women on sexual and reproductive health, encouraging planned births only among couples with favorable conditions to be parents. Lastly, this warrants epidemiological sociodemographic-economic models in line with the currently available data, aiming to design up-to-date strategies focused on the control of virus outbreaks, the protection of economic prosperity against possible upcoming public health crises, and an increase in birth rates.

Conflict of Interests

The authors have no conflict of interests to declare.

References

1 Ministerio de Salud Protección Social de Colombia. Vigilancia demográfica de la mortalidad por COVID-19 en Colombia 2020 [Internet]. Bogotá: Dirección de Epidemiología y Demografía 2021 [cited 2021 Feb 12]. Available from: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/VS/ED/VSP/vigilancia-demografica-mortalidad-covid-19-colombia2020.pdf
2 Nicola M, Alsafi Z, Sohrabi C, Kerwan A, Al-Jabir A, Iosifidis C, et al. The socio-economic implications of the coronavirus pandemic (COVID-19): A review. Int J Surg. 2020;78:185–193. Doi: 10.1016/j.ijsu.2020.04.018
3 Micelli E, Cito G, Cocci A, Polloni G, Russo GI, Minervini A, et al. Desire for parenthood at the time of COVID-19 pandemic: an insight into the Italian situation. J Psychosom Obstet Gynaecol. 2020;41(03):183–190. Doi: 10.1080/0167482X.2020.1759545
4 Di Toro F, Gjoka M, Di Lorenzo G, De Santo D, De Seta F, Maso G, et al. Impact of COVID-19 on maternal and neonatal outcomes: a systematic review and meta-analysis. Clin Microbiol Infect. 2021; 27(01):36–46. Doi: 10.1016/j.cmi.2020.10.007

5 Delcea C, Chirila VI, Săuchea AM. Effects of COVID-19 on sexual life–a meta-analysis. Sexologies. 2021;30(01):e49–e54. Doi: 10.1016/j.sexol.2020.12.001
6 Adsera A, Menendez A. Fertility changes in Latin America in periods of economic uncertainty. Popul Stud (Camb). 2011;65 (01):37–56. Doi: 10.1080/00324728.2010.530291
7 Hanappi D, Ryser VA, Bernardi L, Le Goff JM. Changes in employment uncertainty and the fertility intention–realization link: an analysis based on the Swiss household panel. Eur J Popul. 2017;33 (03):381–407. Doi: 10.1007/s10680-016-9408-y
8 Prettner K, Bloom DE, Strulik H. Declining fertility and economic well-being: do education and health ride to the rescue? Labour Econ. 2013;22:70–79. Doi: 10.1016/j.labeco.2012.07.001
9 Mamelund SE. Fertility fluctuations in times of war and pandemic influenza. J Infect Dis. 2012;206(01):140–141, author reply 141–143. Doi: 10.1093/infdis/jis315

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FEBRASGO POSITION STATEMENT

Obstetric antiphospholipid syndrome

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The National Specialty Commission for Venous Thromboembolisms of the Brazilian Federation of Gynecology and Obstetrics Associations (FEBRASGO) endorses to this document. The content production is based on scientific studies on a thematic proposal and the findings presented contribute to clinical practice.

Key points

- Antiphospholipid syndrome (APS) is an acquired thrombophilia often associated with adverse obstetric outcomes.
- APS severity depends on the type and complexity of the antibodies. Triple positivity for antiphospholipid antibodies and high antibody titers are commonly associated with a more severe disease.
- The antiphospholipid antibodies described for the diagnosis of the syndrome are: IGG and IGM anticardiolipin, lupus anticoagulant and IGG and IgM antibeta2 GPI.
- The occurrence of venous and/or arterial thrombosis is part of the clinical condition.
- Treatment for APS in pregnancy consists in the use of low molecular weight heparin (LMWH) and low dose aspirin. The dose for anticoagulation depends on the presence or absence of previous thrombosis and the type of obstetric morbidity.
- Patients refractory to anticoagulation treatment may need additional therapies (hydroxychloroquine, prednisone and/or intravenous immunoglobulin).

Recommendations

- Primary prophylaxis of pregnancy adverse outcomes with low dose aspirin may be considered in asymptomatic antiphospholipid antibody (aPL) carriers who present a high-risk profile.
- Primary prophylaxis of pregnancy adverse outcomes with low dose aspirin and LMWH in prophylactic dose is recommended.
- Patients with previous thrombosis and APS: intermediate or full dose anticoagulation with LMWH and low dose aspirin during pregnancy.
- Patients with serious adverse pregnancy outcomes previously treated with aspirin and LMWH should receive hydroxychloroquine started before pregnancy plus aspirin and LMWH. (evidence level 2C).

Background

Antiphospholipid syndrome (APS) is a pro-thrombotic and inflammatory condition characterized by thromboembolic events or obstetric complications combined with the presence of at least one antiphospholipid antibody (aPL): lupus anticoagulant (LAC), anticardiolipin (aCL) or anti-β2glycoprotein I (aβ2GP1).[1]

Diagnosis of antiphospholipid syndrome

Antiphospholipid antibody syndrome (APS) is diagnosed when at least one of the following clinical criteria and one of the following laboratory criteria are present:[1]

Clinical Criteria of APS

Pregnancy Morbidity

- One or more unexplained death of a morphologically normal fetus >10 weeks of gestation.
- One or more premature delivery of a morphologically normal fetus < 34 weeks of gestation because of severe preeclampsia (PE) or eclampsia (defined according to standard definitions) or recognized features of placental insufficiency.
- Three or more unexplained consecutive miscarriages at <10 weeks of gestation with maternal and paternal factors (such as anatomical, hormonal, or chromosomal abnormalities) excluded.

Vascular thrombosis

One or more clinical episode of arterial, venous, or small-vessel thrombosis.

- Thrombosis must be objectively confirmed.
- If histopathological confirmation is used, thrombosis must be present without inflammation of the vessel wall.

Laboratory criteria

1. Positive lupus anticoagulant (LA) in plasma on two or more occasions at least 12 weeks apart.
2. Positive anticardiolipin (aCL) antibody of IgG and/or IgM isotype in serum or plasma at medium or high titer (i.e. >40 GPL or MPL, or >the 99th percentile) on two or more occasions, at least 12 weeks apart.
3. Positive anti-b2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (titers above the 99th percentile) on two or more occasions at least 12 weeks apart.

Antiphospholipid syndrome can lead to a wide spectrum of thrombotic complications, such as venous thromboembolism (VTE), venous thrombosis in unusual sites, and arterial and capillary thrombosis, which are highly susceptible to recurrence.[2] There are several more symptoms and other organs can be involved, partly noncriteria APS manifestations (Figure 1).

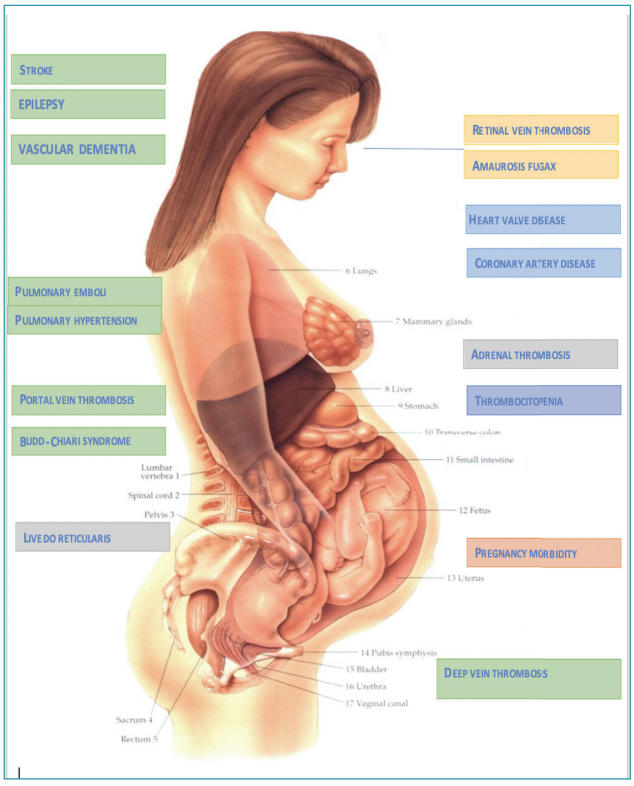

Figure 1. Clinical manifestations of antiphospholipid syndrome in women

Obstetric complications are unexplained recurrent miscarriages (gestational age [GA] < 10 weeks), death of a morphologically normal fetus, premature birth (GA < 34 weeks) due to preeclampsia, eclampsia, HELLP syndrome or intrauterine growth restriction (IUGR).[1,3]

Although antiphospholipid antibodies are at the basis of both thrombotic and obstetric manifestations of APS, different mechanisms are associated with each APS variant.[4] While a pro-thrombotic state is the hallmark of thrombosis in APS, obstetric APS is characterized by defective placentation due to inflammation, activation of the complement system, hypercoagulability and abnormalities in vascular remodeling of the uterine vessels, which may not be related to thrombotic lesions.[4] Although thrombosis and obstetric manifestations are distinct variants of APS, most patients present with both APS complications.[5]

Antiphospholipid syndrome, either primary or associated with systemic lupus erythematosus (SLE), can pose several problems to women's health in terms of contraception, reproduction, and menopause treatment. Patient care in daily medical practice can be compromised by heterogeneous clinical presentation and available therapy approaches. The aim of this review and position paper is to provide useful recommendations on the management of women with APS for the medical community.

Laboratory diagnosis of APS

Antiphospholipid antibodies are widely used as diagnostic markers of antiphospholipid syndrome (APS). The following antibodies and titers are considered laboratory criteria for APS diagnosis: i) positive LAC; ii) IgG or IgM aCL at medium or high titer (> 40 GPL/MPL or > 99th percentile); iii) IgG or IgM aβ2GP1 at medium or high titer (> 99th percentile). At least one of laboratory criteria most be present on two or more occasions, at least 12 weeks apart.[1]

Besides being used for diagnosis, the profile of these antibodies may also play a role in the clinical presentation of the disease. Miyakis et al.[1] suggested that APS should be categorized according to aPL positivity, as type I (one positive aPL), type IIa (LAC present alone), type IIb (aCL present alone) and type IIc (aβ2GP1 present alone). Recently, Pengo et al.[6] suggested that the positivity for the three aPL antibodies, known as triple positivity, was an independent risk factor for thrombosis in aPL asymptomatic carriers. Clinical studies suggest that LAC positivity alone, double, and triple positivity are associated with high risk of APS complications (Table 1).

Table 1. Definitions of high-risk and low-risk aPL profile

High-risk aPL profile	Low-risk aPL profile
Persistently positive lupus anticoagulant (measured according to ISTH guidelines), or	Isolated aCL or antibeta2 glycoprotein I antibodies at low/ medium titers, particularly if transiently positive
Double aPL positivity (any combination of lupus anticoagulant, aCL antibodies or antibeta2 glycoprotein I antibodies), or	
Triple aPL positivity, or	
Persistently high aPL titers	

ISTH International Society of thrombosis and Hemostasis; aCL anticardiolipin; aPL antiphospholipids
Source: Modified from: Tektonidou et al. (2019).[7]

Prevention and treatment of obstetric complications: how to manage these patients?

Obstetric complications in asymptomatic aPL carriers

The first evidence linking aPL positivity with adverse pregnancy outcomes emerged in the early 90's. In a prospective observational study, Lynch et al.[8] evaluated 389 first time pregnant women, of whom 95 (24%) presented with positive aPL. During follow-up, fetal loss was observed in about 16% of aPL carriers and 6.5% of aPL negative patients, which yielded a 2.5 times higher risk for fetal loss in aPL carrier women than in non-aPL.[8] These results were confirmed by cohort and case-controls studies later evaluated in a meta-analysis that revealed LA carriers were 2 to 4 times more likely to develop late placenta-mediated adverse pregnancy outcomes, such as preeclampsia, IUGR and late fetal loss.[9]

The most beneficial approach to prevent obstetric complications in aPL carrier women has not yet been defined, as evaluation of clinical data on primary prophylaxis of obstetric complications are scarce and based on observational data or low numbers of cases, which renders low-quality evidence. A randomized study that included 19 asymptomatic patients with positive aPL showed no benefits in using low dose aspirin (85 mg OD) compared to usual care, even though the number of adverse events such as fetal loss and IUGR were low and the study was underpowered to show actual differences between treatments.[10] More recently, in a retrospective study, Del Ross et al.[11] described the effect of low dose aspirin (100mg OD) on the outcomes of 139 pregnancies in aPL positive women not fulfilling criteria for APS. The risk of miscarriage, prematurity and IURG was similar between women who used or not low dose aspirin, and the frequency of live birth was high (above 92%) regardless of the treatment.[11]

Although no clinical evidence supports primary prophylaxis of obstetric complications among aPL carriers, available studies have not evaluated prophylaxis in patients with high-risk profile (LAC positivity alone, double, and triple positivity) in which the risk of adverse outcomes may justify early medical intervention. For that, experts on APS from the European League Against Rheumatism (EULAR) recently agreed that it is reasonable to consider using low dose aspirin (75 – 100mg OD) in asymptomatic pregnant women with high-risk aPL profile (Table 1) and no previous history of thrombosis or obstetric complications.[7] A summary of treatments according to the clinical profile of patients can be seen in table 2.

Table 2. Management of pregnant women with antiphospholipid antibodies or APS

Clinical manifestations	Treatment	Evidence
Persistent presence of aPL without adverse pregnancy outcomes or thrombosis	Close monitoring of fetus and mother during pregnancy with or without LDA treatment.	No studies performed on APS. Risk factors to be considered: age > 35 y, presence of autoimmune diseases, chronic hypertension.
Persistent positivity for antiphospholipid antibodies and history of recurrent first trimester pregnancy loss	LDA with prophylactic LMWH	Low-quality randomized controlled trials
Previous history of placenta-mediated complications	LDA with prophylactic LMWH	Low-quality randomized controlled trials
Patients with thrombotic APS (venous or arterial)	LDA and intermediate-dose or full-dose LMWH	Based on one prospective observational study
Anticoagulation in postpartum period and APS	LMWH thromboprophylaxis for six weeks postpartum if previous thrombosis; two weeks postpartum if no previous thrombosis or additional risk factors.	Based on case-control studies and cohort studies

APS – antiphospholipid syndrome, aPL- antiphospholipid antibodies; LDA – low dose aspirin; LMWH – low molecular weight heparin. Low dose aspirin -80-150mg/day
Source: Adapted from: Czwalinna and Bergmann (2020).[12]

Obstetric complications in APS patients: is everything solved?

In a large cohort of 1000 patients with APS (many of whom had SLE), which evaluated 188 pregnancies over ten years, the absolute risks for fetal loss, IUGR and prematurity were high, 16%, 26% and 48%, respectively.[5] Interestingly, although the proportion of early pregnancy loss has decreased (from 35% to 16%) combined with higher chances of live birth (from 47% to 73%) over the ten-year period of follow-up, the risk of a live birth with prematurity or IUGR remained extremely high (above 30%).[5] This observation highlights that while the current strategies seem efficient to prevent miscarriages in APS women, there are unmeet clinical needs in the treatment of APS-related late pregnancy complications.

The first randomized studies that evaluated treatment approaches towards the prevention of recurrent miscarriages were performed in the late 90's and early 2000's. In a randomized clinical trial, Rai et al.[13]

demonstrated that the combination of low dose aspirin and low molecular weight heparin was superior to low dose aspirin alone in preventing miscarriages among APS patients. The proportion of live births was roughly two times higher in the group receiving low dose aspirin plus low molecular weight heparin (71%) when compared to the group receiving low dose aspirin alone (42%).[13] Although a subsequent randomized trial failed to demonstrate differences between low dose aspirin alone and low dose aspirin plus low molecular weight heparin therapies in pregnant women with APS and recurrent miscarriages,[14] the study had several methodological issues as the inclusion of pregnant women after the 12th week of gestation and high proportion of protocol violations (25% of women switched study arms). Further observational studies and meta-analysis confirmed the superiority of low dose aspirin and low molecular weight heparin for the prevention of early pregnancy losses. An observational study evaluating 176 aPL/APS women with recurrent miscarriages and 517 women with unexplained miscarriages demonstrated that the chance of a live birth was increased by more than twofold in aPL/APS women using low dose aspirin and low molecular weight heparin as compared to those using low dose aspirin alone.[15] Any treatment effect of low dose aspirin or low dose aspirin plus low molecular weight heparin was observed in pregnant women with previous unexplained miscarriages.[15] In a meta-analysis of five randomized controlled trials, the use of low dose aspirin plus low molecular weight heparin was overall associated with higher rates of live birth than low dose aspirin alone, although there was no difference between the two treatment strategies with regard to the rate of premature labor and preeclampsia.[16]

Treatment strategies to prevent late pregnancy complications were recently evaluated by the FRUIT trial. In this study, 32 APS pregnant women with a history of preeclampsia, eclampsia or HELLP syndrome in previous pregnancies were randomized to receive low dose aspirin plus low molecular weight heparin or low dose aspirin alone during the current pregnancy. The study demonstrated that the absolute risk of these hypertensive disorders of pregnancy was not reduced by the use of low dose aspirin plus low molecular weight heparin, as compared to low dose aspirin alone, suggesting that low dose aspirin plus heparin does not add treatment benefits to standard low dose aspirin alone in terms of preventing late pregnancy complications.[17]

Taking into account the evidence that the use of low dose aspirin plus low molecular weight heparin is superior to low dose aspirin alone in reducing miscarriages but not late pregnancy adverse outcomes in APS, the use of low dose aspirin plus low molecular weight heparin during the entire pregnancy is suggested to prevent recurrent miscarriage and fetal loss among APS women. In APS women with previous premature birth due to preeclampsia, eclampsia, HELLP syndrome or placental insufficiency, either low dose aspirin plus low molecular weight heparin or low dose aspirin alone could be used to prevent the recurrence of these late pregnancy complications.[7]

Management of refractory obstetric APS

A recently published European survey of 1000 consecutive cases of obstetric APS revealed that to date the proportion of fetal loss is still very high (at 27%) among APS women[18] and recurrent miscarriage is the most frequent poor outcome, even though therapy strategies to treat these patients have been improved in recent years.

Indeed, the risk of treatment failure is an important issue that may affect more than 20% of patients with obstetric APS and some risk factors associated with treatment failure have been identified. In a large case-control multicenter study, treatment failure was more likely to occur among women with SLE or other autoimmune diseases, history of both thrombosis and pregnancy complications and triple positivity for aPL.[19] A recent multicenter cohort study confirmed that the presence of autoimmune disease, complement consumption and previous thrombosis were risk factors for the occurrence of adverse pregnancy outcomes in APS, regardless of the treatment approach used to prevent these outcomes.[20]

Various clinical treatments for the treatment of refractory obstetric APS have been described, such as hydroxychloroquine, glucocorticoids, immunoglobulin and plasmaphereses.[21-24] However, these treatments were described mainly in case-series studies and there is no robust clinical evidence to support the use of these therapies. The best available evidence in this regard come from two recent cohort studies. Two multicenter retrospective studies have demonstrated the benefits of adding hydroxychloroquine to conventional treatment in order to increase live birth rates in refractory obstetric APS cases.[25,26] A very recent study, published in 2020, showed that combinations of low dose aspirin with low molecular weight heparin at therapeutic dose could improve pregnancy outcomes in patients with severe pregnancy complications.[26] However, these studies suffer from confounding by indication bias, because the treatment strategy was not randomly assigned but chosen based on the clinical features of patients. Moreover, alternative treatments were mostly compared with historical data, which can result in numerous information biases. Taking together, the available data point towards a possible effect of hydroxychloroquine on improving pregnancy outcomes in APS. To confirm this suspicions, two

randomized clinical trials are being performed to evaluate the impact of hydroxychloroquine in addition to standard therapy in the improvement of pregnancy outcomes in women with obstetric APS,[18,27] but the results of these studies are not yet available.

To date, current suggestions for the treatment of obstetric APS refractory to low dose aspirin plus prophylactic low molecular weight heparin are based on experts' opinion only. Possible therapeutic approaches include: low dose aspirin plus therapeutic dose of low molecular weight heparin, hydroxychloroquine, low dose of glucocorticoids during the first trimester of pregnancy and immunoglobulin.[7]

Patients with APS and pregnancy morbidity are at greater risk for thrombosis?

The following risk factors were associated with a greater risk for having a first thrombosis after a pregnancy morbidity:[28]

- younger age at diagnosis of Ob-APS
- additional cardiovascular risk factors
- superficial vein thrombosis
- heart valve disease
- multiple aPL positivity

Catastrophic antiphospholipid syndrome and pregnancy: a diagnosis that should not be missed

Catastrophic antiphospholipid syndrome (CAPS) is a rare but life-threatening condition that may be precipitated by pregnancy. The condition can be hard to diagnose since it mimics other pregnancy-associated thrombotic microangiopathies. Accurate and timely diagnosis is critical for effective treatment.[29] Criteria for CAPS include multi-organ thrombosis over a one-week period of time that affects at least three organs or tissues. However, these are meant to be guidelines used for classification purposes rather than definitive clinical care. The condition is rare, accounting for less than 1% of APS cases, but can be life threatening and pregnancy may be a trigger. Besides pregnancy, precipitating factors are present in most cases and include infections, surgery, malignancy, contraceptives and drugs. Pregnancy is the precipitating factor in about 8% of cases.[30]

Pregnancy-related CAPS occurs in younger individuals than those who are not pregnant. In addition, CAPS is more likely to present de novo in pregnancy (48.2%) compared to non-pregnancy (26.3%). Pregnancy-related CAPS also is relatively more likely to be associated with liver involvement and previous pregnancy loss. The differential diagnosis includes other thrombotic microangiopathies, many of which are associated with (or specific to) pregnancy. Conditions sharing many features with CAPS include preeclampsia, HELLP syndrome, hemolytic-uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), lupus flare, disseminated intravascular coagulation, and acute fatty liver of pregnancy (AFLP). All these conditions are characterized by microangiopathic hemolytic anemia, thrombocytopenia and potential malignancy.

The treatment basis is therapeutic anticoagulation. Immunosuppression, supportive treatment and removal or treatment of any precipitating factors are recommended. Other treatments focus on immunosuppression. First-line treatment usually include corticosteroids, although efficacy is uncertain. In addition to corticosteroids, intravenous immunoglobulin (IVIG) or plasma exchange is used for additional immunosuppression and treatment. Optimal dosing is uncertain but typically 0.4 g/kg per day for three–five days. In some centers, they proceed with immediate delivery if gestational age is ≥34 weeks' gestation. At earlier gestational ages, proceed to delivery if the patient does not respond to treatment after a reasonable time interval (e.g. 24–48 hours) or if fetal status is compromised.[29] Rituximab is a chimeric monoclonal antibody against CD20 positive B cells. Rituximab has been reported to be useful in improving APS (in patients without CAPS) in uncontrolled cases series. Outcomes were good (75%) in 20 patients with CAPS treated with rituximab.[31] Another monoclonal antibody, eculizumab, is specific for complement protein C5. There are several ongoing studies evaluating the use of eculizumab in APS and CAPS. Meanwhile, the medication should be reserved for refractory cases due to high cost.[32]

Final considerations

- Thrombotic and obstetric APS are two different variants of the same syndrome.
- In pregnant women with APS, the proportion of fetal loss and late obstetric complications are about 15-35% and 5%, respectively.
- Primary prophylaxis of pregnancy adverse outcomes with low dose aspirin may be considered in asymptomatic aPL carriers who present with a high-risk profile.
- Conventional treatment for preventing obstetric complications consists of the association of low dose aspirin and low molecular weight heparin at prophylactic doses.
- Adequate treatment options for refractory cases are not established, although treatment strategies using hydroxychloroquine, prednisone, immunoglobulin and plasmaphereses have been described in case-series studies and the use of hydroxychloroquine seems to be the most promising therapy for refractory obstetric APS.

References

1. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4(2):295-306. doi: 10.1111/j.1538-7836.2006.01753.x
2. Cervera R, Piette JC, Font J, Khamashta MA, Shoenfeld Y, Camps MT, et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. Arthritis Rheum. 2002;46(4):1019-27. doi: 10.1002/art.10187
3. Schreiber K, Hunt BJ. Pregnancy and antiphospholipid syndrome. Semin Thromb Hemost. 2016;42(7):780-8. doi: 10.1055/s-0036-1592336
4. Meroni PL, Borghi MO, Grossi C, Chighizola CB, Durigutto P, Tedesco F. Obstetric and vascular antiphospholipid syndrome: same antibodies but different diseases? Nat Rev Rheumatol. 2018;14(7):433-40. doi: 10.1038/s41584-018-0032-6
5. Cervera R, Serrano R, Pons-Estel GJ, Ceberio-Hualde L, Shoenfeld Y, de Ramón E, et al. Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. Ann Rheum Dis. 2015;74(6):1011-8. doi: 10.1136/annrheumdis-2013-204838
6. Pengo V, Biasiolo A, Pegoraro C, Cucchini U, Noventa F, Iliceto S. Antibody profiles for the diagnosis of antiphospholipid syndrome. Thromb Haemost. 2005;93(6):1147-52. doi: 10.1160/TH04-12-0839
7. Tektonidou MG, Andreoli L, Limper M, Amoura Z, Cervera R, Costedoat-Chalumeau N, et al. EULAR recommendations for the management of antiphospholipid syndrome in adults. Ann Rheum Dis. 2019;78(10):1296-304. doi: 10.1136/annrheumdis-2019-215213
8. Lynch A, Marlar R, Murphy J, Davila G, Santos M, Rutledge J, et al. Antiphospholipid antibodies in predicting adverse pregnancy outcome. A prospective study. Ann Intern Med. 1994;120(6):470-5. doi: 10.7326/0003-4819-120-6-199403150-00004
9. Abou-Nassar K, Carrier M, Ramsay T, Rodger MA. The association between antiphospholipid antibodies and placenta mediated complications: a systematic review and meta-analysis. Thromb Res. 2011;128(1):77-85. doi: 10.1016/j.thromres.2011.02.006
10. Cowchock S, Reece EA. Do low-risk pregnant women with antiphospholipid antibodies need to be treated? Organizing Group of the Antiphospholipid Antibody Treatment Trial. Am J Obstet Gynecol. 1997;176(5):1099-100. doi: 10.1016/s0002-9378(97)70409-5
11. Del Ross T, Ruffatti A, Visentin MS, Tonello M, Calligaro A, Favaro M, et al. Treatment of 139 pregnancies in antiphospholipid-positive women not fulfilling criteria for antiphospholipid syndrome: a retrospective study. J Rheumatol. 2013;40(4):425-9. doi: 10.3899/jrheum.120576
12. Czwalinna A, Bergmann F. Prevention of pregnancy complications in antiphospholipid syndrome. Hamostaseologie. 2020;40(2):174-83. doi: 10.1055/a-1113-0689
13. Rai R, Cohen H, Dave M, Regan L. Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). BMJ. 1997;314(7076):253-7. doi: 10.1136/bmj.314.7076.253
14. Farquharson RG, Quenby S, Greaves M. Antiphospholipid syndrome in pregnancy: a randomized, controlled trial of treatment. Obstet Gynecol. 2002;100(3):408-13. doi: 10.1016/s0029-7844(02)02165-8
15. Cohn DM, Goddijn M, Middeldorp S, Korevaar JC, Dawood F, Farquharson RG. Recurrent miscarriage and antiphospholipid antibodies: prognosis of subsequent pregnancy. J Thromb Haemost. 2010;8(10):2208-13. doi: 10.1111/j.1538-7836.2010.04015.x
16. Mak A, Cheung MW, Cheak AA, Ho RC. Combination of heparin and aspirin is superior to aspirin alone in enhancing live births in patients with recurrent pregnancy loss and positive anti-phospholipid antibodies: a meta-analysis of randomized controlled trials and meta-regression. Rheumatology. 2010;49(2):281-8. doi: 10.1093/rheumatology/kep373
17. van Hoorn ME, Hague WM, van Pampus MG, Bezemer D, de Vries JI; FRUIT Investigators. Low-molecular-weight heparin and aspirin in the prevention of recurrent early-onset pre-eclampsia in women with antiphospholipid antibodies: the FRUIT-RCT. Eur J Obstet Gynecol Reprod Biol. 2016;197:168-73. doi: 10.1016/j.ejogrb.2015.12.011
18. Belizna C, Pregnolato F, Abad S, Alijotas-Reig J, Amital H, Amoura Z, et al. HIBISCUS: Hydroxychloroquine for the secondary prevention of thrombotic and obstetrical events in primary antiphospholipid syndrome. Autoimmun Rev. 2018;17(12):1153-68. doi: 10.1016/j.autrev.2018.05.012
19. Ruffatti A, Tonello M, Visentin MS, Bontadi A, Hoxha A, De Carolis S, et al. Risk factors for pregnancy failure in patients with anti-phospholipid syndrome treated with conventional therapies: a multicentre, case-control study. Rheumatology. 2011;50(9):1684-9. doi: 10.1093/rheumatology/ker139
20. Fredi M, Andreoli L, Aggogeri E, Bettiga E, Lazzaroni MG, Le Guern V, et al. Risk factors for adverse maternal and fetal outcomes in women with confirmed aPL positivity: results from a multicenter study of 283 pregnancies. Front Immunol. 2018;9:864. doi: 10.3389/fimmu.2018.00864
21. Bramham K, Thomas M, Nelson-Piercy C, Khamashta M, Hunt BJ. First-trimester low-dose prednisolone in refractory antiphospholipid antibody-related pregnancy loss. Blood. 2011;117(25):6948-51. doi: 10.1182/blood-2011-02-339234
22. Tenti S, Cheleschi S, Guidelli GM, Galeazzi M, Fioravanti A. Intravenous immunoglobulins and antiphospholipid syndrome: how, when and why? A review of the literature. Autoimmun Rev. 2016;15(3):226-35. doi: 10.1016/j.autrev.2015.11.009
23. Ruffatti A, Favaro M, Hoxha A, Zambon A, Marson P, Del Ross T, et al. Apheresis and intravenous immunoglobulins used in addition to conventional therapy to treat high-risk pregnant antiphospholipid antibody syndrome patients. A prospective study. J Reprod Immunol. 2016;115:14-9. doi: 10.1016/j.jri.2016.03.004
24. El-Haieg DO, Zanati MF, El-Foual FM. Plasmapheresis and pregnancy outcome in patients with antiphospholipid syndrome. Int J Gynaecol Obstet. 2007;99(3):236-41. doi: 10.1016/j.ijgo.2007.05.045
25. Mekinian A, Lazzaroni MG, Kuzenko A, Alijotas-Reig J, Ruffatti A, Levy P, et al. The efficacy of hydroxychloroquine for obstetrical outcome in anti-phospholipid syndrome: data from a European multicenter retrospective study. Autoimmun Rev. 2015;14(6):498-502. doi: 10.1016/j.autrev.2015.01.012
26. Ruffatti A, Tonello M, Hoxha A, Sciascia S, Cuadrado MJ, Latino JO, et al. Effect of additional treatments combined with conventional therapies in pregnant patients with high-risk antiphospholipid syndrome: a multicentre study. Thromb Haemost. 2018;118(4):639-46. doi: 10.1055/s-0038-1632388
27. Schreiber K, Breen K, Cohen H, Jacobsen S, Middeldorp S, Pavord S, et al. HYdroxychloroquine to Improve Pregnancy Outcome in Women with AnTIphospholipid Antibodies

(HYPATIA) protocol: a multinational randomized controlled trial of hydroxychloroquine versus placebo in addition to standard treatment in pregnant women with antiphospholipid syndrome or antibodies. Semin Thromb Hemost. 2017;43(6):562-71. doi: 10.1055/s-0037-1603359

28. de Jesús GR, Sciascia S, Andrade D, Barbhaiya M, Tektonidou M, Banzato A, et al. Factors associated with first thrombosis in patients presenting with obstetric antiphospholipid syndrome (APS) in the APS Alliance for Clinical Trials and International Networking Clinical Database and Repository: a retrospective study. BJOG. 2019;126(5):656-61. doi: 10.1111/1471-0528.15469
29. Silver RM. Catastrophic antiphospholipid syndrome and pregnancy. Semin Perinatol. 2018;42(1):26-32. doi: 10.1053/j.semperi.2017.11.006
30. Rodríguez-Pintó I, Moitinho M, Santacreu I, Shoenfeld Y, Erkan D, Espinosa G, et al. Catastrophic antiphospholipid syndrome (CAPS): descriptive analysis of 500 patients from the International CAPS Registry. Autoimmun Rev. 2016;15(12):1120-4. doi: 10.1016/j.autrev.2016.09.010
31. Berman H, Rodríguez-Pintó I, Cervera R, Morel N, Costedoat-Chalumeau N, Erkan D, et al. Rituximab use in the catastrophic antiphospholipid syndrome: descriptive analysis of the CAPS registry patients receiving rituximab. Autoimmune Rev. 2013;12(11):1085-90. doi: 10.1016/j.autrev.2013.05.004
32. Unlu O, Erkan D. Catastrophic antiphospholipid syndrome: candidate therapies for a potentially lethal disease. Annu Rev Med. 2017;68:287-96. doi: 10.1146/annurev-med-042915-102529

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Instructions to Authors

Scope and policy

The material submitted for analysis cannot be simultaneously submitted for publication in other journals or previously published. In the selection of manuscripts for publication, are evaluated the originality, relevance of the theme, quality of the methodology used, and adequacy to the editorial standards adopted by the journal. The published material becomes intellectual property of the Brazilian Journal of Gynecology and Obstetrics and Febrasgo.

Manuscripts evaluation

The manuscripts submitted to the journal are received by the Editorial Office that checks the mandatory documentation and examines if the editorial norms contained in the Instructions to Authors have been fulfilled. If the process is in compliance, the manuscript is sent to the Editor-in-Chief, who will make a merit evaluation of the material. If the Editor-in-Chief concludes the work is in favorable scientific and technical conditions, the manuscript is forwarded to the Associate Editors, who will designate reviewers (double mind process) to evaluate it. Then, the reviewers' opinions and editor's instructions are sent to authors to inform them about changes to be made. Then, the authors resubmit the text with the suggested changes within the requested deadline. When resubmitting the manuscript, the requested corrections should be highlighted in yellow. In cases of disagreement with the suggestions, observations should be included in the comments balloons. Be assertive and punctual with the inquiry, and support the hypothesis with references.

IMPORTANT! Authors must comply with the deadlines, since non-attendance will result in delay of manuscript publication or even archiving of the process. At any point in the process of analysis and editing of the text, the authors may request the process suspension and withdrawal of the manuscript, except when it is accepted for publication. The concepts and statements contained in the articles are of the authors' responsibility.

Preparing a manuscript for submission

Mandatory submission documents

When submitting a manuscript to RBGO, attach the documents listed below on the ScholarOne submission platform. Note that not attaching the documents will result in cancellation of the submitted process. Mandatory documentation for online submission:

- Authorization of copyright transfer signed by all authors (scanned and attached as supplementary document) **Model;**
- In accordance with chapter XII.2 of Res. CNS 466/2012, in Brazil, research involving human subjects needs to inform the registration number referring to the Certificate of Ethical Assessment (CAAE) or the approval number of the research (CEP/CONEP) in the Ethics Committee. International manuscripts must present local ethical documentation to proceed with the submission process;
- Cover Letter: written to justify the publication. The authors should be identified, together with the title of the team that intends to publish, origin institution of the authors and intention of publication;
- Title page;
- Manuscript.

Title Page

- Title of the manuscript in English with a maximum of 18 words;
- Authors' full name without abbreviations and Orcid ID;
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- **Conflicts of interest:** authors should report any potential conflicts of interest whether political, economic, of resources for research execution or intellectual property;
- **Acknowledgements:** restricted to people and institutions that contributed to research development in a relevant way. Any financial support provided by development agencies or private companies should be mentioned in the section Acknowledgments. For Brazilian authors, RBGO requests the citation of CNPq, Capes, FAPESP and other financing agencies, together with the number of research process or granted scholarships.
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Manuscript

Instructions to Authors

The Brazilian Journal of Gynecology and Obstetrics publishes the following categories of manuscripts:

Original Articles, complete prospective, experimental or retrospective studies. Manuscripts containing original clinical or experimental research results have priority for publication.

Case Reports, of great interest and well documented from the clinical and laboratorial point of view. In the letter of referral, authors should indicate new or unexpected aspects in relation to already published cases. The text of Introduction and Discussion sections should be based on an updated bibliographic review.

Review Articles, including comprehensive reviews, meta-analysis or systematic reviews. Spontaneous contributions are accepted. The methods and procedures adopted for obtaining the text should be described, and based on recent references, including the current year. As this subject is still subject to controversy, the review should discuss the trends and lines of research under way. In addition to the text of the review, there should be an abstract and conclusions. See the 'Instructions to Authors' section for information on the text body and title page;

Letters to the Editor, dealing with editorial matters or not, but presenting relevant information to readers. Letters can be summarized by the editor, but maintaining the main points. In case of criticism to published works, the letter is sent to the authors so their reply can be published simultaneously;

Editorial, only at the publisher's invitation.

Title

When writing a scientific article, the researcher should focus on the manuscript title, which is the business card of any publication. It should be elaborated very carefully, and preferably written only after the article finalization. A good title adequately describes the manuscript content. Generally it is not a phrase, because it does not contain the subject, only verbs and arranged objects. Titles rarely contain abbreviations, chemical formulas, adjectives, names of cities, among others. The title of manuscripts submitted to RBGO must contain a maximum of 18 words.

Abstract

The abstract should provide the context or basis for the study, establish the objectives, basic procedures, main outcomes and key findings. It should emphasize new and important aspects of the study or observations. Since the abstract is the only substantive part of the article indexed in many electronic databases, authors should ensure it reflects the article content in an accurate and highlighted manner. Do not use abbreviations, symbols and references in the abstract. In case of original articles from clinical trials, authors must inform the registration number at the end of the text.

Informational abstract of structured type of original articles

Abstracts of original articles submitted to RBGO must be structured in four sections and contain a maximum of 250 words:

Objective: What was done; the question posed by the investigator.

Methods: How it was done; the method, including the material used to achieve the objective.

Results: What was found, the main findings and, if necessary, the secondary findings.

Conclusion: The conclusions; the answer to the question asked.

Informational abstract of structured type of systematic review articles

Among the included items are the review objective to the question asked, data source, procedures for selecting the studies and data collection, the results and conclusions. The abstracts of systematic review articles submitted to RBGO must be structured in six sections and contain a maximum of 250 words:

Objective: Declare the main purpose of the article.

Data sources: Describe the data sources examined, including the date, indexing terms, and limitations.

Selection of studies: Specify the number of studies reviewed and the criteria used in their selection.

Data collection: Summarize the conduct used for data extraction and how it was used.

Data synthesis: State the main results of the review and the methods used to obtain them.

Conclusions: Indicate the main conclusions and their clinical usefulness.

Informational abstract of unstructured type of review articles, except systematic reviews and case studies

It shall contain the substance of the article, covering the purpose, method, results and conclusions or recommendations. It exposes enough details so readers can decide on the convenience of reading the full text (Limit of words: 150).

Keywords

The keywords of a scientific paper indicate the thematic content of the text they represent. The main objectives of the aforementioned terms are the thematic content identification, indexing of the work in databases, and rapid location and retrieval of contents. The keyword systems used by RBGO are DeCS (Health Sciences Descriptors - Lilacs Indexer) and MeSH (Medical Subject Headings - MEDLINE-PubMed Indexer). Please choose five descriptors that represent your work on these platforms.

Manuscript body (Manuscripts submitted to RBGO must have a maximum of 4000 words. Note that tables, charts and figures in the Results section and References are not counted).

Introduction

The **Introduction** section of a scientific article has the purpose of informing what was researched and the reason for the investigation. This part of the article prepares the reader to understand the investigation and justification of its realization. The content informed in this section should provide context or basis for the study (i.e. the nature of the problem and its importance); state the specific purpose, research objective, or hypothesis tested in the study or observation. The study objective usually has a more precise focus when formulated as a question. Both the primary and secondary objectives should be clear, and any analyzes in a pre-specified subgroup should be described; provide strictly relevant references only and do not include data or conclusions of the work being reported.

Methods

According to the Houaiss dictionary, **Methods** "is an organized, logical and systematic process of research". The method comprises the material and procedures adopted in the research in order to respond to the central research question. Structure the Methods section of RBGO starting with the study design; research scenario (place and period in which it was performed); sample of participants; data collection; intervention to be evaluated (if any) and the alternative intervention; statistical methods used and the ethical aspects of the study. When thinking about the writing of the study design, reflect if it is appropriate to achieve the research objective, if the data analysis reflects the design, and if what was expected with use of the design was achieved to research the theme. Following, the guidelines used in clinical or epidemiological research that should be included in the section Methods of manuscripts sent to RBGO:

Types of study (adapted from Pereira, 2014*):

Case Report (Case study): In-depth investigation of a situation in which one or a few people are included (usually up to ten);

Case series: A set of patients (for example, more than ten people) with the same diagnosis or undergoing the same intervention. In general, these are consecutive series of patients seen in a hospital or other health institution for a certain period. There is no internal control group formed simultaneously. The comparison is made with external controls. The name of external or historical control is given to the group used to compare the results, but that was not constituted at the same time within the study: for example, the case series is compared with patients from previous years.

Transversal (or Cross-sectional) study: Investigation to determine prevalence; examine the relationship between events (exposure, disease, and other variables of interest) at any given time. Cause and effect data are collected simultaneously: for example, the case series is compared with patients from previous years.

Case-control study: Particular form of etiological investigation of retrospective approach in which the search of causes starts from the effects. Groups of individuals, respectively with and without a particular health problem are compared in relation to past exposures in order to test the hypothesis that exposure to certain risk factors is the contributing cause of the disease. For example, individuals afflicted with low back pain are compared with an equal number of individuals (control group) of the same sex and age, but without low back pain.

Cohort study: Particular form of investigation of etiological factors in which the search of effects starts from the cause; therefore, the opposite of case-control studies. A group of people is identified, and pertinent information on the exposure of interest is collected, so the group can be monitored over time, checking those who do not develop the disease in focus, and if the prior exposure is related to occurrence of disease. For example, smokers are compared to nonsmoker controls; the incidence of bladder cancer is determined for each group.

Randomized study: This has the connotation of an experimental study to evaluate an intervention hence the synonym of *intervention study*. Can be performed in a clinical setting; sometimes referred to simply as clinical trial or clinical study. It is also conducted at the community level. In clinical trials, participants are randomly assigned to form groups called study (experimental) and control (or testimony), whether submitted or not to an intervention (for example, a drug or vaccine). Participants are monitored to verify the occurrence of outcome of interest. This way, the relationship between intervention and effect is examined under controlled observation conditions, usually with double-blind evaluation. In the case of a **randomized study**, inform the number of the Brazilian Registry of Clinical Trials (REBEC) and/or the number of the International Clinical Trials Registration Platform (ICTRP/OMS) on the title page.

Ecological study: Research performed with statistics: the unit of observation and analysis is not constituted of individuals, but of groups of individuals hence the synonyms: study of groups, aggregates, clusters, statistics or community. For example, research on the variation of mortality coefficients for diseases of the vascular system and per capita consumption of wine among European countries.

Systematic Review and Meta-analysis: Type of review in which there is a clearly formulated question, explicit methods are used to critically identify, select and evaluate relevant research, and also to collect and analyze data from the studies included in the review. There is use of strategies to

limit bias in the localization, selection, critical evaluation and synthesis of relevant studies on a given topic. Meta-analysis may or may not be part of the systematic review. Meta-analysis is the review of two or more studies to obtain a global, quantitative estimate of the question or hypothesis investigated; and employs statistical methods to combine the results of the studies used in the review.

Source: *Pereira MG. Artigos Científicos – Como redigir, publicar e avaliar. Rio de Janeiro: Guanabara-Koogan; 2014.

Script for statistical review of original scientific papers

Study objective: Is the study objective sufficiently described, including pre-established hypotheses?

Design: Is the design appropriate to achieve the proposed objective?

Characteristics of the sample: Is there a satisfactory report on the selection of people for inclusion in the study? Has a satisfactory rate of responses (valid cases) been achieved? If participants were followed up, was it long and complete enough? If there was a pairing (eg. of cases and controls), is it appropriate? How did you deal with missing data?

Data Collection (measurement of results): Were the measurement methods detailed for each variable of interest? Is there a description of comparability of the measurement methods used in the groups? Was there consideration of the validity and reproducibility of the methods used?

Sample size: Has adequate information on sample size calculation been provided? Is the logic used to determine the study size described, including practical and statistical considerations?

Statistical Methods: Was the statistical test used for each comparison informed? Indicate if the assumptions for use of the test were followed. Was there information about the methods used for any other analysis? For example, subgroup analysis and sensitivity analysis. Are the main results accompanied by accuracy of the estimate? Inform the p value and confidence interval. Was the alpha level informed? Indicate the alpha level below which the results are statistically significant. Was the beta error informed? Or indicate the statistical power of the sample. Has the adjustment been made to the main confounding factors? Were the reasons that explained the inclusion of some and the exclusion of others described? Is the difference found statistically significant? Make sure there are sufficient analyzes to show the statistically significant difference is not due to any bias (eg. lack of comparability between groups or distortion in data collection). If the difference found is significant, is it also relevant? Specify the clinically important minimal difference. Make clear the distinction between statistically relevant difference and relevant clinical difference. Is it a one- or two-tailed test? Provide this information if appropriate. What statistical program is used? Inform the reference where to find it, and the version used.

Abstract: Does the abstract contain the proper article synthesis?

Recommendation on the article: Is the article in acceptable statistical standard for publication? If not, can the article be accepted after proper review?

Source: *Pereira MG. Artigos Científicos – Como redigir, publicar e avaliar. Rio de Janeiro: Guanabara-Koogan; 2014.

IMPORTANT!

RBGO joined the initiative of the International Committee of Medical Journal Editors (ICMJE) and the EQUATOR Network, which are aimed to improve the presentation of research results. Check the following international guides:

Randomized clinical trial:
http://www.consort-statement.org/downloads/consort-statement

Systematic reviews and meta-analysis: http://www.scielo.br/pdf/ress/v24n2/2237-9622-ress-24-02-00335.pdf

Observational studies in epidemiology: strobe-statement.org/fileadmin/Strobe/uploads/checklists/STROBE_checklist_v4_combined.pdf

Qualitative studies: http://intqhc.oxfordjournals.org/content/19/6/349.long

Results

The purpose of the Results section is to show the study findings. It is the original data obtained and synthesized by the author with the aim to answer the question that motivated the investigation. For the writing of the section, present the results in logical sequence in the text, tables and illustrations, first mentioning the most important findings. Do not repeat all information of the tables or illustrations in the text. Emphasize or summarize only important observations. Additional or supplementary materials and technical details may be placed in an appendix where they will be accessible without interrupting the flow of the text. Alternatively, this information may be published only in the electronic version of the Journal. When data are summarized in the results section, provide numerical results not only in derived values (eg. percentages), but also in absolute values from which the derivatives were calculated, and specify the statistical methods used for their analysis. Use only the tables and figures necessary to explain the argument of the work and evaluate its foundation. When scientifically appropriate, include data analysis with variables such as age and sex. Do not exceed the maximum limit of five tables, five charts or five figures. Tables, charts and/or figures should be included in the body of the manuscript and do not count the requested limit of 4000 words.

ATTENTION!

In Case Studies, the Methods and Results sections should be replaced by the term Case Description.

Discussion

In the **Discussion** section, emphasize the new and important aspects of the study and the conclusions derived therefrom. Do not repeat details of data or other information presented in the introduction or results sections. For experimental studies, it is useful to begin the discussion by briefly summarizing the main findings, comparing and contrasting the results with other relevant studies, stating the limitations of the study, and exploring the implications of the findings for future research and clinical practice. Avoid claiming precedence and referring to incomplete studies. Do not discuss data not directly related to the results of the presented study. Propose new hypotheses when justifiable, but qualify them clearly as such. In the last paragraph of the Discussion section, cite which information of your work contributes relatively to advancement of knowledge.

Conclusion

The **Conclusion** section has the function of relating the conclusions to the objectives of the study, but authors should avoid unfounded statements and conclusions not adequately supported by data. In particular, authors should avoid making statements about economic benefits and costs unless their original includes economic analysis and appropriate data.

References

A study is based on the results of other research that preceded it. Once published, it becomes support for future work on the subject. In the report of their research, authors state the references of prior works consulted that they deem pertinent to inform readers, hence the importance of choosing good References. Properly chosen references lend credibility to the report. They are a source for convincing readers of the validity of facts and arguments presented.

Attention! For manuscripts submitted to RBGO, authors should number the references in order of entry into the manuscript and use those numbers for text citations. Avoid excessive references by selecting the most relevant for each statement and giving preference to the most recent work. Do not use hard-to-reach quotations, such as abstracts of papers presented at congresses, theses or restricted publications (non-indexed). Seek to cite the primary and conventional references (articles in scientific journals and textbooks). Do not use references such as 'unpublished observations' and 'personal communication'. Authors' publications (self-citation) should be used only if there is a clear need and relationship with the topic. In this case, include in bibliographical references only original works published in regular journals (do not cite chapters or revisions). The number of references should be 35, in exception review articles. Authors are responsible for the accuracy of data contained in the references.

Please check the Vancouver Citation Style to format your references.

*The Instructions to Authors of this journal were elaborated based in the literary work ***Artigos Científicos: Como redigir, publicar e avaliar de Maurício Gomes Pereira, Editora Guanabara Koogan, 2014.***

Submission of papers

The articles must, necessarily, be submitted electronically, according to the instructions posted on the site: http://mc04.manuscriptcentral.com/rbgo-scielo

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