ISSN 0100-7203
eISSN 1806-9339

RBGO Gynecology & Obstetrics

Revista Brasileira de Ginecologia e Obstetrícia
Number 10 • Volume 43 • Pages 725–802 • October 2021

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Federação Brasileira das Associações de Ginecologia e Obstetrícia

OPEN ACCESS

Thieme

ISSN 0100-7203

RBGO Gynecology and Obstetrics
Revista Brasileira de Ginecologia e Obstetrícia

ISSN 0100-7203

Federação Brasileira das Associações de Ginecologia e Obstetrícia

Brazilian Federation of Gynecology and Obstetrics Associations

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Volume 43, Number 10/2021

RBGO Gynecology and Obstetrics
Revista Brasileira de Ginecologia e Obstetrícia

Thieme Revinter Publicações Ltda

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 RBGO Gynecology and Obstetrics/Revista Brasileiro de Ginecologia e Obstetrícia is published monthly by Thieme-Revinter Publicações Ltda., Rua do Matoso, 170, Rio de Janeiro 20270-135, Brazil.

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Revista Brasileiro de Ginecologia e Obstetrícia is an official publication of the Federação Brasileira das Associações de Ginecologia e Obstetrícia (Brazilian Federation of Association of Gynecology and Obstetrics, Febrasgo), It is listed in Isi - Web of Science, Web of Knowledge (*Emerging*), MEDLINE / PubMed, Index Medicus, Scopus (Sci Verse), SCImago, SciELO (Scientific Electronic Library Online), LILACS (Literatura Latino-Americana e do Caribe em Ciências da Saúde, Index Medicus Latino Americano), and Portal de Periódicos Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). Thieme Medical Publishers is a member of the CrossRef initiative.

ISSN 0100-7203

Editorial

Pink October and Breast Cancer in Brazil

Marcos Felipe Silva de Sá[1]

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Rev Bras Ginecol Obstet

The National Cancer Institute of the Brazilian Ministry of Health has launched this October, as it has been done since 2010, the Pink October campaign. Since 1990, this campaign has been performed worldwide annually, when there is extensive dissemination through the media, social events and educational programs which aims to alert women and society about the importance of prevention and early diagnosis of breast cancer. There is also an important participation of medical societies and health professionals.

Excluding non-melanoma skin tumors, breast cancer is the tumor that most affects the Brazilian female population and represents ~24.5% of all types of diagnosed neoplasms. In Brazil, in the 2020–2022 triennium, the estimated incidence is around 66,280 new cases of breast cancer per year, which represents a rate of 61.61 cases/100,000 women. In 2019, 18,068 deaths from breast cancer were recorded, being the leading cause of death from cancer among women.[1,2]

In the United States, the incidence of breast cancer has been increasing by ~3% per year, considering the 2012–2016 period.[3] In 2021, 281,550 new cases of invasive breast cancer and also 49,290 new cases of non- invasive cancer (in situ) are estimated. One out each eight American women will develop breast cancer during their lifetime.[4] In developing countries, the incidence has increased substantially in recent decades.[5] In 2020, more than 2.3 million women had the diagnosis of breast cancer around the world.[1]

Considering the importance of this disease for public health, the United Nations have established as one of the Sustainable Development Goals, to reduce by a third the number of premature deaths from chronic noncommunicable diseases, including breast cancer, until year 2030. Projections by the National Cancer Institute of Brazil point to stability in mortality rates between 30 and 69 years until 2030, although these numbers are still far from the 30% reduction established by the United Nations.[1]

Like many other types of cancer, it is known that prevention or early identification and rapid institution of tumor treatment for breast cancer lead to better therapeutic results and higher patient survival rates. In fact, campaigns to reduce breast cancer mortality rates can gain strength if services are expanded to provide quality prevention, early detection and timely treatment so that all women who need such services have quick access to them. In many countries, well-organized screening programs have led to a reduction in breast cancer mortality rates.[6,7] Considering the whole world, the incidence/mortality rate is 3.3.[8] In the United States, the breast cancer mortality rate has decreased and fallen 40% between 1989 to 2017, which represents ~375,900 avoided deaths.[3]

Unfortunatelly, in Brazil, despite these periodic campaigns, there has been an increase in incidence and mortality rates associated with breast cancer.[9–11] During these campaigns, a lot is focused on publicizing the need for screening for breast cancer, but the effective measures taken to facilitate the access of patients to medical care or imaging exams for this purpose are unclear. More than that, very little disclosure about the expected flow for patients, in a regionalized and hierarchical manner, for their referral to treatment and follow-up centers. There are not enough specialized services and mammography equipment to meet the demands. Diagnosis appointments take a long time and once diagnosed, a true "via crucis" begins for patients and health professionals until their effective referral to tertiary centers for treatment. As there are few service centers, patients are forced to long waits until they start the treatment.

In 2017, the main Brazilian specialty medical societies involved in breast cancer diagnosis and treatment programs recommended annual mammography as screening for breast cancer in women in the medium-risk population, i.e., those aged between 40 and 74 years.[12] They anticipated the ages for the screening examination based on a peculiarity of breast cancer in Brazil, different from other developed countries, a proportionally higher incidence in women between 40 and 50 years of age.[13,14] However, this suggestion was not implemented by the Ministry of Health, considering the lack of resources for this initiative. Mammography has been described as an effective method for the early detection of breast cancer, with a significant impact on patient survival,[15] although this impact varies in different populations with a clear decline in developing countries compared with developed countries. The differences in this impact have

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DOI https://doi.org/10.1055/s-0041-1739451.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

been attributed to late diagnosis and technological differences used in cancer therapy.[16] In Brazil, from 2000 to 2018, 40% of women between 50 and 69 years old, were diagnosed with cancer when the tumor was locally advanced or already metastatic. Furthermore, when analyzing patients' 10-year survival rates according to the tumor stage, the data showed how significant the worsening is when the tumor was treated in stages 3 and 4.[17] This suggests that the diagnosis when the tumor is already advanced is a consequence of the lack of guidance to patients for screening or delay in the referral of patients to treatment.[17]

Data published by the Oncological Observatory in 2020 show that the average time to start the diagnostic procedure for breast cancer in Brazil was 38 days, ranging from 19 to 90 days between different states of the federation. In 29.7% of patients, the time elapsed between diagnosis and treatment ranged from 0–30 days; in 20.3% between 30–60 days, and in 24% the time was longer than 60 days. It is worth considering that despite these worrying numbers, they have been stable over the last decade.[18] We still do not have data for this period of the COVID-19 pandemic, but these statistics have certainly become much more worrisome. Breast cancer has increased its incidence among black and mixed-race individuals, especially in the poorest regions of Brazil[19] and this is exactly the population that most needs assistance in public health services. It is certainly this poorer population that makes the Brazilian statistics so negative with regard to breast cancer, since, on many occasions, they are left unattended in the search for this care.

Other factors must be considered for the improvement of breast cancer indicators. In Brazil, in 2020, according to National Cancer Institute data, around eight thousand cases of breast cancer were directly related to behavioral factors, such as alcohol consumption, overweight, not having breastfed and physical inactivity[1]. Therefore, in addition to care services to patients, the public health policies must pay attention to the need for permanent campaigns to publicize preventive measures for the population at risk for breast cancer.

The Pink October campaign will be senseless if there is no effective organization of the system to guide patients to seek a service adequately prepared for screening the disease and, above all, specialized services for the more agile treatment and monitoring of patients with a diagnosis or suspected diagnosis. The Brazilian Public Health Service (Brazilian SUS) has been implemented based on provision of organized services in a regionalized and hierarchical manner, but the lack of resources and inadequate management have shown important flaws in the service. The results achieved in the diagnosis and treatment of cancer of breast can be seen as a reflection of the system disorganization. Pink October needs to stop being just another fleeting and repetitive month of campaigning and effectively become a call for health managers to get truly involved to provide the results that society expects from the country's health authorities.

Conflict of Interest
None declared.

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Investigating the Relationship between Childbirth Type and Breastfeeding Pattern Based on the LATCH Scoring System in Breastfeeding Mothers

Investigando a relação entre o tipo de parto e o padrão de amamentação com base no sistema de pontuação LATCH em mães que amamentam

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Rev Bras Ginecol Obstet 2021;43(10):728–735.

Abstract

Objective The role of breast milk in the physical and mental health of infants and in the prevention of infant death is widely known. The benefits of breastfeeding for mothers and infants have been proven, but several factors can affect breastfeeding. Childbirth is one of the most influential factors. The present study aimed to investigate the effect of the type of delivery (natural childbirth and cesarean section) on breastfeeding based on the latch, audible swallowing, type of nipple, comfort, hold (LATCH) scoring system.

Methods The present cross-sectional observational study was performed using the census method among women who referred to Afzalipour Hospital for delivery in May 2020; the breastfeeding pattern was completed by observation and the in-case information, by LATCH checklist. Data were analyzed using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, United States) software, version 19.0, analysis of variance (ANOVA), and the Chi-squared statistical test.

Results Out of a total of 254 deliveries (127 natural childbirths and 127 cesarean deliveries), there was no statistically significant difference between the 2 study groups in terms of age, maternal employment status, and infant weight, but there was a statistically significant relationship between the type of delivery, the maternal level of schooling, and the appearance, pulse, grimace, activity, and respiration (Apgar) score in the first minute. The mean score of breastfeeding patterns among the natural childbirth group (9.33) was higher than that of the cesarean section group (7.21).

Keywords
- cesarean section
- natural childbirth
- LATCH
- breastfeeding

received
November 13, 2020
accepted
August 5, 2021

DOI https://doi.org/10.1055/s-0041-1735985.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Conclusion The type of delivery affects the mother's performance during breastfeeding, and mothers submitted to cesarean sections need more support and help in breastfeeding.

Resumo

Objetivo É sabido o papel do leite materno na saúde física e mental dos bebês e na prevenção da mortalidade infantil. Os benefícios da amamentação para mães e bebês foram comprovados, mas vários fatores podem afetar a amamentação. O parto é um dos fatores mais influentes. Este estudo teve como objetivo investigar o efeito do tipo de parto (parto Natural e cesariana) na amamentação com base no sistema de pontuação agarramento, deglutição audível, tipo de mamilo, conforto, segurar (*latch*, *audible swallowing*, *type of nipple*, *comfort*, *hold*, LATCH, em inglês).

Métodos Este estudo transversal e observacional foi realizado pelo método do censo entre mulheres que buscaram atendimento no Hospital Afzalipour para parto em maio de 2020; o padrão de amamentação foi completado por observação e in-case, pela lista de verificação do LATCH. Os dados foram analisados usando o programa Statistical Package for the Social Sciences (IBM SPSS for Windows, IBM Corp., Armonk, NY, Estados Unidos), versão 19.0, análise de variância (*analysis of variance*, ANOVA, em inglês) e o teste estatístico do qui-quadrado.

Resultados De um total de 254 partos (127 parto naturais e 127 cesarianas), não houve diferença estatisticamente significativa entre os dois grupos de estudo em termos de idade, situação laboral materna e peso do bebê, mas houve uma relação estatisticamente significativa entre os tipo de parto, a escolaridade materna e o índice de aparência, frequência cardíaca, irritabilidade reflexa, tônus muscular, e respiração *appearance*, *pulse*, *grimace*, *activity*, *and respiration* (Apgar), no primeiro minuto. A pontuação média do padrão de amamentação no grupo do parto natural (9,33) foi maior do que a do grupo da cesariana (7,21).

Conclusão O tipo de parto afeta desempenho da mãe durante a amamentação, e as mães submetidas a cesariana necessitam de mais apoio e ajuda na amamentação.

Palavras-chave
- cesariana
- parto natural
- LATCH
- amamentação

Introduction

About 800 thousand infant deaths worldwide are due to the late onset of breastfeeding and the absence of exclusive breastfeeding.[1] One of the United Nations' Sustainable Development Goals (SDGs) is to reduce infant mortality by 12 per 1,000 live births, and deaths under the age of 5 to less than 25 per 1,000 live births by eliminating infant mortality by 2030.[2] Starting breastfeeding in the first hour after birth can reduce the risk of infant mortality by $\sim$ 45%, and infants who are exclusively breastfed are 14 times more likely to survive than those who are not breastfed.[3] Malnutrition is one of the leading causes of child mortality, and according to the United Nations Children's Fund (UNICEF, 2010),[4] $\sim$ 40% to 60% of children under the age of 5 years were affected by late-onset breastfeeding. According to various studies[3] and reports by the World Health Organization (WHO) and UNICEF,[4,5] breastfeeding is related to social, economic, demographic, behavioral, and cultural factors of mothers, the place and method of delivery, counseling and education about breastfeeding, and factors related to obstetric services. The method of delivery is one of the factors that affects breastfeeding. There is an increase in studies[3,6] examining the negative effects of cesarean section on well-being, maternal behavior, and breastfeeding physiology at the beginning of the postpartum period. To clarify this issue, it can be said that the anesthesia and painkillers used in cesarean sections have a negative effect on oxytocin secretion and milk production. Morphine has also been shown to stop breast milk secretion.[7] Cesarean delivery seems to have little effect on the mother-newborn relationship.[8] Women submitted to natural childbirth are 4.8 times more likely to continue breastfeeding exclusively for 30 days after delivery than women submitted to cesarean delivery.[9] Breastfeeding is associated with the early initiation of breastfeeding, and, as recommended in the steps of the Baby-Friendly Hospital Initiative (BFHI), breastfeeding should occur immediately after birth.[9] In a survey conducted in Saudi Arabia, that mothers who had a caesarean delivery had about 1.4 times higher odds for delaying Breast Feeding by $>$1 h when compared to mothers who had a vaginal delivery.[10] In one study,[11] the prevalence of late-onset breastfeeding in women submitted to natural childbirth was of 35.34% against 50.49% of women submitted to cesarean section, who were at a higher risk of performing non-exclusive breastfeeding during the 3 days after delivery. Cesarean section is a major abdominal surgery that can delay exclusive

feeding. During the first few hours after a cesarean section, mothers are expected to begin caring for their baby at the same time as they are coping with postoperative problems, including pain. However, a cesarean section can have a negative effect on breastfeeding physiology, and can cause side effects that prevent the mother from being in contact with the newborn. Unbearable pain after the surgery and an increase in the newborn's need for intensive care negatively affect breastfeeding.[12]

It has been shown that cesarean delivery is a risk factor for incomplete breastfeeding, and the rate of breastfeeding in the early hours after cesarean delivery is lower than that in the early hours after vaginal birth.[13] So far, the effect of childbirth on breastfeeding has been analyzed in countless studies, but no decisive result has been obtained.[14]

Since breastfeeding is an important and effective factor in the growth of the newborn, is directly related to infant mortality, and is one of the indicators of global health, the present study aimed to investigate the pattern of breastfeeding among mothers submitted to natural childbirth and cesarean section to diagnose the issues regarding breastfeeding and their causes according to the type of delivery, to provide more counseling and supportive strategies to mothers to improve this process.

Methods

Study Design

The present cross-sectional observational study was conducted using the census method among women who referred to Afzalipour Hospital, in Kerman, in May 2020, for delivery. The women who underwent natural childbirth and cesarean section were included in the study regardless of previous pregnancy history and age. During the data collection, we excluded women who had undergone emergency cesarean section, those who had given birth preterm (under 34 weeks of gestational age) and whose newborns did not develop effective sucking power due to lack of proper coordination between sucking, swallowing and breathing,[15] and mothers whose newborns had been hospitalized or died in the neonatal ward. After delivery, the mothers received postpartum care performed by expert midwives and gynecologist professionals and were transferred to the Postpartum Surgery and Natural Childbirth Unit of the Department of Gynecology and Midwifery, , the researcher, who was trained in midwifery, went ot the same unit after obtaining the necessary permits and receiving the approval code from the Ethics in Reasearch Committee (IR.MUBAM.REC.1399.016). Then, the researcher explained to the mothers that participation in the research was completely voluntary. They were also assured that if they refused to participate in the research, they would not be deprived of diagnostic and therapeutic care, and even after agreeing to participate in the investigation, they could withdraw from it at any time, and would not be required to pay a fine or damages. They were informed that all of their information was kept confidential, and that the general and group results of the research would be published without mentioning the names and details of the participants. Then, the mothers' written consent was obtained through the questionnaire forms.

Participant and Study Sampling

The sample size was calculated as 197 subjects based on similar studies[16] and a literature review. However, considering the period of the study (1 month), we decided to evaluate all available participants by census, and finally included 254 women.

The data collection tools were the latch, audible swallowing, type of nipple, comfort, hold (LATCH) Score for Breastfeeding Success Assessment and the personal data collection form, which included age, occupation, level of schooling, place of residence, number of children, gestational age, and previous breastfeeding history. The data collection form was filled out through questions and observation, as well as information contained in the medical records. The LATCH was used to assess breastfeeding patterns.

The LATCH was first published in 1994, and was used to identify the need for breastfeeding-related interventions.[17,18] The method of scoring is based on the appearance, pulse, grimace, activity, and respiration (Apgar) score, and results range from 0 to 10. A score < 10 indicates the mother's need for more support during breastfeeding. The system consists of five components, including how the baby latches, audible swallowing, the type of nipple, the mother's level of comfort (regarding the breast and nipple), and the level of assistance needed by the mother in order to hold the newborn while breastfeeding; the scores for each component can be 0 (weak), 1 (relatively good), or two (good).

The validity and reliability of this tool have been studied and confirmed in several studies.[16–19] In a 2011 study by Karimi et al.,[16] the Cronbach α coefficient was of 0.71.

The study data were entered into the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, United States) software, version 19.0, and descriptive and analytical statistics were used to analyze the results and the correlations among the variables.

Ethical Consideration

The present article is the result of a dissertation approved by the Bam University of Medical Sciences under the code of ethics number (IR.MUBAM.REC.1399.016) and supported by the university's research deputy. The purpose of the study was explained to the subjects, and they were included after signing the written informed consent.

Statistical Analysis

The data were analyzed using the SPSS version 19.0. Descriptive statistics (frequency, percentage, mean, and standard deviation) were used to describe the characteristics of the sample. The Chi-squared test and the parametric equivalent (analysis of variance, ANOVA) was also used. *P* value less than 0.05 was considered as statistically significant.

Results

The sample of the present study was compsed of 254 participants: 127 women submitted to natural childbirth, and 127 who underwent cesarean section. The mean age of the sample was 27.5 ± 2.3 years. The weight of the newborns

in both groups ranged from 1,100 g to 5,200 g. The mean newborn weight in the natural childbirth group was of 3,021 g, and, in the cesarean section group, of 2,855 g, which was not statistically significant ($p = 0.38$). The majority of the participants were housewives. In terms of the level of schooling, most of the participants in Natural Childbirth group had not graduated from high school, and, in the cesarean section group, most participants had graduated from high school. Statistically, there was no difference between the two groups in terms of employment status, place of residence, newborn gender, mean age of mothers, and newborn weight. In both groups, the average number of previous children was two. In total, 26% of the newborns examined had been born preterm ($\geq$ 34 weeks of gestational age) by cesarean section. However, in the natural childbirth group, only 13.4% of infants were preterm ($p = 0.006$) (►**Table 1**).

The results of the statistical tests show that the type of delivery was related to the mother's level of schooling, the rate of cesarean delivery was higher in mothers with a high school degree ($p = 0.03$) (►**Table 2**).

For 99.2% of the natural childbrith group and 95.9% of the cesarean section group, the Apgar score in the first minute was ≥ 9 ($p = 0.03$). However, regarding the Apgar score in the fifth minute, no statistically significant differences between the two groups were found ($p = 0.13$) (►**Table 3**).

In total, 52.8% of the natural childbirth group and 53.5% of the cesarean section group had previous breastfeeding experience, but no statistically significant relationships were found between that and the type of delivery ($p = 0.52$) (►**Table 4**).

An examination of the breastfeeding pattern and a Comparison of breastfeeding pattern score based on LATCH scores in both groups revealed statistically significant differences in all subsets of the LATCH score, with the mean score of the natural childbirth group (9.33) being higher than that of the cesarean section (7.21) group ($p \leq 0.0001$) (►**Table 5**).

Discussion

As shown by the findings of the present study, the type of delivery was directly related to the mother's level of schooling. The rate of cesarean delivery was higher in mothers with a high school degree. In the study by Ahmad-Nia et al. (2009),[20] the level of schooling was higher among mothers in the cesarean section group. In the studies by Hassanzadeh et al. (2019),[21] Valiani and Heshmat (2018),[22] and Islami et al. (2008),[23] the cesarean section group had complete secondary education, which is in line with with the present study.[21–23] Perhaps the reason for the lower level of schooling of the mothers in the present studyis the referral of people with low education and therefore average income,

Table 1 Comparison of employment status, place of residence, newborn gender and time of birth according to the type of delivery

		Cesarean section: *n* (%)	Natural childbirth: *n* (%)	*p*-value*
Employment status	Housewife	119 (93.7)	125 (98.4)	0.05
	Employed	8 (6.3)	2 (1.6)	
Place of residence	Urban center	89 (70.1)	78 (61.4)	0.14
	Countryside	38 (29.9)	49 (38.6)	
Newborn gender	Girl	66 (52)	58 (45.7)	0.19
	Boy	61 (48)	69 (54.3)	
Time of birth	Term	93 (73.2)	110 (86.6)	0.006
	Preterm	34 (26.8)	17 (13.4)	
Total		127 (100)	127 (100)	

Note: *Chi-squared test.

Table 2 Comparison of the mother's level of schooling and the type of delivery

		Cesarean section: *n* (%)	Natural childbirth: *n* (%)	*p*-value*
Level of schooling	Illiterate	12 (9.4)	13 (10.2)	0.03
	Incomplete high school	42 (33)	61 (48.0)	
	Complete high school	48 (37.8)	44 (34.6)	
	Associate degree	6 (4.7)	2 (1.6)	
	Bachelor's degree	18 (14.2)	6 (4.7)	
	Master's degree or higher	1 (0.8)	1 (0.8)	
Total		127 (100)	127 (100)	

Note: *Chi-squared test.

Table 3 Comparison of the Apgar scores and the type of delivery

		Cesarean section: *n* (%)	Natural childbirth: *n* (%)	*p*-value*
1-minute Apgar score	7	5 (3.9)	1 (0.7)	0.03
	8	16 (12.5)	9 (7.09)	
	9	106 (83.4)	117 (92.2)	
	10	0	0	
Total		127 (100)	127 (100)	
5-minute Apgar score	7	0	0	0.03
	8	2 (1.6)	1 (0.8)	
	9	6 (4.7)	1 (0.8)	
	10	119 (93.7)	125 (98.4)	
Total		127 (100)	127 (100)	

Abbreviation: Apgar, appearance, pulse, grimace, activity, and respiration.
Note: *Chi-squared test.

Table 4 Comparison of previous breastfeeding experience and the type of delivery

		Cesarean section: *n* (%)	Natural childbirth: *n* (%)	*p*-value*
Previous breastfeeding experience	Yes	68 (53.5)	67 (52.8)	0.52
	No	59 (46.5)	60 (47.8)	
Total		127 (100)	127 (100)	

Note: *Chi-squared test.

Table 5 Comparison of LATCH scores and the type of delivery

LATCH	Cesarean section: mean ± standard deviation	Natural childbirth: mean ± standard deviation	*p*-value *
Latch	1.54 ± 0.67	1.75 ± 0.54	0.009
Audible nwallowing	0.61 ± 0.73	1.69 ± 0.55	< 0.0001
Type of Nipple	1.83 ± 0.43	1.94 ± 0.22	0.012
Comfort (breast/nipple)	1.94 ± 0.22	1.94 ± 0.12	0.009
Hold (positioning)	1.28 ± 0.76	1.96 ± 0.13	< 0.0001
Total (average score)	7.21	9.33	< 0.0001

Abbreviation: LATCH, latch, audible swallowing, type of nipple, comfort, hold.
Note:*Analysis of variance (ANOVA).

to the Afzalipour Hospital and the use of free and low-cost insurance and childbirth services in this center compared with private and expensive medical centers.

In the present study, the Apgar score among the natural childbirth group was higher than that of the cesarean section group, which is consistent with the results of the study by Raafati et al. (2006).[24] However, in the study by Jafari et al. (2016),[25] there was no statistically significant relationship between the Apgar score and the type of delivery. During natural childbirth, it is easier to breathe because of the outflow of fluid from the lung of the fetus due to the pressure exerted by labor contractions and the passage of the fetus through the birth canal, and the Apgar score is expected to be higher.[26]

In the present study, the number of preterm newborns delivered by cesarean section was higher than the number of those delivered by natural childbirth, which is in line with the studies by Namakin et al. (2011)[27] and Goldenberg et al. (2008).[28] However, this result is not consistent with the study by Maroufizadeh et al. (2016),[29] in which the authors mention that there was no separation between emergency and elective cesarean sections, on the other hand their study was performed in Tehran, where treatment status and healthcare facilities are better than in other provinces. In the present study, due to the referral of Afzalipour Medical Center, perhaps one of the reasons for the increase in preterm infant statistics in cesarean delivery is the occurrence of maternal and fetal problems that lead to rapid termination of labor and increase in preterm infants.

In the present study, the mean total LATCH score of the natural childbirth group was 9.33, and that of the cesarean section group was 7.21, and this difference was statistically significant. It should be noted that the mentioned scores

were not related to pregnancy ranking and breastfeeding experience.

In the study by Parsay et al. (2005),[30] although the mean duration of breastfeeding in the natural childbirth was shorter than that of the cesarean section group, the breastfeeding pattern did not different much between the groups. In the study by Islami et al. (2008),[23] breastfeeding during the first hour of birth was higher among the natural childbirth group than among the cesarean section group, which was statistically significant. Karimi et al. (2011)[16] observed a significant difference in breastfeeding patterns between the natural childbirth and cesarean section groups. Cakmak and Kuguoglu (2007)[31] compared the breastfeeding pattern after both types of deliveries based on the LATCH score, and found a statistically significant difference. They also showed that the lower LATCH scores among mothers in the cesarean section group indicated that these individuals needed more support during hospitalization and at the beginning of breastfeeding, especially in terms of holding the baby.[31] A study conducted in Turkey by Gungor et al. (2004)[32] also confirmed that mothers submitted to cesarean section experienced several problems such as fatigue, insomnia, and breastfeeding problems that affected both their recovery process and neonatal care. In a more detailed study,[33] the mean score of how to breastfeed a baby in the vaginal delivery group was higher than in the cesarean section, and the position of the nipple, significantly better, in the natural childbirth group than in the cesarean section group. This can be due to the mother's general physical status for breastfeeding, which tends to be better after natural childbirth. The anesthesia and analgesics used in cesarean sections, such as morphine sulfate, can affect the secretion of oxytocin and stop the secretion of milk from the breast.[33] Intravenous injection of narcotics during cesarean delivery affects the newborn's normal reflexes in the first hours of life, and reduces the likelihood of the onset of breastfeeding.[34] However, when natural childbirth occurs spontaneously, without the use of tools and drugs, and the newborn is immediately exposed to the mother's skin, the chances that breastfeeding will begin then increase.[35]

A study conducted in Tehran by Ekhtiari and Emami (2008) [36] showed that breastfeeding until the newborn is 3 months old is significantly more likely to occur among mothers in the natural childbirth group. Newborns delivered by cesarean section experience a delay in breastfeeding due to maternal problems.[37] In a study conducted in the United States by Evans et al. (2003),[7] the authors found that cesarean delivery reduced the rates of success in breastfeeding. The reasons were the mother's despair about unplanned and emergency cesarean section, the longer recovery time, higher levels of pain, and the greater risks posed by cesarean delivery. During the first few weeks after delivery, mothers submitted to cesarean sections should be taught about proper breastfeeding. Increasing the rate of exclusive breastfeeding requires more support and assistance to mothers submitted to cesarean sections, especially to create the right conditions for the mother to sit and hug the newborn to begin breastfeeding.[38]

According to Morton et al.,[39] "The increased rate of cesarean delivery is mainly attributable to a greater number of procedures performed for slow progress in labor, breech presentation or repeat cesarean section." Puhl et al.[40] reported that induction of labor at 34 weeks of gestation is often linked to an increased risk of cesarean section. Byerly et al. (2020)[41] showed no independent association between prematurity and the likelihood of the onset of breastfeeding right after birth. Cesarean section is associated with in-hospital formula feeding and a higher proportion of breastfeeding difficulties.[42] The World Health Organization (WHO)[43] recommends that health education for women is an essential component of antenatal care, implementation of evidence-based clinical practice guidelines with timely second opinion and collaborative midwifery-obstetrician model of care. A model of supporting based on care provided primarily by midwives.[43] We also believe that mothers need to be educated by health care providers to select natural childbirth for improving their health and the health of their newborns.

Conclusion

Natural childbirth is associated with a better maternal performance during breastfeeding, and mothers submitted to cesarean sections need more support and assistance in breastfeeding. Moreover, mothers should be encouraged to choose natural childbirth and start and continue breastfeeding.

Author Contributions

Tayebeh Mokhtari Sorkhani: investigation, writing of the original draft, formal analysis, and visualization. Elahe Namazian: formal analysis. Samaneh komsari: methodology, project administration, review and editing of the text. Shima Arab: verification of resources. All of the authors have approved the final version of the manuscript for submission.

Conflict of Interests

The authors have no conflict of interests to declare.

Acknowledgments

The present article was supported by Bam University of Medical Sciences, Bam, Iran. The authors consider it necessary to appreciate and thank the esteemed management of Afzalipour Hospital in Kerman University, Bam University of Medical Sciences, the mothers who participated in the study, and all those who cooperated and participated in this research.

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Thyroid Function of Pregnant Women and Perinatal Outcomes in North Macedonia

Função tireoidiana de mulheres grávidas e resultados perinatais na Macedônia do Norte

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Rev Bras Ginecol Obstet 2021;43(10):736–742.

Abstract

Objective Thyroid diseases are the second most common endocrine disorders in the reproductive period of women. They can be associated with intrauterine growth restriction (IUGR), preterm delivery, low Apgar score, low birthweight (LBW) or fetal death. The aim of the present study is to explore thyroid dysfunction and its relationship with some poor perinatal outcomes (Apgar Score, low birthweight, and preterm delivery).

Methods Dried blood spot samples from 358 healthy pregnant women were analyzed for thyroid stimulating hormone (TSH), total thyroxine (TT4), and thyroglobulin (Tg). Neonatal data were collected upon delivery. Four groups were formed based on thyroid function tests (TFTs).

Results Of the 358 tested women, 218 (60.72%) were euthyroid. Isolated hypothyroxinemia was present in 132 women (36.76%), subclinical hyperthyroidism in 7 women (1.94%), and overt hypothyroidism in 1 (0.28%). The perinatal outcomes IUGR ($p = 0.028$) and Apgar score 1 minute ($p = 0.015$) were significantly different between thyroid function test [TFT]-distinct groups. In the multiple regression analysis, TT4 showed a statistically significant inverse predictive impact on LBW ($p < 0.0001$), but a positive impact of Tg on LBW ($p = 0.0351$).

Conclusion Thyroid hormones alone do not have a direct impact on neonatal outcomes, but the percentage of their participation in the total process cannot be

Keywords
- thyroid stimulating hormone
- total thyroxine
- thyroglobulin
- perinatal outcomes
- low birth weight

received
December 4, 2020
accepted
August 9, 2021

DOI https://doi.org/10.1055/s-0041-1736172.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

neglected. Based on the regression analysis, we can conclude that TT4 and Tg can be used as predictors of neonatal outcome, expressed through birthweight and Apgar score. The present study aims to contribute to determine whether a test for thyroid status should become routine screening during pregnancy.

Resumo

Objetivo As doenças da tireoide são as segundas doenças endócrinas mais comuns no período reprodutivo das mulheres. Elas podem estar associadas à restrição de crescimento intrauterino (RCIU), parto prematuro, baixo índice de Apgar, baixo peso ao nascer (BPN) ou morte fetal. O objetivo do presente estudo é explorar a disfunção tireoidiana e sua relação com alguns resultados perinatais insatisfatórios (índice de Apgar, baixo peso ao nascer e parto prematuro).

Métodos Amostras secas de sangue em 358 gestantes saudáveis foram analisadas para hormônio estimulador da tireoide (TSH), tiroxina total (TT4) e tireoglobulina (Tg). Os dados neonatais foram coletados no momento do parto. Quatro grupos foram formados com base em testes de função tireoidiana (TFT).

Resultados Das 358 mulheres testadas, 218 (60,72%) eram eutireoidianas. Hipotiroxinemia isolada estava presente em 132 mulheres (36,76%), hipertireoidismo subclínico em 7 mulheres (1,94%) e hipotireoidismo evidente em 1 (0,28%). Os resultados perinatais RCIU ($p = 0{,}028$) e índice de Apgar de 1 minuto ($p = 0{,}015$) foram significativamente diferentes entre os grupos distintos de TFT. Na análise de regressão múltipla, TT4 mostrou impacto preditivo inverso estatisticamente significativo no BPN ($p < 0{,}0001$), mas impacto positivo da Tg no BPN ($p = 0{,}0351$).

Conclusão Isoladamente, os hormônios tireoidianos não têm impacto direto no desfecho neonatal, mas o percentual de sua participação no processo total não pode ser desprezado. Com base na análise de regressão, podemos concluir que TT4 e Tg podem ser usados como preditores do resultado neonatal, expressos por meio do peso ao nascer e do índice de Apgar. O presente estudo tem como objetivo contribuir para que um teste para verificar o estado da tireoide deva se tornar um rastreamento de rotina durante a gravidez.

Palavras-chave
- hormônio estimulante da tireoide
- tiroxina total
- tireoglobulina
- resultados perinatais
- baixo peso de nascimento

Introduction

Thyroid diseases are the second most common endocrine disorders affecting women in the reproductive period.[1] Early diagnosis and treatment of thyroid diseases before and during pregnancy is important for maintaining the health of the mother and the baby.[2] The maternal thyroid gland undergoes physiological changes during pregnancy, including increased thyroid gland vascularity, increased iodine clearance and fluctuation in thyroxine metabolism, which may impair the maternal-fetal transfer of thyroxine despite optimal thyroid status.[3]

Hypothyroidism is relatively uncommon in pregnancy, occurring in between 2 and 3% of pregnant women, mainly as subclinical form.[1] Hyperthyroidism in pregnancy, also with very low prevalence ranging between 0.1% and 1%,[4] has been associated with intrauterine growth restriction (IUGR) and low birthweight (LBW).[5] The study of the Consortium on Safe Labor found that neonates of women with hyperthyroidism were more likely to need resuscitation in the delivery room and neonatal intensive care unit (NICU) admission. Also, they had an 1.6- to 2.0-fold odds of respiratory distress syndrome, transient tachypnea, apnea, sepsis, as well as increased odds of cardiomyopathy, retinopathy of prematurity, and neonatal thyroid disease.[5]

Isolated maternal hypothyroxinemia has been reported in $\sim$ 1.3% of pregnant women; however, its incidence can be as high as 25.4%.[6] An American study of 17,298 women examined the association between maternal isolated hypothyroxinemia and adverse neonatal outcomes, including LBW, Apgar score < 3, and fetal or neonatal mortality.[7] The same study suggested that preterm delivery may be associated with maternal hypothyroxinemia, characterizing the condition as detrimental during pregnancy.[7]

According to the guideline of the American Thyroid Association (ATA) for the diagnosis and management of thyroid disease during pregnancy and the postpartum period,[8,9] the reference values for thyroid stimulating hormone (TSH) and total thyroxine (TT4) range from 0.1 to 3.7 mIU/L and 65/97.5–165–247.5 nmol/L, respectively. Based on the above, the study population was categorized into four groups.

The largest decrease in TSH is observed during the 1st trimester because of elevated levels of serum hCG directly

stimulating the TSH receptor, therefore increasing thyroid hormone production.[8] Consequently, serum TSH and its reference range gradually rise in the 2nd and 3rd trimesters but are still lower than in nonpregnant women.

The aim of our study is 2-fold: first, to examine the correlation between thyroid function tests (TFTs) and neonatal outcome; and second, to evaluate the predictive potential of TSH, TT4 and Tg on delivery and some of the perinatal outcomes (Apgar Score and low birth weight).

Methods

The present prospective study included 358 healthy pregnant women without known thyroid disorders. For recruitment, the following inclusion criteria were adopted: singleton pregnancy at any gestational age, with the following exclusion criteria: no previous history of thyroid disease or treatment with any thyroid-related therapy, cigarette smoking, and no history of other chronic diseases, in particular diabetes mellitus or hypertension, and any fetal anomaly diagnosed with amniocentesis or ultrasound. Pregnant women were divided into 3 subgroups depending on the gestational age at the time of recruitment: 1st trimester (up to the 12th gestation week [g.w.], second trimester (between the 12th and 28th g.w.), and 3rd trimester (29 g.w. to the end of pregnancy).

Participant recruitment and collection of blood samples were performed between April and July 2017. Data on maternal age, weight and height, gestational age at the time of recruitment, as well as eligibility check against exclusion criteria, were collected using a recruitment questionnaire.

Dried blood spot (DBS) samples were collected upon recruitment onto filter paper cards using the standard lancet finger prick method. The samples were dried for 24 hours and stored at - 20°C. The TSH and TT4 levels were analyzed with a time-resolved fluoroimmunoassay method (GSP 2021–0010; PerkinElmer, Turku, Finland) at the University Children's Hospital, Zurich, Switzerland. The Tg concentration was assessed using a DBS Tg sandwich enzyme-linked immunosorbent assay[10] at Eidgenössische Technische Hochschule Zürich (ETH Zurich).

Postpartum data was retrieved for each of the labored women from their medical histories. Birthweight and birth length were measured by the midwife attending the birth, while condition of the newborn after delivery and Apgar scores were determined by the neonatologist. In addition, obstetric history, gestational age at the time of birth, as well as the way of birth were noted in the medical history.

Intrauterine growth restriction (IUGR) was defined as birthweight $< 10^{th}$ percentile for the gestational age. Low birthweight (LBW) was defined as weight $\leq$ 2,500 g, regardless of the gestational age.[11] Preterm delivery was defined as delivery before 37 completed g.ws.. Low Apgar score was considered if Apgar score at 1 minute was < 7.[12]

All participants signed an informed consent form after reading the project information sheet, and the ethics approval for the study was obtained from the Etic Committee at Medical Faculty – Skopje, Ss. Cyril and Methodius University – Skopje at its XVI session on January 24, 2019, with N * 03–242/3.

Data analyses were performed using MedCalc Statistical Software version 19.1.3 (MedCalc Software, Ostend, Belgium). Continuous variables were presented as mean ± standard deviation (SD), median (interquartile range [IQR]) (when the frequency distribution for our data was skewed) and percentages (%). One-way analysis of variance (ANOVA) was used to test the difference between the means of the six subgroups. Prior to the ANOVA test, the Levene test for equality of variances was performed. We applied a logarithmic transformation if the Levene test was positive ($p < 0.05$). Multiple backward regression analysis was used to show predictable values of independent variables (TT4 and Tg as predictors) on the dependent variable (LBW).

Results

In total, 358 women were included in the analysis. The mean maternal age was 29.27 ± 5.5 years old (range: 25 to 33 years old), their BMI was overweight, preobesity state according to the World Health Organization (WHO) classification[13] (27.12 ± 4.4 kg/m^2; range: 23.44–29.63 kg/m^2), delivered approximately at 38.43 ± 2.54 g.ws. Of the total sample, 64 women were classified at the 1st trimester according to g.w. (17.82%), 100 were in the 2nd (27.85%), and 194 were in the 3rd trimester (54.03%) (►**Table 1**). The median (IQR) TSH of the women in the 1st trimester did not deviate from the reference range (0.4 mIU/mL; range 0.3 mIU/mL), whereas the TT4 values of only 2 pregnant women (3.12%) deviated from it. In the 2nd trimester, from all the cohort, the median TSH was within the reference values (0.4 mIU/mL; range 1.1 mIU/L), while the TT4 values of 31 pregnant women (31%) deviated from the reference range. In the 3rd trimester, the median TSH of 0.56 mIU/L; (range 0.4 mIU/L) did not deviate

Table 1 Demographic and clinical characteristics of the study population at recruitment

Demographic values	*n*	% of total
1st trimester	64	17.82%
2nd trimester	100	27.85%
3rd trimester	194	54.03%
	Mean ± SD	25th – 75th P (min – max)
Age (years old)	29.27 ± 5.5	25 – 33
BMI (kg/m^2)	27.14 ± 4.7	23.44–29.63
Gestational week at birth	38.43 ± 2.54	37.7–40.0
Thyroid function values	**Normal**	**Range**
TSH (mU/L)	0.54 ± 0.33	0.3–0.7
TT4 (nmol/L)	103.92 ± 28.7	24 – 195.2
Tg (μg/L)	11.6 ± 9.01	5.53–15.49

Abbreviations: BMI, body mass index; SD, standard deviation; Tg, thyroglobulin; TSH, thyroid stimulating hormone; TT4, total thyroxine. The results are expressed as median (interquartile range) or mean ± SD.

Table 2 Categorization of pregnant women based on gestational age and TFT

Group	TSH level	TT4 level	No. (%) of pregnant women in the group
1. Normal thyroid function	normal (0.1 to 3.7 mIU/L)	normal, slightly increased	218 (60.72%)
2. Isolated hypo thyroxinemia	normal (0.1 to 3.7 mIU/L)	depressed	132 (36.76%)
3. Subclinical hyperthyroidism	suppressed (<0.1 mIU/L)	normal	7 (1.94%)
4. Subclinical hypothyroidism	elevated (3 to 10 mIU/L)	normal	0 (0.0%)
5. Overt hypothyroidism	elevated (> 3.7 mIU/L)	low (for TSH < 10mIU/L) any level (for TSH ≥ 10 mIU/L	1 (0.27%)
6. Overt hyperthyroidism	suppressed (< 0.1 mIU/L)	high (or irrelevant)	0 (0.0%)

Abbreviation: TSH, thyroid stimulating hormone.

from reference range; however, the TT4 values of 54 (27.83%) pregnant women deviated from the reference range. ►**Table 1** summarizes the clinical and demographic characteristics of the study population at the time of recruitment.

Of the cohort of 358 tested women, 193 pregnancies were terminated with normal vaginal delivery (53.91%), while 165 women underwent cesarean section (46.08%). From all the births, 41 delivered prematurely (before the 37th g.w.) corresponding to 11.45%, and only 21 infants had an Apgar score value at 1 minute < 7 (5.86%). Low birthweight was noted in 47 cases (13.12%). When we categorized the pregnant women according to their gestational age and TFT, we noticed that, from the total of the group, 218 mothers (60.72%) were euthyroid. Isolated hypothyroxinemia was present in 132 women (36.76%), subclinical hyperthyroidism in 7 women (1.94%), overt hypothyroidism in 1 (0.28%), and no cases (0%) with subclinical hypothyroidism or overt hyperthyroidism (►**Table 2**).

►**Table 3** shows the way of birth in each group. A total of 58.25% of the pregnant women with normal thyroid function, 46.21% of the isolated hypothyroxinemia group, and 71.42% of the subclinical hyperthyroidism group had normal spontaneous delivery. Also, the fetal outcome in each group is shown in ►**Table 3**. Adverse fetal outcome in isolated hypothyroxinemia included preterm delivery (9.84 versus 0 versus 12.84%), IUGR (2.27 versus 0 versus 4.12%), and LBW (12.87 versus 14.28 versus 13.30%) as compared with the group of subclinical hyperthyroidism and normal thyroid function group, consequently. Apgar score (1 minute) < 7 was seen in the group of subclinical hyperthyroidism in 28.57% of the cases, compared with the group of normal thyroid function, which appears with 5.96%. and the

Table 3 Mode of delivery and fetal outcomes in different groups

Groups	1	2	3	4		
Mode of delivery	**Normal thyroid function (*n* = 218)**	**Isolated hypothyroxinemia (*n* = 132)**	**Subclinical hyperthyroidism (*n* = 7)**	**Overt hypothyroidism (*n* = 1)**	**F**	***p-value***
Normal spontaneous delivery	127 (58.25)	61 (46.21)	5 (71.42)	0	2.6975	0.068
CS total	91 (41.74)	71 (53.78)	2 (28.57)	1		
- CS for fetal distress	28 (30.76)	10 (7.57)	1 (14.28)	1	1.1334	0.323
- CS for dystotia/ dysproprtion	25 (27.47)	11(8.33)	1 (14.28)	0	4.175	0.024
Fetal outcomes						
Preterm births	28 (12.84)	13 (9.84)	0	0	0.804	0.448
IUGR	9 (4.12)	3 (2.27)	0	0	3.594	0.028
LBW	29 (13.30)	17 (12.87)	1 (14.28)	0	0.517	0.597
Apgar score (1 minute) < 7	13 (5.96)	6 (4.54)	2 (28.57)	/	4.252	0.018
Mother characteristics						
Median age (years old)	29	29	32	26	1.299	0.274
Median BMI, kg/m^2	26.44	27.43	23.23	27.12	4.091	0.018

Abbreviations: BMI, body mass index; CS, cesarean section; IUGR, intrauterine growth restriction; LBW, low birthweight.

Table 4 Multiple backward regression analysis of low birthweight according to thyroid status categorization groups

Dependent Y	LBW						
Regression Equation							
Independent variables	βst coefficient	Std. Error	t	*p-value*	$r_{partial}$	$r_{semipartial}$	exp (βst)
(Constant)	2.5225						
TT4	– 0.01124	0.0009484	– 11.854	< 0.0001	– 0.5391	0.5388	0.98767
Tg	0.006294	0.002975	2.116	0.0351	0.1135	0.09616	1.00631
Significance level							$p < 0.0001$

Abbreviations: βst, β standardized; LBW, low birthweight; Std, standard; Tg, thyroglobulin; TT4 = total thyroxine.

isolated hypothyroxinemia group, with 4.54%. The median BMI was 26.44 kg/m^2 for women with normal thyroid function, 27.96 ± 5.05 kg/m^2 for women with isolated hypothyroxinemia, and 23.23 kg/m^2 for those with subclinical hyperthyroidism ($p = 0.018$). Obese women had depressed TT4 range and were prone to hypothyroxinemia. Substantial differences between the four groups in accordance with their impact on the neonatal outcome are presented by ANOVA results: F-ratio (F) and p-value of significance (p) are shown in ►**Table 3**. The statistical significance of each group differs according to certain variables shown: Apgar score (1 minute) ($F = 4.252$; $p = 0.015$), IUGR ($F = 3.594$; $p = 0.028$), and mean BMI ($F = 4.091$; $p = 0.018$). A greater F-ratio value and A smaller p mean a higher difference between the groups according to outcomes, according to thyroid status that defines groups.

The results of the Levene test show significance only for Apgar score (1 minute). Levene statistic ($LS = 7.897$) and p-value ($p < 0.001$). The Levene test for IUGR ($LS = 1.643$; $p = 0.179$) and BMI ($LS = 1.3$; $p = 0.274$) do not show statistical significance. Assessments (standardized coefficient β [βst], standard error of βst [Std. Error], t, p-value, partial r [$r_{partial}$], semipartial r [$r_{semipartial}$], and exp [βst]) of the dependent predictor LBW or determinants (TT4 and Tg) for increasing the incidence of LBW in TFT-categorized groups after backward multiple regression analysis are shown in ►**Table 4**.

Groups 3 and 4 were excluded from the regression analysis due to small group size. The p-values followed the order of statistical significance: TT4 (< 0.0001) and Tg (0.0351). Due to the regression model criterion (remove variable if $p > 0.5$), TSH was not included in the regression analysis. There was an inverse correlation (negative βst coefficient; βst = - 0.01124) between TT4 and LBW, and a positive correlation (positive βst = 0.006294) between Tg and LBW. The regression parameter exp (βst) for TT4 (1.012484 = 1/0.98767) signified that with each increase of 1 unit (nmol/L) in TT4, the LBW score decreased by 1.01248 g. The regression parameter exp (βst) for Tg (1.00631) signified that with each increase of 1 unit (μg/L) in Tg, the LBW score increased by 1.00631 g. The coefficient of determination R^2 (0.2914) and the multiple correlation coefficients (0.5398) showed that 29.14% from LBW was dependent on TT4 and Tg as the predictors. Only 29.14% of the changes in LBW were a result of TT4 changes (accompanied by Tg changes), and the remaining from the total variability between them were not explained (70.86% of LBW were dependent on other factors, which were not covered with the regression model. Partial coefficient of regression βst for Tg affects the LBW, because there is a simple linear relationship between Tg and LBW and it can be used for predicting. In addition, TT4 is in a simple linear relationship with LBW, and it can also be used for predicting LBW.

Discussion

Attention to thyroid dysfunction during pregnancy has increased in the past decade, particularly in the area of subclinical thyroid dysfunction, as thyroid disease is the second most common endocrine disorder complicating pregnancy.[14] We examined the thyroid function through TSH and TT4 and its relationship with perinatal outcomes in 358 healthy pregnant women divided in subgroups created by ATA guidelines.

We revealed that thyroid dysfunction during pregnancy was associated with preterm delivery, low Apgar score and low birth weight (< 2,500 g), even in the forms of isolated hypothyroxinemia. The study of Saki et al.[15] showed that both hyperthyroidism and hypothyroidism are associated with IUGR, contrary to our results, in which isolated hypothyroxinemia was connected with IUGR. They found that hypothyroidism was associated with IUGR ($p = 0.017$) and low Apgar score in the first minute ($p = 0.04$); thus, the risk for IUGR was increased by 2.2 times, and the low Apgar score increased the risk by 1.95 times. Intergroup comparison in our study showed statistically significant differences with respect of IUGR ($p = 0.028$) and Apgar score [(1 minute) < 7] ($p = 0.018$), results similar and close to the above-mentioned studies.

Clinical hypothyroidism also showed a statistically significant correlation with premature labor ($p = 0.045$) in the study by Saki et al.[15] According to Davis et al.,[16] premature labor occurred in 44% of pregnant women diagnosed with hypothyroidism and in 17% of pregnant women with subclinical hypothyroidism, which is similar to our results for preterm births (18% of women with subclinical hypothyroidism labored prematurely).

Millar et al.[17] also reviewed pregnancy outcomes in 181 women with hyperthyroidism and demonstrated that uncontrolled hyperthyroidism was associated with a 9-fold

higher LBW rate compared with the control population, which is not different from our study results. We found that TT4 ($p < 0.0001$) and Tg ($p = 0.0351$) have a simple linear relationship with LBW and can be used for predicting it. Based on the multiple correlation coefficient in our study, it is calculated that 53.98% from LBW was dependent on TT4 and Tg as the predictors.

In our study, isolated hypothyroxinemia was associated with increased risk of preterm delivery (3.62% of all pregnancies) and with increased rate of cesarean section delivery (51.58%). Also, LBW (< 2,500 g) was found in 11.90% of the newborns and lower Apgar score (1 minute) < 7 (2.38%). Subclinical hyperthyroidism had association with increased risk of preterm delivery (18%), as well as low Apgar score at the 1st minute (25%) and LBW < 2,500 g (25%).

According to Davis et al.,[16] perinatal mortality and morbidity were also increased due to placental abruption (19%), as well as to postpartum hemorrhage and anemia (19%), with consequent LBW (31%) or even fetal death (12%). Leung at al.[18] found that the pregnancies with overt hypothyroidism had significant increase in the incidence of LBW of the neonates (< 2,500 g) compared with controls. Others found no association between thyroid hormonal status or thyroid antibody positivity and preterm delivery or other obstetrical complications, like in the study of Lejeune et al.[19] In a study of 233 pregnant women with isolated hypothyroxinemia, Casey et al.[20] reported no increased adverse perinatal outcomes associated with the condition.

The present study has limitations. First, although the results of the study were statistically significant for some of the analyses, the number of women with thyroid dysfunction was limited, especially in groups with subclinical hyperthyroidism and overt hypothyroidism. Second, neither thyroid peroxidase antibodies (TPOAb), nor thyroglobulin antibodies (TgAb) were evaluated by us, which are also connected to negative neonatal outcomes. In the future, a bigger cohort plus TPOAb and TgAb evaluation is a logical next step.

Conclusion

Thyroid hormones alone do not have a direct impact on neonatal outcome. However, even if it was small, the percentage of their participation in the total process that affect the final outcome cannot be neglected. Based on the regression analysis, we can conclude that TT4 and Tg can be used as determinants for predicting the neonatal outcome, expressed through birthweight and Apgar score. These results are similar to the data that had been evaluated over the past 10 years, although there are still ongoing studies trying to clarify whether maternal subclinical hyperthyroidism and isolated hypothyroxinemia in pregnancy are associated with adverse outcomes. The present study aims to contribute to the scientific debate as to whether a test for thyroid status should become routine screening during pregnancy.

Contributions

All authors participated in the concept and design of the present study; in the analysis and interpretation of data; in the elaboration of the draft or in the revision of the manuscript; and they have approved the manuscript as submitted. All authors are responsible for the reported research.

Conflict of Interests

The authors have no conflict of interests to declare.

Acknowledgments

The present study is supported by funding from the European Union's Horizon 2020 research and innovation program under Grant agreement no.634453. It is part of International EUthyroid project task 3.3.

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The Assessment of Vitamin D Levels in Pregnant Women is not Associated to Fetal Growth Restriction: A Cross Sectional Study

A avaliação dos níveis de vitamina D em gestantes não está associada à restrição do crescimento fetal: um estudo transversal

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Rev Bras Ginecol Obstet 2021;43(10):743–748.

Abstract

Objective To assess maternal serum levels of vitamin D in fetuses appropriate for gestational age (AGA), small for gestational age (SGA), and with fetal growth restriction (FGR) according to estimated fetal weight (EFW).

Methods This cross-sectional study included 87 pregnant women between 26 and 36 weeks of gestation: 38 in the AGA group, 24 in the SGA group, and 25 in the FGR group. Maternal serum vitamin D levels were assessed using the chemiluminescence method. The Fisher exact test was used to compare the results between the groups.

Results The mean ± standard deviation (SD) of maternal age (years) and body mass index (kg/m^2) in the AGA, SGA, and FGR groups were 25.26 ± 8.40 / 26.57 ± 4.37; 25.04 ± 8.44 / 26.09 ± 3.94; and 25.48 ± 7.52 / 26.24 ± 4.66, respectively ($p > 0.05$). The maternal serum vitamin D levels (mean ± SD) of the AGA, SGA, and FGR groups were 22.47 ± 8.35 ng/mL, 24.80 ± 10.76 ng/mL, and 23.61 ± 9.98 ng/mL, respectively, but without significant differences between the groups ($p = 0.672$).

Conclusion Maternal serum vitamin D levels did not present significant differences among pregnant women with AGA, SGA, or FGR fetuses between 26 and 36 weeks of gestation according to EFW.

Keywords
- pregnancy
- maternal serum levels
- vitamin D
- small for gestational age
- fetal growth restriction

received
January 21, 2020
accepted
July 21, 2021

DOI https://doi.org/10.1055/s-0041-1735158.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Resumo

Objetivo Avaliar o nível sérico materno de vitamina D em fetos adequados para idade gestacional (AIG), pequenos para idade gestacional (PIG) e com restrição de crescimento (RCF) de acordo com a estimativa de peso fetal (EPF).

Métodos Realizou-se um estudo transversal envolvendo 87 gestantes entre 26 e 36 semanas, sendo: 38 do grupo AIG, 24 do grupo PIG e 25 do grupo RCF. A dosagem sérica materna de vitamina D foi realizada pelo método de quimiluminescência. Para as comparações entre os grupos, utilizou-se o teste exato de Fisher.

Resultados A média ± desvio-padrão (DP) da idade materna (anos) e do índice de massa corporal (kg/m^2) nos grupos AIG, PIG e RCF foram 25,26 ± 8,40 / 26,57 ± 4,37; 25,04 ± 8,44 / 26,09 ± 3,94; e 25,48 ± 7,52 / 26,24 ± 4,66, respectivamente ($p > 0,05$). A concentração sérica materna de vitamina D (médias ± desvios-padrão) dos grupos AIG, PG e RCF foram 22,47 ± 8,35 ng/ml; 24,80 ± 10,76 ng/ml; e 23,61 ± 9,98 ng/ml, respectivamente, contudo, sem diferenças significativas entre os grupos ($p = 0,672$).

Conclusão A concentração sérica materna de vitamina D não apresentou diferenças significantes entre gestantes com fetos AIG, PIG ou RCF entre 26 e 36 semanas de acordo com a EPF.

Palavras-chave

- gestação
- concentração sérica materna
- vitamina D
- pequeno para idade gestacional
- restrição de crescimento fetal

Introduction

Fetal growth restriction (FGR) affects ~ 5 to 10% of pregnancies and is the second obstetric complication with higher perinatal mortality, responsible for ~ 30% of stillbirths, as well as a cause of higher frequency of premature births and intrapartum asphyxia.[1] Small for gestational age (SGA) fetuses are those with prediction of weight below the 10th percentile for gestational age, without impairing their genetic potential for growth.[2]

Currently, the classification of FGR follows the Delphi consensus, in which the fetuses are classified with early (< 32 weeks) and late fetal growth restriction (≥ 32 weeks), excluding congenital anomalies.[3] Hypertrophy of fetal cells begins approximately at 32 weeks, and the importance given to the abdominal circumference (AC) is justified by the reduction of the liver, with reduction of glycogen storage associated with a decrease in abdominal fatty tissue.[4]

Vitamin D is a steroid involved in intestinal absorption and regulation of calcium homeostasis and is essential for the formation and maintenance of healthy and strong bones. Vitamin D deficiency may be due to inadequate exposure to the sun, inefficient food intake, decrease in absorption, and abnormal metabolism.[5] Recent studies have related vitamin D deficiency during pregnancy to preeclampsia,[6] gestational diabetes mellitus,[7] and prematurity;[8] yet, the relationship with FGR or SGA fetuses remains uncertain.

Bodnar et al.[9] sought to elucidate the association between maternal serum concentrations of 25-hydroxyvitamin D (25(OH)D) and the risk of SGA fetuses. They observed a relationship between maternal 25(OH)D serum level and risk of SGA in white women but not in black women, suggesting that vitamin D has a complex relationship with fetal growth that may vary according to race. Gernand et al.[10] evaluated the association between maternal 25(OH)D levels and increased risk of placental insufficiency and observed a relationship between 25(OH)D and vascular damage, with 25(OH)D ≥ 80 nmol/L associated with 49% lower risk of FGR in male newborns.

Therefore, the objective of the present study is to assess the vitamin D serum levels of mothers with SGA and FGR fetuses, comparing them with those of mothers with fetuses appropriate for gestational age (AGA) between 26 and 36 weeks of gestation according to estimated fetal weight (EFW).

Methods

A cross-sectional study was conducted between November 2016 and July 2019. The study was approved by the local research ethics committee under protocol No. 2.004.104, and all participants signed an informed consent form. The study was conducted at two university hospitals.

The inclusion criterion was pregnancy with a single fetus between 26 and 36 weeks of gestation, and the exclusion criteria were women in labor, fetuses with congenital anomalies detected on ultrasound, and chronic diseases such as hypertension, diabetes mellitus, autoimmune diseases, and heart diseases. Gestational age was determined by the date of the last menstrual period (LMP) and confirmed by ultrasonography performed up to 13 weeks.

The pregnant women were divided into 3 groups: 1) AGA (control); 2) SGA; and 3) FGR. Appropriate for gestational age was defined if the EFW was between 10th and 90th percentile according to the respective gestational age,[11] following normal values of pulsatility index (PI) of the umbilical artery (UA), PI of the middle cerebral artery (MCA) and mean PI of the uterine artery (UtA). Fetuses were considered to have early-onset FGR when the gestational age was < 32 weeks

and the following criteria were present: EFW or AC < 3rd percentile for the gestational age or absent end-diastolic flow in the UA; EFW or AC < 10th percentile for the gestational age, associated with a mean PI of the UtA or PI of the UA > 95th percentile for the gestational age.[3] Fetuses were considered to have late-onset FGR when the gestational age was > 32 weeks and the following criteria were present: EFW or AC < 3rd percentile for the gestational age; EFW or AC < 10th percentile for the gestational age, associated with a mean PI of the UA > 95th percentile for the gestational age, cerebroplacental ratio (CPR) < 5th percentile for the gestational age, or AC/EFW ratio crossing centiles > 2 quartiles on growth centiles.[3] Fetuses were considered SGA when EFW was between 3rd and 10th percentile and the criteria for early- and late-onset FGR diagnosis were not met.

The ultrasound examinations were performed using a diagnostic WS80 Ultrasound System (Samsung Corp., Seoul, South Korea) by experienced examiners. Biometric measurements and EFW were determined, according to the equation by Hadlock et al.[12] The Doppler parameters of the MCA and UA arteries were evaluated according to the curve of Arduini and Rizzo.[13] These were considered altered when the MCA PI < 5th percentile and/or the UA PI > 95th percentile for gestational age. The UtA Doppler parameters were evaluated according to the curve reported by Gómez et al.[14] and were considered abnormal when the mean PI > 95th percentile for gestational age. The volume of the amniotic fluid was evaluated by the four quadrants technique, according to the amniotic fluid index (AFI),[15] with AFI < 5 cm being considered oligohydramnios.

Maternal blood samples were collected during prenatal consultations only once and when the EFW < 10th percentile by ultrasound evaluation. Peripheral venous punctures were performed by two trained investigators, and the material was homogenized by inversion 5 to 8 times, accommodated in a sealed tube, and kept in a vertical position for 30 minutes. After complete blood coagulation, centrifugation was performed at 3,000 rpm for 15 minutes, and the samples were sent for laboratory analysis.

The ADVIA Centaur Vitamin D Total test (Siemens Healthineers, Erlangen, Germany) was used in the in vitro quantitative determination of total vitamin D 25(OH) in human serum and plasma. This is an 18-minute single pass competitive immunoassay using mouse monoclonal acridine ester (AE) labeled anti-vitamin D 25(OH) antibody and a fluorescein-labeled vitamin D analog. The ADVIA Centaur and ADVIA Centaur XP systems automatically perform the following steps: 1) dispenses 20 µL of sample into a cuvette and incubates for 15 seconds; 2) dispenses 200 µL auxiliary reagent and incubates for 4.5 minutes at 37° C; 3) dispenses 50 µL of lite reagent and incubates for 5.5 minutes at 37° C; 4) dispenses 100 µL of solid phase and 50 µL of auxiliary container reagent and incubates for 2.75 minutes at 37° C; 5) separates the solid phase from the mixture and aspirates unbound reagent; 6) washes the cuvette with wash solution 1; and 7) dispenses 300 µl of acid reagent and base reagent to initiate the chemiluminescent reaction.

The ADVIA Centaur systems communicate the results by e-mail, which, according to an analysis of the literature, recommends the following classification for 25(OH)D levels: 1) deficiency < 20 ng/mL (50 nmol/L); 2) insufficiency between 20 and 30 ng/mL (50–75 nmol/L); 3) sufficiency between 30–100 ng/mL (75–250 nmol/L); and 4) toxicity > 100 ng/mL (250 nmol/L).

A power analysis was performed to calculate the sample size on the basis of the Cohen effect of 0.35 to achieve a power of 80% and an α of 5% to detect the differences in the evaluated parameters.[16] Using the software G 3.1, the results suggested at least a sample size of 84 fetuses distributed homogeneously.

The data were transferred to an Excel 2010 spreadsheet (Microsoft Corp., Redmond, WA, USA) and analyzed with the SPSS for Windows, Version 15.0 (SPSS Inc., Chicago, IL, USA). The variables analyzed in the study were acquired on the day of the prenatal care consultation, when a questionnaire was applied with the following data: maternal age (years), weight (Kg), height (m), body mass index (BMI) (m/Kg^2), gestational age (weeks), consumption of fish (150 g at least 3 times a week), sun exposure (at least 20 minute per day), sun protection (yeas or no), vitamin D replacement therapy (yes or no) and smoking (at least 1 cigarette per day). From the point of view of inferential statistics, to compare the groups (AGA, SGA, and FGR) with regard to the numerical variables of the study, we applied the analysis of variance model with a fixed factor and Tukey multiple comparisons method. For categorical variables, the Fisher exact test was used. In all analyses, a significance level of $p < 0.05$ was set.

Results

Initially, blood samples from 100 pregnant women were collected; however, 13 samples were excluded due to the unavailability of the material at the time of analysis. Therefore, the final samples included 38 from the AGA group, 24 from the SGA group, and 25 in the FGR group. ►**Table 1** presents the descriptive analysis of maternal characteristics of the three groups.

Regarding categorical variables, the consumption of fish was low in all three groups. In relation to sunlight exposure, a more balanced result was observed, with 59.8% of pregnant women not sunbathing regularly. In relation to vitamin D and smoking, 94.3% and 86.2%, respectively, did not supplement this vitamin and did not smoke. No pregnant woman reported using sunscreen. ►**Table 2** shows the comparison between the groups, with no statistical differences between them in any of the categorical variables.

The mean ($\pm$SD) levels of maternal serum vitamin D for the AGA, SGA, and FGR groups (22.47 ± 8.35 ng/mL, 24.80 ± 10.76 ng/mL, and 23.61 ± 9.98 ng/mL, respectively) showed no significant differences between the groups ($p = 0.672$) (►**Table 2**). The results were compatible with vitamin D insufficiency (20–30 ng/mL) in the 3 groups.

Considering all cases included in the study, there was no significant correlation between vitamin D levels and gestational age (r = - 0.01, $p = 0.891$) (►**Fig. 1**). Furtheremore, there was no significant correlation between vitamin D levels and EFW (r= -0.06, $p = 0.551$) (►**Fig. 2**).

Table 1 Comparison of numerical maternal variables in all three groups evaluated

	AGA ($n = 38$)				SGA ($n = 24$)				FGR ($n = 25$)				*p*-value*
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	
Age (years)	25.3	8.4	15.0	42.0	25.0	8.4	15.0	41.0	25.5	7.5	15.0	42.0	0.983
GA (weeks)	31.1	2.9	26.0	35.9	33.0	2.6	27.7	36.7	32.2	3.2	26.3	36.0	0.556
Maternal weight (Kg)	69.4	14.6	49.3	105.8	65.3	11.0	51.0	84.6	65.2	13.1	39.0	85.2	0.659
Maternal height (m)	1.6	0.1	1.5	1.8	1.6	0.1	1.5	1.8	1.6	0.1	1.5	1.7	0.690
Maternal BMI (Kg/m^2)	26.6	4.4	19.8	37.1	26.1	3.9	20.7	35.2	26.2	4.7	16.4	34.6	0.922
EFW (grams)	1725	526,6	958	2788	1678,0	408,7	879	2261,0	1409	455,4	630	2053	0.030

Abbreviations: AGA, appropriate for gestational age; BMI, body mass index; EFW, estimated fetal weight; FGR, fetal growth restriction; GA, gestational age at blood sample collection; SD, standard deviation; SGA, small for gestational age.
*Tukey's multiple comparisons, $p < 0.05$.

Table 2 Comparison of maternal variables and vitamin D level in the three analyzed groups

	AGA ($n = 38$)	SGA ($n = 24$)	FGR ($n = 25$)	*p*-value
Fish consumption	7 (18.4%)	1 (4.2%)	5 (20%)	0.233*
Exposure to sunlight	15 (39.5%)	9 (37.5%)	11 (44%)	0.925*
Smoking	8 (21.1%)	2 (8.3%)	2 (8%)	0.325*
Vitamin D supplement	3 (7.9%)	1 (4.2%)	1 (4%)	0.999*
Vit D level (ng/ml)	22.5 ($\pm$8.3)	23.6 ($\pm$9.9)	24.8 ($\pm$10.8)	0.672**

Abbreviations: AGA, appropriate for gestational age; FGR, fetal growth restriction; SGA, small for gestational age, Vit, vitamin.
*Fisher exact test: frequency (centile).
** ANOVA: mean (standard deviation), $p < 0.05$.

Discussion

The inability of a fetus to attain the weight corresponding to its genetic potential increases morbidity and perinatal mortality; thus, FGR and obstetrical pathology must be diagnosed and managed early and adequately.[17] Fetal growth restriction increases the risk of long-term sequelae, such as coronary heart disease, diabetes mellitus type 2, arterial hypertension, and metabolic syndrome.[18–20] Therefore, the knowledge of extrinsic predisposing factors could help in the early diagnosis of this pathology. Accordingly, we evaluated the effect of maternal parameters such as BMI on FGR, as a study found that pregnant women with FGR fetuses have low BMI compared with pregnant women with AGA fetuses.[21]

Classic obstetric complications have been associated with serum Vitamin D levels,[22–25] even though the challenge of establishing a value specifically attributable to the pregnancy and puerperal period is recognized. However, the tendency of the available literature is to adopt indices from the non-pregnant population, which as can be seen in a recent systematic review with meta-analysis of 54

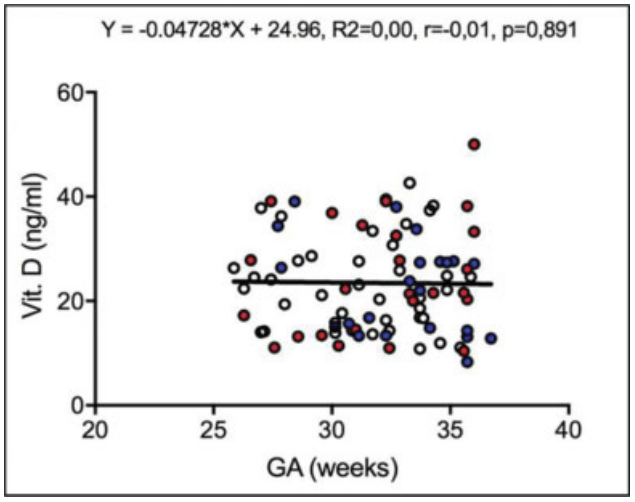

Fig. 1 Scatter plot of the vitamin D level (ng/ml) according to gestational age (weeks) in all pregnant women included in the study. Open dots: adequate for the gestational age; blue dots: small for the gestational age; red dots: fetal growth restriction. Pearson correlation coefficient, $p < 0.05$.

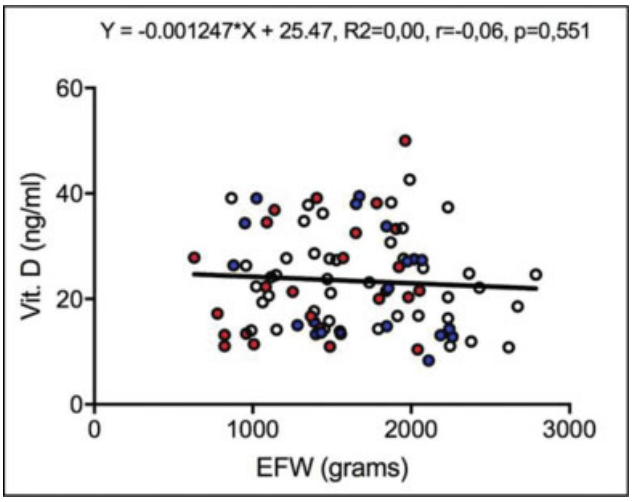

Fig. 2 Scatter plot of the vitamin D level (ng/ml) according to estimated fetal weight (grams) in all pregnant women included in the study. Open dots: adequate for the gestational age; blue dots: small for the gestational age; red dots: fetal growth restriction. Pearson correlation coefficient, $p < 0.05$.

articles.[26] Furthermore, we did not find any significant correlation between vitamin D level and gestational age, as well vitamin D level and EFW. This condition supported our choice for the Vitamin D scores that classified the group of women studied.

Vitamin D insufficiency is associated with obstetric pathologies such as preeclampsia and diabetes mellitus.[6,7] During pregnancy, supplementation of this vitamin can be a viable strategy to prevent fetuses with low birth weight and SGA;[22] for this reason, the present study aimed at assessing the correlation of vitamin D with fetal growth.

Vitamin D deficiency in pregnant women is a major concern due to the risk of adverse obstetric pathologies and perinatal outcomes.[23,24] The level of 25(OH)D, which is the main form of vitamin D storage in humans, can, therefore, be measured in maternal blood to determine overall vitamin D status.

In the current study, low levels of vitamin D were observed in pregnant women between 26 and 36 weeks of gestation, living in a tropical country like Brazil, with abundant sunshine. In the south of China, which also has a tropical climate and where women were believed to have sufficient exposure to ultraviolet B radiation and regular vitamin supplementation in prenatal care, a high prevalence of low levels of vitamin D between 16 and 20 weeks of gestation was also observed.[25] No significant differences in adverse perinatal outcomes were observed between pregnant women with different vitamin D levels, except for a higher prevalence of gestational diabetes mellitus and preterm delivery in women with high serum vitamin D levels.[25]

The present study demonstrated a high prevalence (75.9%) of low serum vitamin D levels (deficient and insufficient levels) in pregnant women evaluated, regardless of the group assessed. A systematic review and meta-analysis, which included 54 eligible studies, reported that vitamin D deficiency (< 30 ng/mL) was associated with SGA,[26] unlike our study, which did not identify this association. This systematic review also identified the occurrence of preterm birth and deficits in mental development and language when vitamin D insufficiency was present.[26]

In this study, five women received vitamin D supplementation, with three pregnant women having AGA fetuses, one having a SGA fetus, and the other one having a FGR fetus. In this study, we did not assess the differences associated with the skin color of pregnant women.

As limitations of the study are cross-sectional character, which did not evaluate the neonatal outcomes, thus making it impossible to compare with birthweight. Furthermore, although the sample size is within the statistical calculation, the small number of cases may have impacted our results. Futures studies with a higher number of cases are necessary to prove our results.

Conclusion

In summary, maternal serum concentration of vitamin D assessed between 26 and 36 weeks of pregnancy showed no significant differences between cases identified through EFW as AGA, SGA, or FGR.

Contributions

All authors participated in the conception and design of the present study; analysis and interpretation of data; draft or revision of the manuscript; and they have approved the manuscript as submitted. All authors are responsible for the reported research.

Conflict of Interests

The authors have no conflict of interests to declare.

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Improving Implantation Rate in 2nd ICSI Cycle through Ovarian Stimulation with FSH and LH in GNRH Antagonist Regimen

Melhorando a taxa de implantação no 2° ciclo de ICSI através do estímulo ovariano com FSH e LH no regime com antagonista do GnRH

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Rev Bras Ginecol Obstet 2021;43(10):749–758.

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Abstract

Objective To investigate whether patients with a previous recombinant follicle stimulating hormone (rFSH)-stimulated cycle would have improved outcomes with rFSH + recombinant luteinizing hormone (rLH) stimulation in the following cycle.

Methods For the present retrospective case-control study, 228 cycles performed in 114 patients undergoing intracytoplasmic sperm injection (ICSI) between 2015 and 2018 in an in vitro fertilization (IVF) center were evaluated. Controlled ovarian stimulation (COS) was achieved with rFSH (Gonal-f, Serono, Geneva, Switzerland) in the first ICSI cycle (rFSH group), and with rFSH and rLH (Pergoveris, Merck Serono S.p.A, Bari, Italy) in the second cycle (rFSH + rLH group). The ICSI outcomes were compared among the groups.

Results Higher estradiol levels, oocyte yield, day-3 high-quality embryos rate and implantation rate, and a lower miscarriage rate were observed in the rFSH + rLH group compared with the rFSH group. In patients < 35 years old, the implantation rate was higher in the rFSH + rLH group compared with the rFSH group. In patients ≥ 35 years old, higher estradiol levels, oocyte yield, day-3 high-quality embryos rate, and implantation rate were observed in the rFSH + rLH group. In patients with ≤ 4 retrieved oocytes, oocyte yield, mature oocytes rate, normal cleavage speed, implantation rate, and miscarriage rate were improved in the rFSH + rLH group. In patients with ≥ 5 retrieved oocytes, higher estradiol levels, oocyte yield, and implantation rate were observed in the rFSH + rLH group.

Conclusion Ovarian stimulation with luteinizing hormone (LH) supplementation results in higher implantation rates, independent of maternal age and response to COS when compared with previous cycles stimulated with rFSH only. Improvements were also observed for ICSI outcomes and miscarriage after stratification by age and retrieved oocytes.

Keywords

- follicle-stimulating hormone
- intracytoplasmic sperm injection implantation
- luteinizing hormone
- ovarian stimulation

received
December 1, 2020
accepted after revision
August 26, 2021

DOI https://doi.org/10.1055/s-0041-1736306.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Resumo

Objetivo: Investigar se há algum efeito da suplementação com hormônio luteinizante (LH, na sigla em inglês) no regime com antagonista do hormônio liberador de gonadotropina (GnRH, na sigla em inglês) sobre os resultados dos ciclos consecutivos de injeção intracitoplasmática de espermatozoides (ICSI, na sigla em inglês).

Métodos Para o presente estudo retrospectivo de caso-controle, foram avaliados 228 ciclos de microinjeção intracitoplasmática de espermatozoides (ICSI, na sigla em inglês) realizados em 114 pacientes entre 2015 e 2018 em um centro privado de fertilização in vitro (FIV) afiliado a uma universidade. O estímulo ovariano controlado (EOC) foi feito com hormônio folículo- estimulante recombinante (rFSH, na sigla em inglês) (Gonal-f, Serono, Genebra, Suíça) no primeiro ciclo de ICSI (grupo rFSH), e com rFSH e rLH (Pergoveris, Merck Serono S.p.A, Bari, Itália) no segundo ciclo (grupo rFSH + rLH). Os desfechos dos ciclos de ICSI foram comparados entre os grupos.

Resultados Níveis mais elevados de estradiol, de recuperação oocitária, taxa de embriões de alta qualidade no 3° dia e taxa de implantação, e menor taxa de aborto foram observados no grupo rFSH + rLH. Em pacientes < 35 anos, a taxa de implantação foi maior no grupo rFSH + rLH em comparação com o grupo rFSH. Em pacientes com $\geq$ 35 anos, maiores níveis de estradiol, recuperação oocitária, a taxa de embriões de alta qualidade no 3° dia e a taxa de implantação foram observados no grupo rFSH + rLH. Em pacientes com baixa resposta ao EOC ($\leq$ 4 oócitos recuperados), a recuperação oocitária, a taxa de oócitos maduros, a taxa de velocidade normal de clivagem, a taxa de implantação e a taxa de aborto foram melhoradas no grupo rFSH + rLH. Em pacientes com resposta normal ao EOC ($\geq$ 5 oócitos recuperados), níveis mais elevados de estradiol, recuperação oocitária e taxa de implantação foram observados no grupo rFSH + rLH.

Conclusão A estimulação ovariana com suplementação de LH resultou em taxas de implantação mais altas, independentemente da idade materna e da resposta ao EOC, em comparação com os ciclos anteriores estimulados apenas com rFSH. Melhorias também foram observadas nos resultados da ICSI e na taxa de aborto quando as pacientes foram estratificadas por idade e número de oócitos recuperados.

Palavras-chave

- hormônio folículo-estimulante
- microinjeção intracitoplasmática de espermatozoides
- hormônio luteinizante
- estímulo ovariano

Introduction

For assisted reproductive technology (ART), gonadotropin-releasing hormone (GnRH) and gonadotropins are routinely administered for controlled ovarian stimulation (COS). For that, recombinant follicle stimulating hormone (rFSH or follitropin alfa) and recombinant luteinizing hormone (rLH or lutropin alfa) are the key hormonal stimulus, which can be used individually or in combination. Follicle stimulating hormone and LH play distinct but complementary roles in follicle regulation, leading to synergistic actions in stimulating the recruitment and development of ovarian follicles, increasing follicle estradiol secretion, and completing oocyte maturation and subsequent ovulation.[1]

Although ovarian stimulation is essential for the success of ART, it is also known to reduce endogenous FSH and LH releases.[2] Particularly, GnRH antagonists induce a profound pituitary supression, avoiding premature LH surge. Consequently, recruited follicles are radically deprived of LH sustenance. Exogenous FSH stimulation will support follicular development in most patients undergoing ART; however, up to 12% of the patients will not respond to FSH stimulation alone, which can happen due to the absence of LH.[3] For this subpopulation of patients, there is evidence that LH supplementation to FSH administration could be advantageous.[3–10]

In fact, rLH was originally commercialized to supplement follitropin alfa administration for specific patients, especially those presenting with severe LH and FSH deficiency, namely hypogonadotropic hypogonadism. More recently, new products were developed in a fixed combination of 2:1 (150 IU of rFSH and 75 IU of rLH), under the presupposition that this is the optimal FSH:LH ratio for the purpose of stimulating follicular development. Then, it was agreed that patients (i) with previous poor response to ovarian stimulation, (ii) inadequate ovarian response in the treatment in progress, (iii) aged $\geq$ 35 years old could also benefit from LH supplementation.[10]

A recent meta-analysis that included 36 randomized controlled trials investigated the effectiveness of rLH combined with rFSH for COS compared with rFSH alone in 8,125 women undergoing ART. Moderate quality evidence that the use of rLH combined with rFSH may lead to more ongoing

pregnancies than rFSH alone was observed. No evidence of a difference between the two regimens was observed in terms of live birth rate. The authors concluded that the evidence was insufficient to encourage or discourage stimulation regimens that include rLH combined with rFSH in ART.[11]

To date, there is no evidence that ovarian simulation with rLH improves ART outcomes in an unselected subpopulation. In addition, there is only one previous study that investigated cycles in which patients acted as their own controls. The objective of the present study was to investigate whether patients with a previous rFSH-stimulated cycle would have improved outcomes with rFSH + rLH stimulation in the following cycle.

Methods

Experimental Design, Patients, and Inclusion and Exclusion Criteria

The present case-control within-subject study included data obtained via chart review of 228 cycles performed in 114 patients undergoing ICSI between 2015 and 2018 in a private university-affiliated IVF center. For all patients, rFSH (Gonalf, Serono, Geneva, Switzerland) was used for COS in the first ICSI cycle (rFSH group, $n = 114$), followed by ovarian stimulation with rFSH and rLH (Pergoveris, Merck Serono S.p.A, Bari, Italy) in the next cycle (rFSH + rLH group, $n = 114$). Pituitary suppression was achieved with GnRH antagonist (cetrorelix acetate, Cetrotide; Merck KGaA, Darmstadt, Germany) in both groups.

The inclusion criteria were: couples with primary infertility undergoing their first rFSH-stimulated ICSI cycle, with intended fresh embryo transfer on day 5 of embryo development, who underwent a second rFSH + rLH stimulated ICSI cycle, also intending fresh embryo transfer on day 5 of embryo development.

The exclusion criteria were as follows: Female patients undergoing ICSI cycles with vitrified/thawed or donated oocytes, surgical sperm retrieval, cryopreserved sperm, and vitrified/thawed embryo transfer.

Ovarian response to COS and ICSI outcomes were compared between the groups.

All patients signed a written informed consent form. The present study was approved by the local Institutional Review Board.

Controlled Ovarian Stimulation

For the first ICSI cycle of the patients, COS was started on the 3rd day of the cycle, with the administration of daily doses of r-FSH. For the second ICSI cycle, on the 3rd day of the cycle, COS was started with the administration of r-FSH + r-LH.

The following steps were the same for both the first and the second ICSI cycles. When at $\geq$ 1 follicle $\geq$ 14 mm was visualized, pituitary blockage was performed using GnRHa. When $\geq$ 3 follicles attained a mean diameter $\geq$ 17 mm and adequate serum estradiol levels were observed, final follicular maturation was triggered by the administration of 250 µg of r-hCG (Ovidrel, Merck KGaA, Geneva, Switzerland) or GnRH agonist (triptorelin 0.2 mg, Gonapeptyl; Ferring GmbH, Kiel, Germany; or leuprolide acetate 2.0mg, Lupron Kit, Abbott S.A Societé Française des Laboratoires, Paris, France). Oocyte retrieval was performed 35 hours later.

Intracytoplasmic Sperm Injection and Embryo Quality and Transfer

Intracytoplasmic sperm injection was performed according to Palermo et al.[12] Embryos were cultured in 50-µL drops culture medium (Global, LifeGlobal, Guilford, USA) covered with paraffin oil, in a humidified atmosphere under 6% CO_2, at 37°C, for 5 days. The embryos were morphologically evaluated on days 3 and 5 of development. On day 5, 1 to 2 embryos were transferred per patient, depending on maternal age and embryo quality, using a soft catheter with transabdominal ultrasound guidance.

Clinical Follow-Up

A serum pregnancy test was performed 10 days after embryo transfer. Women with a positive β human Chorionic Gonadotropin (βhCG) test underwent a transvaginal ultrasound scan after 2 weeks. Clinical pregnancy was confirmed when at least one intrauterine gestational sac with fetal heartbeat was detected. Implantation rate was calculated per transferred embryos. Clinical pregnancy rates were calculated per embryo transfer. Miscarriage was defined as pregnancy loss before 20 weeks of gestation.

Data Analysis and Statistics

The sample size calculation suggested that 200 cycles would be enough to demonstrate a 20% effect with 80% power and 5% significance level considering as primary outcome the implantation rate.

In the first analysis, response to COS, and the outcomes of ICSI were compared between the rFSH and rFSH + rLH groups ($n = 228$), using generalized linear models followed by the Bonferroni post hoc test. Then, data were stratified according to female age ($<$ 35 years old, $n = 50$, and $\geq$ 35 years old, $n = 178$) and response to COS (poor response: $\leq$ 4 retrieved oocytes, $n = 102$, and normal response: $\geq$ 5 retrieved oocytes, $n = 126$), and were reanalyzed as mentioned above. In all models, female age, body mass index (BMI) and total FSH dose were included as covariates. No patient has shifted age categories from the 1st to the 2nd ICSI cycle. Patients that became pregnant in the 1st ICSI cycle and returned for a 2nd cycle desiring another child were not excluded from the analysis, to avoid bias.

Data are expressed as mean ± standard error for continuous variables or as percentages for dichotomous variables, and p-values. P-value was significant at 5% level (< 0.05). The analysis was performed using IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, NY, USA).

Results

All patients completed the follow-up (20 weeks of gestation), and there were no data missing regarding the reported variables. Higher estradiol levels (1151.73 ± 194.34 pg/mL versus 1909.11 ± 194.34 pg/mL, $p = 0.006$), oocyte yield

Table 1 Descriptive analysis of demographics, response to COS and laboratorial ICSI outcomes of patients in repeated cycles ($n = 228$)

Variables	rFSH group ($n = 114$)	rFSH + rLH group ($n = 114$)	*p-value*
Female age	37.19 ± 0.35	37.89 ± 0.35	0.160
Male age	39.23 ± 0.63	39.96 ± 0.64	0.416
BMI	24.88 ± 0.42	24.68 ± 0.42	0.740
FSH dose (IU)	2826.92 ± 199.67	2693.64 ± 198.79	0.636
LH dose (IU)	0.0	1346.82 ± 34.50	NA
Estradiol level (pg/mL)	1151.73 ± 194.34	1909.11 ± 194.34	0.006
Cycles triggered with GnRHa	9/114 (7.9)	10/114 (8.8)	0.811
Follicles (n)	9.99 ± 0.70	10.38 ± 0.70	0.695
Retrieved oocytes (n)	6.37 ± 0.49	7.30 ± 0.49	0.185
Oocyte yield (%)	63.41 ± 2.24	69.78 ± 2.24	0.045
MII oocyte rate (%)	67.72 ± 2.53	71.48 ± 2.52	0.293
Fertilization rate (%)	77.33 ± 2.41	73.02 ± 2.37	0.202
Normal cleavage speed rate (%)	67.16 ± 3.16	73.07 ± 3.11	0.182
D3 high –quality embryos rate (%)	34.13 ± 4.37	47.71 ± 4.40	0.029
Blastocyst development rate (%)	36.72 ± 6.68	42.68 ± 5.73	0.499
Frozen embryos (n)	2.21 ± 0.61	3.05 ± 0.57	0.308
Endometrial thickness (mm)	10.32 ± 0.27	10.71 ± 0.25	0.288
Embryos transferred (n)	2.08 ± 0.09	2.04 ± 0.09	0.759
Cycles with embryo transfer (%)	70/114 (61.4)	69/114 (60.5)	0.892
Implantation rate (%)	18.57 ± 0.52	26.47 ± 0.62	< 0.001
Pregnancy rate (%)	15/70 (21.4)	20/69 (29.0)	0.303
Miscarriage rate (%)	5/15 (33.0)	1/20 (5.0)	0.031
OHSS rate (%)	3/114 (2.6)	6/114 (5.3)	0.308

Abbreviations: BMI, body mass index; COS, controlled ovarian stimulation; D3, day 3 of embryo development; ICSI, intracytoplasmic sperm injection; IU, international unit; MII, metaphase II; NA, not applicable; OHSS, ovarian hyper stimulation syndrome; rFSH, recombinant follicle stimulating hormone; rLH, recombinant luteinizing hormone.
Note: values are mean ± standard error, unless otherwise noted.

(63.41 versus 69.78%, $p = 0.045$), day-3 high-quality embryos rate (34.13 versus 47.71%, $p = 0.029$) and implantation rate (18.57 versus 26.47%, $p < 0.001$), and lower miscarriage rate (33.0 versus 5.0, $p = 0.031$) were observed in the rFSH + rLH group compared with the rFSH group (►**Table 1**).

In patients < 35 years old, the implantation rate was significantly higher in the rFSH + rLH group compared with the rFSH group (21.43 versus 38.46%, $p < 0.001$) (►**Table 2**).

In patients aged ≥ 35 years old, higher estradiol levels (1161.80 ± 215.94 pg/mL versus 1966.55 ± 220.13 pg/mL, $p = 0.009$), oocyte yield (61.28%versus 68.62%, $p = 0.038$), day-3 high-quality embryos rate (32.01 versus 48.81%, $p = 0.013$), and implantation rate (17.35 versus 23.64%, $p < 0.001$) were observed in the rFSH + rLH group compared with the rFSH group (►**Table 3**).

In patients with poor response to COS (≤ 4 retrieved oocytes), oocyte yield (56.82 versus 63.29%, $p = 0.001$), mature oocytes rate (69.87 versus 78 + 12%, $p < 0.001$), normal cleavage speed (62.5 versus 75.83%, $p < 0.001$), implantation rate (10.00 versus 20.45%, $p < 0.001$) and miscarriage rate (100 versus 0.00%, $p < 0.001$) were improved in the rFSH + rLH group compared with the rFSH group (►**Table 4**).

In patients with normal response to COS (≥ 5 retrieved oocytes), higher estradiol levels (1725.74 ± 303.65 pg/mL versus 2788.37 ± 281.12 pg/mL, $p = 0.010$), oocyte yield (75.37 versus 82.69%, $p = 0.006$), and implantation rate (23.33 versus 29.35%, $p < 0.001$) were observed in the rFSH + rLH group compared with the rFSH group (►**Table 5**).

Discussion

In the present study, we observed that COS with rFSH + rLH resulted in higher estradiol levels, oocyte yield, day-3 high-quality embryos rate and implantation rate, and lower miscarriage rate compared with COS with rFSH only. The only previous study that has investigated the effect of adding rLH to stimulation in patients with a previous cycle stimulated with rFSH alone showed lower fertilization rates associated with rLH supplementation.[13]

The use of rLH during COS is a matter of debate in the literature that has produced controversial results. In studies

Table 2 Descriptive analysis of demographics, response to COS and laboratorial ICSI outcomes of patients < 35 years old in repeated cycles ($n = 50$)

Variable	rFSH group ($n = 25$)	rFSH + rLH group ($n = 25$)	*p-value*
Female age	32.00 ± 0.48	32.05 ± 0.54	0.942
Male age	36.26 ± 1.39	36.31 ± 1.66	0.981
BMI	25.54 ± 0.98	24.58 ± 1.03	0.500
FSH dose (IU)	2521.50 ± 138.76	2471.05 ± 159.17	0.811
LH dose (IU)	0.0	1235.53 ± 401.07	NA
Estradiol level (pg/mL)	1085.05 ± 399.49	1916.20 ± 357.31	0.322
Cycles triggered with GnRHa	1/25 (4.0)	2/25 (8.0)	0.551
Follicles (n)	12.12 ± 1.49	13.74 ± 1.71	0.475
Retrieved oocytes (n)	8.12 ± 1.09	9.84 ± 1.25	0.298
Oocyte yield (%)	70.98 ± 4.49	75.54 ± 5.16	0.505
MII oocyte rate (%)	68.08 ± 4.75	67.36 ± 5.44	0.920
Fertilization rate (%)	81.08 ± 4.47	72.83 ± 5.2	0.229
Normal cleavage speed rate (%)	71.79 ± 5.13	75.32 ± 5.97	0.654
D3 high-quality embryos rate (%)	42.97 ± 9.99	40.41 ± 12.24	0.871
Blastocyst development rate (%)	41.60 ± 12.12	47.44 ± 11.06	0.859
Frozen embryos (n)	1.50 ± 0.56	2.83 ± 0.64	0.118
Endometrial thickness (mm)	10.30 ± 0.59	10.87 ± 0.66	0.520
Embryos transferred (n)	2.33 ± 0.14	2.07 ± 0.17	0.223
Cycles with embryo transfer (%)	21/25 (84.0)	18/25 (72.00)	0.409
Implantation rate (%)	21.43 ± 1.01	38.46 ± 1.72	< 0.001
Pregnancy rate (%)	6/21 (28.57)	9/18 (50.00)	0.197
Miscarriage rate (%)	2/6 (33.33)	0/9 (0.0)	0.083
OHSS rate (%)	0/25 (0.0)	1/25 (4.0)	0.999

Abbreviations: BMI, body mass index; COS, controlled ovarian stimulation; D3, day 3 of embryo development; ICSI, intracytoplasmic sperm injection; IU, international unit; MII, metaphase II; NA, not applicable; OHSS, ovarian hyper stimulation syndrome; rFSH, recombinant follicle stimulating hormone; rLH, recombinant luteinizing hormone.
Note: values are mean ± standard error, unless otherwise noted.

that investigated the benefits of adding LH to FSH stimulus in women with normal response to COS, higher levels of estradiol[14–18] and progesterone,[15] higher rate of high-quality embryos,[15] a smaller number of cycles cancelled,[14] increased pregnancy rate,[14] and less incidence of OHSS[14] were observed compared with stimulus with rFSH alone. One study demonstrated a negative impact of LH supplementation on oocyte maturation and fertilization.[13] Conversely, several studies reported no difference in the outcomes of cycles when rFSH alone was compared with rFSH + rLH.[8,19–22]

In patients with poor response to COS, stimulation with rFSH + rLH resulted in higher pregnancy, implantation, and live birth rates when compared with stimulation with rFSH alone or human menopausal gonadotropin.[23] Another study showed that stimulation with rFSH + rLH yielded higher rate of high-quality embryos.[19] These results suggest that poor response to COS could be related to LH insufficiency, and rLH supplementation might rescue oocyte competence that, in turn, could lead to the development of viable embryos, thus increasing pregnancy outcomes. On the other hand, some studies reported no significant differences in ICSI outcomes when comparing the two stimulation regimens in poor responder patients.[24,25]

Significantly increased implantation ratse[6,8,9] and live birth rates[9] have been observed in older women stimulated with rFSH + rLH when compared with their nonsupplemented counterparts.[8] Moreover, treatment with rLH significantly reduced total FSH consumption,[8] confirming that FSH and LH act synergistically. Conversely, Fábregues et al.[26] showed that rLH supplementation did not increase ovarian response to COS and implantation rates in patients of older reproductive ages. Marrs et al.[21] observed similar pregnancy rates in young and older women receiving rFSH + rLH; however, pregnancy rates in women ≥ 35 years old receiving rFSH alone significantly declined when compared with those of women < 35 years old, suggesting that these patients might benefit from the addition of rLH. It has been suggested that younger women possess a higher number of LH receptors compared with older women and, therefore, do not require LH supplementation, while LH supplementation in older women secures a sufficient LH-induced response.[8]

Table 3 Descriptive analysis of demographics, response to COS and laboratorial ICSI outcomes of patients aged ≥ 35 years old in repeated cycles in patients ($n = 178$)

Variable	rFSH group ($n = 89$)	rFSH + rLH group ($n = 89$)	p-value
Female age	38.65 ± 0.29	39.06 ± 0.28	0.303
Male age	40.10 ± 0.68	40.67 ± 0.66	0.549
BMI	24.70 ± 0.47	24.70 ± 0.45	0.995
FSH dose (IU)	2913.69 ± 248.86	2738.16 ± 2369.51	0.611
LH dose (IU)	0.0	1369.08 ± 359.66	NA
Estradiol level (pg/mL)	1161.80 ± 215.94	1966.55 ± 220.13	0.009
Cycles triggered with GnRHa	8/89 (9.0)	8/89 (9.0)	> 0.999
Follicles (n)	9.39 ± 0.77	9.71 ± 0.75	0.772
Retrieved oocytes (n)	5.88 ± 0.54	6.79 ± 0.53	0.227
Oocyte yield (%)	61.28 ± 2.54	68.62 ± 2.46	0.038
MII oocyte rate (%)	67.61 ± 2.95	72.33 ± 2.83	0.248
Fertilization rate (%)	76.20 ± 2.81	73.06 ± 2.65	0.417
Normal cleavage speed rate (%)	65.72 ± 3.78	72.65 ± 3.57	0.184
D3 high-quality embryos rate (%)	32.01 ± 4.83	48.81 ± 4.69	0.013
Blastocyst development rate (%)	39.06 ± 7.34	45.10 ± 6.24	0.531
Frozen embryos (n)	2.42 ± 0.75	3.09 ± 0.67	0.508
Endometrial thickness (mm)	10.33 ± 0.30	10.68 ± 0.26	0.386
Embryos transferred (n)	1.98 ± 0.11	2.04 ± 0.10	0.712
Cycles with embryo transfer (%)	49/89 (55.06)	51/89 (57.30)	0.698
Implantation rate (%)	17.35 ± 0.60	23.64 ± 0.66	< 0.001
Pregnancy rate (%)	9/49 (18.37)	12/51 (23.53)	0.508
Miscarriage rate (%)	3/9 (33.33)	1/12 (8.33)	0.140
OHSS rate (%)	3/89 (3.4)	5/89 (5.6)	0.469

Abbreviations: BMI, body mass index; COS, controlled ovarian stimulation; D3, day 3 of embryo development; ICSI, intracytoplasmic sperm injection; IU, international unit; MII, metaphase II; NA, not applicable; OHSS, ovarian hyper stimulation syndrome; rFSH, recombinant follicle stimulating hormone; rLH, recombinant luteinizing hormone.
Note: values are mean ± standard error, unless otherwise noted.

Table 4 Descriptive analysis of demographics, response to COS and laboratorial ICSI outcomes in repeated cycles in patients with poor response to COS (≤ 4 retrieved oocytes) ($n = 102$)

Variable	rFSH group ($n = 51$)	rFSH + rLH group ($n = 51$)	*p-value*
Female age	38.37 ± 0.54	38.93 ± 0.58	0.481
Male age	39.90 ± 0.89	39.97 ± 0.98	0.959
BMI	25.11 ± 0.45	24.23 ± 0.50	0.194
FSH dose (IU)	3051.60 ± 456.34	2536.05 ± 492.09	0.442
LH dose (IU)	0.0	1268.02 ± 442.97	NA
Estradiol level (pg/mL)	596.24 ± 101.87	725.51 ± 111.24	0.391
Cycles triggered with GnRHa	0/51 (0.0)	0/51 (0.0)	> 0.999
Follicles (n)	4.65 ± 0.30	4.33 ± 0.33	0.472
Retrieved oocytes (n)	2.29 ± 0.16	2.47 ± 0.18	0.481
Oocyte yield (%)	56.82 ± 1.31	63.29 ± 1.34	0.001
MII oocyte rate (%)	69.87 ± 1.34	78 + 12 ± 1.56	< 0.001
Fertilization rate (%)	79.46 ± 1.68	81.0 ± 1.8	0.533
Normal cleavage speed rate (%)	62.5 ± 1.08	75.83	< 0.001

Table 4 (*Continued*)

Variable	rFSH group ($n = 51$)	rFSH + rLH group ($n = 51$)	*p-value*
D3 high-quality embryos rate (%)	32.47 ± 6.44	49.14 ± 7.32	0.087
Blastocyst development rate (%)	32.81 ± 7.23	33.20 ± 6.41	0.967
Frozen embryos (n)	0.71 ± 0.17	0.69 ± 0.19	0.957
Endometrial thickness (mm)	10.22 ± 0.45	10.10 ± 0.50	0.861
Embryos transferred (n)	1.68 ± 0.12	1.68 ± 0.13	0.992
Cycles with embryo transfer (%)	25/51 (49.02)	26/51 (50.98)	0.836
Implantation rate (%)	10.00 ± 0.63	20.45 ± 0.96	< 0.001
Pregnancy rate (%)	3/25 (12.00)	6/26 (23.08)	0.332
Miscarriage rate (%)	3/3 (100)	0/6 (0.0)	< 0.001
OHSS rate (%)	0/51 (0.0)	0/51 (0.0)	0.999

Abbreviations: BMI, body mass index; COS, controlled ovarian stimulation; D3, day 3 of embryo development; ICSI, intracytoplasmic sperm injection; IU, international unit; MII, metaphase II; NA, not applicable; OHSS, ovarian hyper stimulation syndrome; rFSH, recombinant follicle stimulating hormone; rLH, recombinant luteinizing hormone.
Note: values are mean ± standard error, unless otherwise noted.

Table 5 Descriptive analysis of demographics, response to COS and laboratorial ICSI outcomes in repeated cycles in patients with normal response to COS (≥ 5 retrieved oocytes) ($n = 126$)

Variable	rFSH group ($n = 63$)	rFSH + rLH group ($n = 63$)	*p-value*
Female age	36.24 ± 0.44	37.27 ± 0.42	0.092
Male age	38.78 ± 0.86	39.95 ± 0.83	0.322
BMI	24.68 ± 0.66	24.94 ± 0.60	0.770
FSH dose (IU)	2648.61 ± 75.42	2789.08 ± 71.04	0.175
LH dose (IU)	0.0	1394.54 ± 308.62	NA
Estradiol level (pg/mL)	1725.74 ± 303.65	2788.37 ± 281.12	0.010
Cycles triggered with GnRHa	9/63 (14.3)	10/63 (15.9)	0.803
Follicles (n)	14.32 ± 0.91	14.04 ± 0.86	0.826
Retrieved oocytes (n)	9.67 ± 0.61	10.23 ± 0.57	0.503
Oocyte yield (%)	75.37 ± 1.99	82.69 ± 1.78	0.006
MII oocyte rate (%)	73.33 ± 1.43	71.8 ± 1.69	0.489
Fertilization rate (%)	78.31 ± 2.65	72.40 ± 2.51	0.105
Normal cleavage speed rate (%)	70.02 ± 3.16	72.54 ± 3.00	0.565
D3 high-quality embryos rate (%)	35.80 ± 5.96	46.78 ± 5.45	0.174
Blastocyst development rate (%)	41.79 ± 11.63	53.29 ± 9.59	0.445
Frozen embryos (n)	3.71 ± 0.99	4.23 ± 0.80	0.680
Endometrial thickness (mm)	10.40 ± 0.33	10.98 ± 0.27	0.182
Embryos transferred (n)	2.29 ± 0.107	2.21 ± 108	0.603
Cycles with embryo transfer (%)	45/63 (71.43)	42/63 (66.67)	0.513
Implantation rate (%)	23.33 ± 0.72	29.35 ± 0.80	< 0.001
Pregnancy rate (%)	12/43 (27.91)	13/42 (30.95)	0.579
Miscarriage rate (%)	2/12 (16.67)	1/13 (7.69)	0.425
OHSS rate (%)	3/63 (4.76)	6/63 (9.52)	0.299

Abbreviations: BMI, body mass index; COS, controlled ovarian stimulation; D3, day 3 of embryo development; ICSI, intracytoplasmic sperm injection; IU, international unit; MII, metaphase II; NA, not applicable; OHSS, ovarian hyper stimulation syndrome; rFSH, recombinant follicle stimulating hormone; rLH, recombinant luteinizing hormone.
Note: values are mean ± standard error, unless otherwise noted.

Moreover, ovarian androgen secretion is also diminished in older women, suggesting an age-related decline in ovarian response to stimulation with LH.[8]

Few studies investigated the potential benefits of adding rLH to rFSH in patients with reduced serum LH concentrations. Lisi et al.[27] showed an increased implantation rate in women with LH concentration < 1.0 IU/l at downregulation who received rLH supplementation, suggesting that, in the experience of profound LH downregulation, rLH supplementation might be beneficial. In opposition, Humaidan et al.[8] observed increased implantation rates when LH supplementation was used in patients with endogenous LH concentrations $\geq$ 1.99 IU/l. One study failed to demonstrate association between rLH supplementation and improved outcomes.[28]

The lack of consensus in the aforementioned literature has led to the publication of several meta-analyses, which also came to conflicting conclusions. The meta-analyses suggested that rFSH+rLH results in shorter stimulation length and fewer rFSH consumption,[29] and yields higher estradiol levels[29,30] and higher number of mature oocytes.[30] While several meta-analysis showed no significant differences in implantation,[2,29,30] pregnancy,[2,29,30] and live-birth rates,[2,11,30,31] others have demonstrated higher implantation rates,[3] pregnancy rates,[3,32] ongoing pregnancy rates,[11] and lower miscarriage rates[11] in the recombinant LH-supplemented regimen.

For poor responder patients, an increase in clinical pregnancy rate was observed in favor of supplementing rLH.[2,32] In addition, poor responders showed significantly more retrieved oocytes with rFSH + rLH compared with rFSH alone.[32]

The disparity found in the literature may be due to (i) LH administration start (beginning of treatment or late phase), (ii) type of GnRH analogue used (agonist or antagonist), (iii) starting dose of gonadotropin and gonadotropin dose adjustment during COS, (iv) heterogeneous definition of poor ovarian response, and (v) heterogeneous cutoff values for advanced maternal age (35 or 36 years old).

The possible mechanisms behind the benefits offered by rLH supplementation are improved oocyte competence and endometrial receptivity. Lower levels of cumulous cell apoptosis have been demonstrated in cycles with rLH supplementation as compared with cycles with rFSH alone,[33] which can reflect enhanced oocyte competence. In addition, LH stimulates CYP17 to convert progesterone into androgens, which in turn can be aromatized to estrogens. The supplementation with LH decreases the chance of a premature progesterone rise prior to luteinization, thus benefitting the endometrium and increasing the chance of implantation and clinical pregnancy.[34] Finally, the addition of rLH may improve follicular insulin sensitivity, leading to decreased androgen levels through a cascade mediated by increased production of adiponectin. This favorable setting may culminate in enhanced follicular maturation, ovulation, and fertilization capacity.[35]

This is a retrospective study with its inherent limitations and bias. In addition, although the sample size was adequate for the analysis of the general group, the present study is underpowered for subgroups analyses. The present study was limited by its small sample size but creates a rationale to conduct randomized studies with larger casuistic to draw concrete conclusions about the use of rFSH and rLH for ovarian stimulation in patients with cycles stimulated with rFSH alone. The results presented here might provide another tool for the clinician to use in the decision-making process regarding the trigger regimen. The most important take home message is that the outcomes of ICSI cycles from unselected patients can be improved in a following cycle with the use of LH supplementation for ovarian stimulation.

Conclusion

In conclusion, ovarian stimulation with LH supplementation results in higher implantation rates, regardless of maternal age and response to COS, compared with cycles stimulated with rFSH only. Improvements were also observed for ICSI laboratory outcomes and miscarriage rate when the patients were stratified by age and number of retrieved oocytes. Despite being encouraging, due to the retrospective nature of the present study, these results should be confirmed in randomized controlled trials.

Conflict of Interests

The authors have no conflict of interests to declare.

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Intraoperative Assessment of Endogenous Microbiota in the Breast

Avaliação intraoperatória da microbiota endógena da mama

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Rev Bras Ginecol Obstet 2021;43(10):759–764.

Abstract

Objective: Breast surgery is considered a clean surgery; however, the rates of infection range between 3 and 15%. The objective of the present study was to intraoperatively investigate the presence of autochthonous microbiota in the breast.

Methods: Pieces of breast tissue collected from 49 patients who underwent elective breast surgery (reconstructive, diagnostic, or oncologic) were cultured. The pieces of breast tissue were approximately 1 cm in diameter and were removed from the retroareolar area, medial quadrant, and lateral quadrant. Each piece of tissue was incubated in brain heart infusion (BHI) broth for 7 days at 37°C, and in cases in which the medium became turbid due to microorganism growth, the samples were placed in Petri dishes for culturing and isolating strains and for identifying species using an automated counter.

Results: Microorganism growth was observed in the samples of 10 of the 49 patients (20.4%) and in 11 of the 218 pieces of tissue (5%). The detected species were *Staphylococcus lugdunensis, Staphylococcus hominis, Staphylococcus epidermidis, Sphingomonas paucimobilis*, and *Aeromonas salmonicida.* No patient with positive samples had clinical infection postoperatively.

Conclusion: The presence of these bacteria in breast tissue in approximately 20% of the patients in this series suggests that breast surgery should be considered a potential source of contamination that may have implications for adverse reactions to breast implants and should be studied in the near future for their oncological implications in breast implant-associated large-cell lymphoma etiology.

Keywords

- surgery infection
- capsular contracture
- nipple-sparing mastectomy
- breast conservative surgery
- biofilm

received
January 25, 2021
accepted after revision
August 26, 2021

DOI https://doi.org/10.1055/s-0041-1736300.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Resumo

Objetivo: A cirurgia de mama é considerada uma cirurgia limpa; entretanto, as taxas de infecção variam entre 3 e 15%. O objetivo deste estudo foi investigar no intraoperatório a presença de microbiota autóctone na mama.

Métodos: Pedaços de tecido mamário coletados de 49 pacientes submetidas à cirurgia eletiva da mama (reconstrutiva, diagnóstica ou oncológica) foram cultivados. Os pedaços de tecido mamário tinham aproximadamente 1 cm de diâmetro e foram removidos da área retroareolar e dos quadrantes medial e lateral. Cada pedaço de tecido foi incubado em caldo BHI (*brain heart infusion*) por 7 dias a 37 ° C, e nos casos em que o meio ficou turvo devido ao crescimento de microrganismos, as amostras foram colocadas em placas de Petri para cultivo e isolamento de cepas e para identificação de espécies usando um contador automatizado.

Resultados: O crescimento do microrganismo foi observado nas amostras de 10 das 49 pacientes (20,4%) e em 11 dos 218 pedaços de tecido (5%). As espécies detectadas foram *Staphylococcus lugdunensis*, *Staphylococcus hominis*, *Staphylococcus epidermidis*, *Sphingomonas paucimobilis* e *Aeromonas salmonicida*. Nenhum paciente com amostras positivas apresentou infecção clínica no pós-operatório.

Conclusão: A presença dessas bactérias no tecido mamário em aproximadamente 20% das pacientes desta série sugere que a cirurgia mamária deve ser considerada uma fonte potencial de contaminação que pode ter implicações nas reações adversas aos implantes mamários e deve ser estudada em um futuro próximo por suas implicações oncológicas na etiologia do linfoma de células grandes associado ao implante de mama.

Palavras-chave

- infecção cirúrgica
- contratura capsular
- mastectomia com preservação do mamilo
- cirurgia conservadora da mama
- biofilme

Introduction

Elective breast surgery, mastoplasty, and partial and total mastectomies are traditionally considered clean surgeries, according to their potential for contamination.[1] However, the rate of infection in patients who undergo elective breast surgery ranges between 3 and 15%,[2] which is above that expected for a surgery that is considered as clean. In patients with risk factors for infection, such as smoking, neoadjuvant chemotherapy, and previous radiotherapy, the infection rate may be as high as 25%.[3] The use of prophylactic antibiotics has reduced the infection rate from 15 to 9%, but this percentage is still above that expected for a surgery that is considered a clean one.[4]

In addition to postoperative clinical infections, another common but late complication related to infection or to the presence of bacteria in the surgical area is capsular contracture.[5] This may occur in up to 30% of patients undergoing plastic or reconstructive surgery of the breast with implant placement.[6] The causes of capsular contracture are yet to be clarified, but the presence of subclinical infection is one of the involved factors.[7] One hypothesis is that the presence of bacteria in the surgical area from the breast ducts and parenchyma causes contamination of the implant, with the formation of a biofilm that is resistant to antibiotics.[8] The formation of a biofilm leads to chronic inflammation, which causes capsular contracture. In addition, this chronic inflammation around the implant covered by biofilm may lead to the development of breast implant-associated large-cell lymphoma.[9]

The objective of the present study was to determine the presence of endogenous microbiota in the breast tissue intraoperatively, at different locations in the breast. The presence of endogenous bacteria in the breast tissue can explain why infection rates are higher than expected in breast surgery.

Methods

Patients

The present prospective study was approved by the internal review board (IRP) of Universidade Positivo. The study subjects were female patients undergoing elective breast surgery (mastoplasty and partial and total mastectomies with immediate breast reconstruction) at the Breast Unit of Hospital Nossa Senhora das Graças (Curitiba, PR); all patients were operated on by the same surgical team. The patients received information about the study and signed an informed consent form agreeing to participate in it. Forty-nine patients were recruited, with a total of 78 breasts (29 bilateral and 20 unilateral surgeries). Tissue samples were collected from each operated breast of the study patients. The pieces of tissue were representative of the retroareolar or central areas, medial , and lateral (axillary) quadrants, with the aim of comparing bacterial growth among the various samples in different positions of the breast.

In addition, the influence of the following clinical variables on the results of the cultures was investigated: diabetes mellitus, neoadjuvant chemotherapy, radiotherapy, menopause, breastfeeding, body mass index (BMI), purpose of the

surgery (oncologic, cosmetic, or reconstructive), and presence of malignancy.

Sample Collection

All the patients received prophylactic antibiotics preoperatively (cefazolin 1 g, intravenously during anesthesia induction). The skin was thoroughly prepared with 2% antimicrobial chlorhexidine (antisepsis) and with an alcohol-based solution of 0.5% chlorhexidine (asepsis) and subsequently covered with sterile surgical drapes, exposing only the area of the skin involved in the surgery. Intraoperative samples were collected from each breast in the first 30 min of surgery, from 3 different locations: retroareolar tissue (region 1), medial gland tissue (region 2), and axillary gland tissue (region 3). The samples consisted of pieces of tissue of at least 1 cm each, excised by a cold scalpel. The samples were placed in sterile flasks with saline solution (also sterile), stored in a cold room (4°C), and sent for culture in the microbiology laboratory of Universidade Positivo.

The samples were incubated in brain heart infusion (BHI) broth at 37°C in a shaker incubator, with constant shaking (150 RPM), for 7 days, and then they were subsequently assessed for turbidity. The samples that became turbid due to microorganism growth were inoculated in Petri dishes and placed in an incubator at 37°C for 7 days. Each turbid broth was used to inoculate two dishes using different techniques: the pour-plate technique for facultative/microaerophilic anaerobes, and the spread-plate technique for aerobes. Different strains were isolated from each dish and were inoculated in new dishes, with each strain placed in one quadrant of the dish. The cultures were sent to a private clinical analysis laboratory to identify the species using a Vitek 2 automated counter.

Statistical Analysis

Data analysis started with the evaluation of the normality condition of the quantitative variables using the D'Agostino-Pearson test. Subsequently, the Student t-test was used for the quantitative variables that passed the normality test and Fisher exact test was used for the qualitative variables to detect differences between patients with positive and negative culture results (Zar, 2009).[10] The statistical analyses were performed using the GRAPHPAD PRISM statistical package, and the level of significance was set at 5% ($\alpha = 0.05$).

Results

The patients' ages ranged from 33 to 74 years (mean, 49 years; standard deviation, 8.48). Twenty-eight patients (57%) underwent oncologic surgery, 10 (20%) underwent diagnostic surgery, 8 (16%) underwent delayed reconstructive surgery, 2 (4%) underwent risk-reduction surgery, and 1 (2%) underwent one-stage oncologic surgery and risk-reduction surgery (oncologic surgery in one breast and risk-reduction surgery in the contralateral breast) (►**Table 1**).

A total of 218 pieces of breast tissue were removed and processed from 78 breasts of 49 patients. Two patients had tumors in both breasts, 21 patients had a tumor in the right breast, and 23 patients had a tumor in the left breast. Regarding neoadjuvant therapies, 12 patients underwent chemotherapy before surgery and 6 underwent radiotherapy before surgery. Three patients were diabetic, 17 were menopausal, and 31 had breastfed. The patients' BMI varied between 18 kg/m^2 and 36 kg/m^2 (mean, 24.8 kg/m^2; standard deviation, 5.66).

Table 1 Type of surgery performed

Type of surgery	Frequency
Oncologic surgery	28 (57.1)
Diagnostic surgery	10 (20.4)
Reconstructive surgery	8 (16.3)
Risk-reduction surgery	2 (4)
Oncologic + risk-reduction contralateral surgery	1 (2)
Total	49 (100)

The culture media of 13 samples from 12 different patients exhibited turbidity (positive result). Of these, two samples from two different patients did not exhibit bacterial growth in the laboratory. Ten of the 49 patients (20.4%) exhibited bacterial growth in at least one of the cultures of the sampled pieces. Eleven of the 218 samples (5%) presented cultures with bacterial growth. The identified bacteria were *Sphingomonas paucimobilis* (three patients), *Staphylococcus hominis* (one patient), *Staphylococcus lugdunensis* (three patients), *Staphylococcus epidermidis* (two patients, one of them had the microorganism in two different samples), and *Aeromonas salmonicida* (one patient) (►**Table 2**). No patient with positive samples for bacterial growth had clinical infection in the postoperative period. There were no significant differences between patients with and without a positive culture result with regard to the nine assessed variables (►**Table 3**).

Among the samples with bacterial growth, four were from retroareolar tissue (three in the left breast and one in the right breast), four were from axillary tissue (one left axillary, one right axillary plus right medial, and two right axillary), and three were from medial tissue (one right medial, one left medial, and one right medial plus right axillary) (►**Table 4**).

Discussion

Conceptually, clean surgeries are those performed in tissues that are sterile or susceptible to decontamination, in the

Table 2 Identified species

Species	Frequency (n = 10)
Staphylococcus lugdunensis	30.0%
Sphingomonas paucimobilis	30.0%
Staphylococcus epidermidis	20.0%
Staphylococcus hominis	10.0%
Aeromonas salmonicida	10.0%

Table 3 Relationship between the clinical findings and purpose of surgery and the presence of endogenous microbiota in the breast

Variable analyzed		Positive (n = 10)	Negative (n = 38)	*P*-value
Age (years)		**50.9 ± 9.2**	**48.6 ± 11.1**	**0.694**
Purpose of surgery	Reconstruction	20.0%	15.8%	> 0.999
	Diag + RR	0.0%	2.6%	
	Oncologic	80.0%	81.6%	
Tumor location	Right	45.9%	44.4%	0.716
	Left	51.4%	44.4%	
	Bilateral	2.7%	11.1%	
DM (yes)		10.0%	5.4%	0.521
NC (yes)		10.0%	28.9%	0.414
RTX (yes)		20.0%	10.5%	0.591
BMI (kg/m^2)		24.5 ± 1.6	24.9 ± 4.7	0.684
Menopause (yes)		44.4%	36.1%	0.711
Breastfeeding (yes)		70.0%	64.9%	> 0.999

Abbreviations: BMI, body mass index; Diag + RR, diagnostic and risk-reduction surgeries; DM, diabetes mellitus; NC, neoadjuvant chemotherapy; RTX, radiotherapy.

Table 4 Locations of the positive cultures

Location of the positive culture	Frequency (n)
Retroareolar	4
Lateral	4
Medial	3

absence of a local infectious and inflammatory process or gross technical errors, elective and traumatic surgeries with first-intention wound healing and without drainage, and surgeries wherein there is no entry into the digestive, respiratory, or urinary tracts. Breast surgeries are traditionally categorized as clean surgeries. However, studies have reported higher rates of breast surgery infection than expected for this category.[2,3] The routine use of prophylactic antibiotics has significantly reduced the rates of infectious complications, but values that are higher than expected in clean operations are still found. In a meta-analysis involving 2,395 patients, Zhang (2014)[11] concluded that the use of prophylactic antibiotics reduces the rates of infection and prevents the development of other surgical complications, such as dehiscence, ischemia, and necrosis.

Capsular contracture is a frequent cause of reoperations, and its etiology is still unclear or lacking in consensus; however, it has been strongly associated with the presence of bacteria in the surgical area,[5] which form a film over an implant, known as biofilm.[12] A biofilm is defined as an adhesion layer between bacterial cells and an extracellular matrix, which is resistant to most antibiotics[5,13,14] and to physical and chemical methods of sterilization, because it blocks the penetration of gases and liquids.[15] The presence of biofilms is confirmed by sonication and implant culture, even in patients without signs of clinical infection.[16] Bacterial contamination is the factor with the greatest impact on capsular contracture formation, regardless of the type of implant lining.[17] Bacterial contamination leads to the formation of a thicker capsule with greater tissue reaction and a higher amount of inflammatory cells.[17,18]

Irrigation of the surgical area with antimicrobial substances reduces the risk of developing capsular contracture,[19] another fact that supports the hypothesis that the presence of microbiota is a factor for the development of capsular contracture, as reported by Yalanis et al. (2015)[19] in a meta-analysis with 5,153 patients. Another late complication of mastoplasty with implants, anaplastic large-cell lymphoma, has been associated with the presence of biofilms. Breast implant-associated anaplastic large-cell lymphoma is a rare T-cell lymphoma that may develop around breast implants in plastic or reconstructive surgeries.[18,20] Chronic inflammation around the implant is thought to be the cause of lymphoma development,[18,20,21] and the presence of a biofilm around the implant is believed to promote inflammatory reactions, which increase the probability of developing lymphoma.[22] Removal of the capsule is the primary treatment for this type of lymphoma.[18] However, the presence of the bacterial component alone does not appear to be sufficient for the inflammatory stimulus required for the development of lymphoma; the surface component of the silicone implant is also needed.[23] Therefore, Santanelli di Pompeo (2015)[24] suggests that the only safe treatment to avoid breast implant-associated lymphoma is autologous flap breast reconstruction instead of implants.

Moreover, the presence of microbiota in nipple aspirate fluid has been demonstrated through the amplification and sequencing of nucleic acids.[25] Chan (2016)[25] compared the microbiota of nipple aspirate fluid of patients with a history of breast cancer with that of control patients and found abundant bacterial populations in both groups.

Similarly, different surgical techniques are associated with different complication rates, with techniques involving incisions near the nipples and implants having higher rates.[26–28] The use of a funnel-shaped device that assists in implant placing to avoid contact between the implant and tissues during its positioning also reduces the rates of reoperation due to capsular contracture.[29] These facts may be explained by the presence of bacteria inside the breast ducts and by the intraoperative contamination of the implant by these bacteria.[7] A similar study with cultures of tissue samples collected from 50 breasts reported bacterial growth in 19 of them, resulting in 38% of breasts with cultures positive for microorganisms.[7] The authors of the study concluded that the breast harbors endogenous microbiota that may be the source of spontaneous or postoperative infections.

In the present study, *Sphingomonas paucimobilis*, a gram-negative bacillus, was found in 30% of the positive cultures. This microorganism is also present in the soil, plants, and potable water. It has been isolated from distilled water tanks, respirators, and hemodialysis equipment in hospital settings. Patients with chronic diseases or immunosuppression may be susceptible to infections by this microorganism. Hospital and community infections have been described, including sepsis, septic pulmonary embolism, peritonitis, septic arthritis, and endophthalmitis.[30] *Staphylococcus lugdunensis* was also detected in 30% of the positive cultures in the present study. It was first described in 1988 and was attributed an important role because of its capacity to cause serious infections, such as endocarditis; intra-abdominal infections; as well as infections of the skin and soft tissues, the central nervous system, and bones and joints. Its penicillin-resistance rate can be as high as 76%, varying between community and nosocomial strains.[31] *Staphylococcus epidermidis*, a typical gram-positive commensal microorganism of the human skin microbiota, was isolated in 20% of the positive cultures. It is a facultative anaerobe that is resistant to various environmental conditions.[7] Its pathogenic capacity is closely related to the capacity of biofilm formation of its strains, which makes it resistant to various hostile environments.[32] *Staphylococcus hominis* was present in 10% of the positive cultures. It is another gram-positive microorganism commonly present in the human skin, in animals of the human biome, and in the environment. It is a causative agent of infections in immunocompromised individuals. It is capable of facultative fermentation, as demonstrated by the isolation of lactate fermentation genes.[33] Finally, *Aeromonas salmonicida* was also isolated in 10% of the positive cultures. The genus comprises gram-negative, oxidase-positive, facultative anaerobes that are rod-shaped and widely distributed in the aquatic environment. It was considered a pathogen in cold-blooded animals only but has increasingly been reported as an opportunistic pathogen in humans, causing mainly gastrointestinal infections, furunculosis, and septicemia.[34]

Conclusion

Despite the preoperative use of prophylactic antibiotics, rigorous skin antisepsis, and adequate sterile surgical techniques, 20.4% of the patients in the present study had positive cultures. This number, although in a limited sample, is similar to that found in the literature, and the majority of the isolated species, which were staphylococci, were the same as those detected in other studies. Some of the detected species are associated with infections in immunocompromised patients. The locations with a higher number of positive cultures were the retroareolar area and the lateral quadrant, which is in line with the findings of other studies (not statistically significant). Thus, the breast harbors endogenous microbiota that may be responsible for the formation of biofilms and the contamination of implants and may even be associated with the pathophysiology of implant-associated large-cell lymphoma. Further studies are necessary to prove this hypothesis.

Conflict of Interests

There are no conflict of interests to declare.

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The Impact of the COVID-19 Pandemic on Depression and Sexual Function: Are Pregnant Women Affected More Adversely?

O impacto da pandemia de COVID-19 na depressão e na função sexual: as mulheres grávidas são afetadas de forma mais adversa?

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Rev Bras Ginecol Obstet 2021;43(10):765–774.

Abstract

Objective To investigate depression and sexual function among pregnant and non-pregnant women throughout the COVID-19 pandemic.

Methods A total of 188 women, 96 pregnant and 92 non-pregnant were included. The Beck Depression Inventory (BDI) and the Arizona Sexual Experience Scale (ASEX) were applied to the participants after obtaining sociodemographic data.

Results The depression scores of pregnant and non-pregnant women were similar ($p = 0.846$). We found that the depression scores were significantly higher among the group of participants who have lower economic status ($p = 0.046$). Moreover, the depression score was significantly higher among women who lost their income during the pandemic ($p = 0.027$). The score on the ASEX was significantly higher, and sexual dysfunction was more prevalent among women who have lower levels of schooling and income ($p < 0.05$). Likewise, the ASEX scores were significantly higher ($p = 0.019$) among the group who experienced greater income loss throughout the pandemic. Upon comparing the pregnant and non-pregnant groups, we detected that sexual dysfunction had a significantly higher rate among pregnant women ($p < 0.001$).

Conclusion In times of global crisis, such as the current pandemic, low-income families have an increased risk of experiencing depression and sexual dysfunction. When we compared pregnant women with non-pregnant women, depression scores were similar, but pregnant women were at a 6.2 times higher risk of developing sexual dysfunction.

Keywords
- COVID-19
- pandemic
- pregnant
- depression
- sexual function

received
November 3, 2020
accepted
August 8, 2021

DOI https://doi.org/10.1055/s-0041-1736174.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Resumo

Objetivo Investigar a depressão e as funções sexuais de mulheres grávidas e não grávidas durante a pandemia de Covid-19.

Métodos Um total de 188 mulheres, 96 grávidas e 92 não grávidas, foram incluídas. O Inventário de Depressão de Beck (Beck Depression Inventory, BDI, em inglês) e a Escala de Experiências Sexuais do Arizona (Arizona Sexual Experience Scale, ASEX, em inglês) foram aplicados aos participantes após a obtenção dos dados sociodemográficos.

Resultados As pontuações de depressão de mulheres grávidas e não grávidas foram semelhantes ($p = 0{,}846$). Verificou-se que as pontuações de depressão foram significativamente maiores no grupo de participantes de menor nível econômico ($p = 0{,}046$). Além disso, a pontuação de depressão foi significativamente maior em mulheres que perderam sua renda durante a pandemia ($p = 0{,}027$). A pontuação na ASEX foi significativamente maior, e a disfunção sexual foi mais prevalente em pessoas com menores escolaridade e nível de renda ($p < 0{,}05$). Da mesma forma, as pontuações na ASEX foram significativamente mais altas ($p = 0{,}019$) no grupo que experimentou maior perda de renda durante a pandemia. Ao comparar os grupos de gestantes e não gestantes, detectou-se que a disfunção sexual apresentava índice significativamente maior entre as gestantes ($p < 0{,}001$).

Conclusão Em tempos de crise global, como a atual pandemia, famílias de baixa renda têm um risco maior de sofrer depressão e disfunção sexual. Quando comparamos mulheres grávidas e mulheres não grávidas, as pontuações de depressão foram semelhantes, mas as mulheres grávidas apresentaram um risco 6,2 vezes maior de desenvolver disfunção sexual.

Palavras-chave

- Covid-19
- pandemia
- grávida
- depressão
- função sexual

Introduction

Caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), coronavirus disease 2019 (COVID-19) began with the first suspicious cases in November 2019 in Wuhan, Hubei, China, in late December. After that, the disease shortly spread throughout the world, in about three months. It was declared a pandemic by the World Health Organization (WHO) on March 12, 2020, and the first case in Turkey was reported on March 11, 2020.[1,2] The entire world is facing an unprecedented crisis due to the rapidness, depth, and scope of the pandemic. The physical, mental, and social well-being of people is being adversely affected throughout the pandemic, and the national and international measures taken against it have directly or indirectly affected people's economic well-being. Movement restrictions, one of the measures to slow down the spread of the pandemic, negatively affected the supply of labor. As has been the case in the global market, Turkey has also experienced an increase in the unemployment rate and decreased incomes.[3] A decrease in people's economic welfare levels in society can increase the rates of depression.[4]

Previous studies[5,6] have revealed that the tendency to develop depression among females is higher than among males. Some studies[6,7] performed during the pandemic found that females were affected more adversely compared with the males. The mother might experience emotional fluctuations during pregnancy due to the anxiety she feels for both herself and the fetus.[8] The physiological and psychosocial changes that occur during pregnancy increase the tendency of developing depression and anxiety. In this regard, pregnant and new mothers are included in the vulnerable and high-risk group for depression.[9,10]

The female sexual response cycle can be divided into four phases: baseline, arousal (excitement), orgasm, and resolution. Regarding these phases, women might experience various sexual dysfunctions, such as lack of sexual drive and arousal, and inability to orgasm during sexual activity. It is an underestimated problem with a general prevalence between 20% and 50%.[11] On the other hand, it has been demonstrated that a significant decrease in sexual activities occurs with pregnancy. Physical discomfort, fear of injuring the fetus, loss of sexual drive, physical awkwardness, painful sexual activity, and perceived lack of attraction are among the reported causes of this decrease in sexual activity during pregnancy.[12]

The present research aimed to investigate the depression and sexual functions of pregnant and non-pregnant women throughout the COVID-19 pandemic.

Methods

The present is a cross-sectional trial conducted from May to August, 2020 with 96 healthy pregnant and 92 nonpregnant women, totalling a sample of 188 women. Most of the pregnant and non-pregnant women whom we invited to the study did not want to participate; they wanted to be in the hospital for a shorter time because they were afraid of

contact transmission by the doctor. In total, 188 healthy women aged between 18 and 45 years who sought care at the Gynecology and Obstetrics Outpatient Clinic of a hospital in Turkey, and who did not have any known disorder, had not received medication for chronic conditions, and voluntarily agreed to participate in our study were included. Pregnant women who had fetal or maternal problems during their pregnancy, women who had a medical history of psychiatric or sexual dysfunction, and participants who submitted incomplete questionnaires were excluded from the study. The participants read and signed the informed consent form; after that, we collected sociodemographic data from them, and applied the Beck Depression Inventory (BDI) and Arizona Sexual Experience Scale (ASEX).

Approval of the Ethics Committee was obtained (2020/514/176/9), with the project name of 2020–05–07T14_43_09, from the Ministry of Health of the Republic of Turkey to conduct the relevant scientific research.

Sociodemographic data, such as age, level of schooling, occupational status, and obstetric status was obtained from the patients. They were asked whether they were pregnant or not, and, if they were, their gestational week was requested, and they were divided into three groups based on the trimester: first trimester (up until the 14th gestational week), second trimester (14th to 28th gestational weeks), and third trimester (28th to 42th gestational weeks) groups. Based on data from May 2020 from the Turkish Statistical Institute, those with a total family monthly income below the hunger limit (2,438 Turkish Lira [TL]) are considered the lower-income class, those below the poverty line (7,942 TL), the middle-income class, and those with a higher income ($>$ 7,942 TL), the upper-income class. Regarding loss of income during the pandemic, the participants were examined in 3 groups:, those who had no income loss, those who lost up to 50% of their income, and those who lost more than 50% of their income.

The BDI was developed to measure the risk of depression, the level of the depressive symptoms, and the fluctuation in the severity of depression among adults. It is a self-administered questionnaire developed by Beck et al.[13], which consists of 21 items. The items of the scale are scored from 0 and 3 points. Higher scores indicate greater symptom severity. In those diagnosed with depression, scores of between 0 and 13 indicate minimal depression, from 14 to 19, mild depression, from 20 to 28, moderate depression and, from 29 to 63, severe depression.[14] The validity and reliability study of the Turkish adaptation of the questionnaire was performed by Hisli.[15]

The Arizona Sexual Experience Scale (ASEX), which has been developed by McGahuey et al.[16] to briefly and efficiently screen and identify the problems experienced by patients in their sexual life, is a five-item rating scale that measures sexual drive, arousal, vaginal lubrication/penile erection, ability to have an orgasm, and satisfaction from orgasm; and it also has separate questionnaires specific to male and female subjects. We used the female form (questionnaire detailed to female patients) in the present study. The total score ranges from 5 to 30, and lower scores manifest that the sexual response is strong, easy, and satisfying, whereas higher scores display sexual dysfunction. The higher the scores, the more sexual dysfunction there is.[16] Based on the ASEX scores, the participants were included in the healthy group (scores from 0 to 10), the group with moderate sexual disorder (scores from 11 to 17), and the group with severe sexual disorder (scores $\geq$ 18). The validity and reliability of the Turkish adaptation of the ASEX have been tested among patients with renal failure.[17]

For the categorical values, *p*-values were calculated using the Chi-squared test. For the continuous variables, the *p*-values were calculated using the Mann Whitney U test the and Kruskall Wallis test. Multivariable logistic regressions were used to calculate odds ratios (ORs) and 95% confidence intervals (95%CIs) for the associations between sexual dysfunction and depressive symptoms. Potential a priori confounders (such as, age, occupational status, and parity) were included in the models. All statistical analyses were performed using The Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, United States) software, version 25.0.

Results

The mean age of the sample was 30.1 ± 6.4 years. The demographics of the participants are presented in ►**Table 1**.

In the univariate analysis of the factors affecting the presence of depression among the participants, severe depression was significantly more prevalent among those who were unemployed compared with the other groups. Besides, as the level of income decreases, the percentage of mild or moderate depression increases significantly ($p = 0.046$) (►**Table 2**).

In the univariate analysis of the BDI scores of the participants, they were significantly higher among those who were unemployed (mean = 13.8) compared with those working in the public sector (mean = 9.5) ($p < 0.001$). We also found that, as the level of economic income decreases, depression scores increase significantly ($p = 0.009$). The BDI score (mean = 13.9) was significantly higher among the participants who lost their income during the pandemic compared with those who did not experienced income loss (mean = 11.4) ($p = 0.001$). Furthermore, the BDI scores (mean = 14.0) of the patients whose relatives were diagnosed with COVID-19 was significantly higher than the scores of those whose relatives were not diagnosed ($p = 0.013$) (►**Table 3**).

We found that the rate of severe sexual disorder among pregnant women was significantly higher than that of the non-pregnant women ($p = 0.006$). On the other hand, the percentage of moderate sexual dysfunction was significantly higher among multiparous women ($p = 0.016$). When the participants were categorized based on their depression status, moderate sexual dysfunction was determined to be high in patients with mild depressive symptoms, whereas the rate of severe sexual dysfunction was found to be high in those with severe depressive symptoms ($p = 0.008$) (►**Table 4**).

According to the univariate analysis, ASEX score decreases significantly as the level of schooling increases.

Table 1 Demographics of the study participants

Age (years)		Mean (standard deviation): 30.1 (6.4)	Median (range): 29.0 (18.0–45.0)
		n	%
Level of schooling	Primary school graduate	23	12.2%
	High school graduate	68	36.2%
	University graduate or higher	97	51.6%
Occupational status	Unemployed	100	53.2%
	Working in the public sector	57	30.3%
	Working in the private sector	31	16.5%
Level of income	Low	17	9.0%
	Medium	110	58.5%
	High	61	32.4%
Pregnancy	Absent	92	48.9%
	Present	96	51.1%
Trimester of pregnancy	First	20	20.8%
	Second	58	60.4%
	Third	18	18.8%
Parity	Nulliparous	70	37.2%
	Multiparous	118	62.8%
Type of delivery	Nulliparous	70	37.2%
	Cesarean	69	36.7%
	Vaginal	49	26.1%
Total		188	100.0%

Moreover, the ASEX score was higher among those who were unemployed. As the level of economic income decreases, the ASEX score increases significantly. We found that as the loss of income rose during the COVID-19 pandemic, the ASEX score also rose significantly. In addition to that, as depressive symptoms increased, the ASEX score increased as well ($p = 0.005$) (►**Table 5**).

When the factors affecting sexual dysfunction in women are analyzed by the multivariate analysis, odds ratio increase was found 1.18 times (95% CI 1.07–1.31), corresponding to each unit increase of age. Compared with those without depressive symptoms, the risk of sexual dysfunction in mildly-depressed patients increased 4.31 times (95%CI: 1.57 to 11.82), while this rate was of 5.57 (95%CI: 1.42 to 21.81) among participants with moderate/severe depression. The risk of sexual dysfunction increased 6.40 times (95%CI: 2.09 to 19.54) among unemployed patients compared with those employed in the private sector. Moreover, there was an increase of 6.20 in the OR (95% CI 2.15–17.90) among pregnant women compared with non-pregnant women (►**Table 6**).

When the correlation analysis was performed between the ASEX and BDI scores regarding the pregnancy status, a slightly positive significant correlation was found among pregnant women ($r = 0.365$; $p < 0.001$).

Discussion

The expectant mother may experience emotional fluctuations throughout pregnancy because she is concerned about herself and the fetus.[18] Depression, which may be experienced during pregnancy, may lead to maternal and fetal complications.[8] The stress experienced throughout the pregnancy might trigger preterm labor through the activation of the pituitary and adrenal axis, and is also a risk factor for gestational hypertension and preeclampsia.[8,19] Previous researches have reported that the prevalence of depression during pregnancy ranges from 12% to 36%.[20] In the present study, we found that 42.7% of pregnant women had mild symptoms of depression throughout the pandemic, whereas 21% had moderetely-severe symptoms of depression. Similarly, depression rates in non-pregnant women were found to be significantly higher than studies conducted before the pandemic. In studies of depression in female university students in Turkey prevalence of ranges from 9.2% to 35.2%, in other countries between 18.5% and 52.6% (14a–14c).[6,21,22] In the present study, we found that 44.6% of non-pregnant women had mild symptoms of depression throughout the pandemic, whereas 22% had moderate/severe symptoms. A study[23] performed in Turkey during the pandemic reported that it has a considerable impact

Table 2 Factors impacting depression among the study participants

		Healthy		Mildly depressed		Moderately/ severely depressed		
		n	%	*n*	%	*n*	%	*p*-value
Level of schooling	Primary school graduate	5	21.7%	8	34.8%	10	43.5%	0.115
	High school graduate	21	30.9%	33	48.5%	14	20.6%	
	University graduate or higher	37	38.1%	41	42.3%	19	19.6%	
Occupational status	Unemployed	25	25.0%	45	45.0%	30	30.0%	**0.003**
	Working in the public sector	30	52.6%	20	35.1%	7	12.3%	
	Working in the private sector	8	25.8%	17	54.8%	6	19.4%	
Level of income	Low	3	17.6%	7	41.2%	7	41.2%	**0.046**
	Medium	34	30.9%	47	42.7%	29	26.4%	
	High	26	42.6%	28	45.9%	7	11.5%	
Loss of income during the pandemic	No	46	40.7%	46	40.7%	21	18.6%	0.124
	$\leq$ 50%	9	22.5%	19	47.5%	12	30.0%	
	$>$ 50%	8	22.9%	17	48.6%	10	28.6%	
Pregnancy	Absent	29	31.5%	41	44.6%	22	23.9%	0.846
	Present	34	35.4%	41	42.7%	21	21.9%	
Trimester of pregnancy	First	10	50.0%	7	35.0%	3	15.0%	0.097
	Second	22	37.9%	25	43.1%	11	19.0%	
	Third	2	11.1%	9	50.0%	7	38.9%	
Parity	Nulliparous	24	34.3%	31	44.3%	15	21.4%	0.936
	Multiparous	39	33.1%	51	43.2%	28	23.7%	
Type of delivery	Cesarean	24	34.8%	29	42.0%	16	23.2%	0.986
	Vaginal	15	30.6%	22	44.9%	12	24.5%	
Relative infected with COVID-19	No	56	36.8%	65	42.8%	31	20.4%	0.087
	Yes	7	19.4%	17	47.2%	12	33.3%	

on the levels of depression and anxiety of pregnant women. In a multi-center cross-sectional study[24] performed in China comparing the mental state of pregnant women before and after the declaration of the COVID-19 pandemic, the authors reported that pregnant women who were examined after the announcement of the COVID-19 outbreak had significantly higher levels of anxiety and symptoms of depression.

Previous studies[25,26] have suggested that individuals who are in the lower socioeconomic class have a higher rate of mental disorders such as depression compared with individuals in middle and upper socioeconomic classes. Upon reviewing the literature, we could not detect one study that revealed a correlation between depression and parameters such as economic status and income loss during the COVID-19 pandemic. In the present study, the depression scores were significantly higher among the group with lower economic status. Likewise, the BDI score was significantly higher among patients who lost their income during the pandemic. The depression scores were significantly higher among females whose relatives have been infected with COVID-19.

The present study has demonstrated that the depression scores increased among women during the pandemic, which is in line with reports in the literature.[15,16] In the present study, we found that pregnant and non-pregnant women are adversely affected in a similar manner; on the other hand, the study suggests that circumstances such as being in the low-income class, experiencing loss of income, and having relatives infected with COVID-19 may increase the risk of developing depression.

Sexual life is one of the factors that affect the general health status and quality of life of women. Sexual dysfunction is a multifactorial problem influenced by various biological, psychological, and environmental factors.[27] It has been suggested[28] that sexual dysfunction is a common problem that increases with age and impacts 30% to 50% of women. Women who have an ASEX score $\geq$ 11 are considered to have sexual dysfunction. In a study[29] comparing pregnant and non-pregnant women in Turkey before the pandemic, the authors found high ASEX scores in 55.6% of pregnant women and in 23.2% of non-pregnant women. In the present study the rate of sexual dysfunction

Table 3 Factors impacting the score of Beck Depression Inventory

		Mean	standard deviation	Median	First quartile	Third quartile	*p*-value
Level of schooling	Primary school graduate	15.4	7.8	14.0	10.0	19.0	0.080
	High school graduate	12.3	5.4	12.0	8.0	16.0	
	University graduate or higher	11.6	6.2	11.0	8.0	15.0	
Occupational status	Unemployed	13.8	6.2	13.0	9.5	18.0	< 0.001
	Working in the public sector	9.5	6.0	9.0	5.0	12.0	
	Working in the private sector	12.9	5.2	13.0	8.0	16.0	
Level of income	Low	15.3	4.8	17.0	13.0	18.0	0.009
	Medium	12.7	6.6	12.0	8.0	17.0	
	High	10.9	5.6	11.0	7.0	14.0	
Loss of income during the pandemic	No	11.4	6.4	11.0	7.0	14.0	0.001
	Yes	13.9	5.8	14.0	10.0	18.0	
Pregnancy	Absent	12.5	6.2	12.0	8.0	17.0	0.389
	Present	12.1	6.3	11.0	7.5	16.0	
Trimester of pregnancy	First	11.1	6.4	10.0	7.0	15.0	0.081
	Second	11.9	6.7	10.0	7.0	14.0	
	Third	14.1	4.7	14.5	11.0	17.0	
Parity	Nulliparous	12.4	6.3	11.0	8.0	14.0	0.654
	Multiparous	12.3	6.2	12.0	8.0	17.0	
Type of delivery	Cesarean	12.0	5.8	13.0	8.0	16.0	0.888
	Vaginal	12.7	6.8	12.0	8.0	18.0	
Relative infected with COVID-19	No	11.9	6.4	11.0	8.0	16.0	0.013
	Yes	14.0	5.3	15.0	11.5	18.0	

during the pandemic was of 83.3% among pregnant women, and of 75% among non-pregnant women. This situation indicated that the sexual lives of all women were negatively affected throughout the pandemic. A significantly higher rate of sexual dysfunction was also detected among pregnant women in the present study, and the OR was 6.2, which is in line with previous studies.[29,30]

It is well documented that the level of socioeconomic well-being impacts sexuality. Previous studies[31,32] have reported that sexual dysfunction was more prevalent among women with a lower economic status. Similarly, in the present study, sexual dysfunction was significantly higher among women in the lower-income class and among those who lost their income during the pandemic. The risk of sexual dysfunction among unemployed women is 6.40 times higher than that of employed women. One study[33] demonstrated that sexual dysfunction is less common among women with higher levels of schooling. Likewise, the results of the present study support the aforementioned finding as well the fact that, as the level of schooling increases, the prevalence of sexual dysfunction decreases significantly.

Previous studies[34–37] have suggested that the previous number of births and the type of delivery do not impact the sexual function. In the present study, similar results were found when the ASEX scores were analyzed.

It has been claimed[38] that people under restriction have a high tendency to develop depression and anxiety, and that sexual intercourse while under restriction is a protective factor against depression. One study[39] found that several women who were using short-acting reversible contraception (SARC) discontinued their contraceptive method during the pandemic but continued to engage in sexual activity and had unplanned pregnancies. The BDI and ASEX scores were also observed in parallel in the present study (►**Tables 3**,**4**), and The BDI scores were significantly higher among women with sexual dysfunction.

This study has some limitations; Firstly, it is a cross-sectional trial and the long-term effects are unknown. Secondly, the study compared slightly different women in the same period, not different periods when the same women were pregnant and not pregnant. Finally, the negative impact of the pandemic detected among the participants may have been milder than the impact felt by women in other parts of Turkey, since the district where the present study was performed was considered a low-risk area for infection by SARS-CoV2.

Table 4 Factors impacting sexual dysfunction

		Normal		Moderate sexual dysfunction		Severe sexual dysfunction		
		n	%	*n*	%	*n*	%	*p*-value
Level of schooling	Primary school graduate	1	4.3%	14	60.9%	8	34.8%	0.145
	High school graduate	14	20.6%	40	58.8%	14	20.6%	
	University graduate or higher	24	24.7%	57	58.8%	16	16.5%	
Occupational status	Unemployed	14	14.0%	62	62.0%	24	24.0%	0.088
	Working in the public sector	14	24.6%	34	59.6%	9	15.8%	
	Working in the private sector	11	35.5%	15	48.4%	5	16.1%	
Level of income	Low	0	0.0%	12	70.6%	5	29.4%	0.101
	Medium	21	19.1%	66	60.0%	23	20.9%	
	High	18	29.5%	33	54.1%	10	16.4%	
Loss of income during the pandemic	No	29	25.7%	63	55.8%	21	18.6%	0.123
	Yes	10	13.3%	48	64.0%	17	22.7%	
Pregnancy	Absent	23	25.0%	59	64.1%	10	10.9%	**0.006**
	Present	16	16.7%	52	54.2%	28	29.2%	
Trimester of pregnancy	First	4	20.0%	12	60.0%	4	20.0%	0.579
	Second	11	19.0%	29	50.0%	18	31.0%	
	Third	1	5.6%	11	61.1%	6	33.3%	
Parity	Nulliparous	22	31.4%	34	48.6%	14	20.0%	**0.016**
	Multiparous	17	14.4%	77	65.3%	24	20.3%	
Type of delivery	Cesarean	14	20.3%	43	62.3%	12	17.4%	**0.018**
	Vaginal	3	6.1%	34	69.4%	12	24.5%	
Relative infected with COVID-19	No	32	21.1%	88	57.9%	32	21.1%	0.783
	Yes	7	19.4%	23	63.9%	6	16.7%	
Depression	Normal	20	31.7%	29	46.0%	14	22.2%	**0.008**
	Mildly depressed	15	18.3%	56	68.3%	11	13.4%	
	Moderately/severely depressed	4	9.3%	26	60.5%	13	30.2%	

Table 5 Factors impacting the Arizona Sexual Experience Scale

		Arizona Sexual Experience Scale					
		Mean	Standard deviation	Median	First quartile	Third quartile	*p*-value
Level of schooling	Primary school graduate	16.2	3.3	15.0	14.0	19.0	0.034
	High school graduate	14.4	4.1	14.0	12.0	17.0	
	University graduate or higher	13.8	3.6	14.0	11.0	17.0	
Occupational status	Unemployed	14.9	3.6	15.0	13.0	17.0	0.032
	Working in the public sector	13.8	3.9	13.0	11.0	15.0	
	Working in the private sector	13.4	4.1	14.0	10.0	16.0	
Level of income	Low	15.9	2.6	16.0	15.0	18.0	0.040
	Medium	14.4	3.9	14.0	12.0	17.0	
	High	13.7	3.9	14.0	10.0	15.0	
Loss of income during pandemic	No	13.8	3.8	14.0	10.0	16.0	0.019
	$\leq$ 50%	14.6	4.1	15.0	13.0	16.0	
	$>$ 50%	15.7	3.3	16.0	13.0	18.0	

(*Continued*)

Table 5 (*Continued*)

		Arizona Sexual Experience Scale					
		Mean	Standard deviation	Median	First quartile	Third quartile	*p*-value
Pregnancy	Absent	13.3	3.3	13.0	10.5	15.0	< 0.001
	Present	15.2	4.0	15.0	13.0	18.0	
Trimester of pregnancy	First	14.6	3.8	15.0	13.0	17.0	0.260
	Second	15.1	4.4	15.0	12.0	18.0	
	Third	16.4	2.7	17.0	15.0	19.0	
Parity	Nulliparous	13.8	4.1	14.0	10.0	17.0	0.064
	Multiparous	14.6	3.6	15.0	13.0	17.0	
Type of delivery	Nulliparous	13.8	4.1	14.0	10.0	17.0	0.098
	Cesarean	14.3	4.0	15.0	12.0	17.0	
	Vaginal	15.0	3.1	15.0	13.0	17.0	
Relative infected with COVID-19	No	14.3	3.8	14.0	12.0	17.0	0.888
	Yes	14.2	3.9	14.0	12.0	16.0	
Depression	Normal	13.7	4.4	14.0	10.0	17.0	0.005
	Mildly depressed	14.0	3.1	14.0	12.0	15.0	
	Moderately/severely depressed	15.8	3.8	16.0	14.0	18.0	

Table 6 Multivariate analysis of the factors impacting sexual dysfunction

	B	SE	Wald test	*p*-value	Odds Ratio	95% confidence interval	
						Lower	Upper
Age (years)	0.173	0.051	11.598	0.001	1.189	1.076	1.313
Healthy			10.153	0.006			
Mildly depressed	1.462	0.514	8.084	0.004	4.317	1.575	11.829
Moderately/severely depressed	1.718	0.696	6.089	0.014	5.573	1.424	21.812
Working in the private sector			10.696	0.005			
Unemployed	1.857	0.569	10.634	0.001	6.402	2.098	19.543
Working in the public sector	1.217	0.635	3.670	0.055	3.377	.972	11.731
Pregnant	1.826	0.540	11.421	0.001	6.209	2.153	17.905
Multiparous	0.267	0.539	0.245	0.620	1.306	0.454	3.758
Loss of income	1.040	0.545	3.647	0.056	2.830	0.973	8.230

B = value; SE = standard error.

Conclusion

In the present study, women who lost their income during the pandemic had higher rates of depression and sexual dysfunction. Upon comparing the pregnant and nonpregnant women, we found that the rates of depression were similarly higher in both groups than the before pandemic, whereas the rate of sexual dysfunction was 6.2 times (95%CI: 2.1 to 17.9) higher among pregnant women. Low-income families and women have an increased risk of experiencing depression and sexual dysfunction in times of global crises, such as a pandemic.

Contributions
All authors participated in the concept and design of the present study; in the analysis and interpretation of data; in the draft or revision of the manuscript; and they have approved the manuscript as submitted. All authors are responsible for the reported research.

Ethical considerations

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of Interests

The authors have no conflict of interests to declare.

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Association of the Maternal Folic Acid Supplementation with the Autism Spectrum Disorder: A Systematic Review

Associação da suplementação de ácido fólico materno com o transtorno do espectro do autismo: uma revisão sistemática

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Rev Bras Ginecol Obstet 2021;43(10):775–781.

Abstract

Objective To analyze the scientific production regarding maternal folic acid (FA) supplementation and its relationship with autistic spectrum disorder (ASD).

Data Sources We performed unrestricted electronic searches in the BIREME virtual bank, Virtual Health Library (VHL) and Medical Literature Analysis and Retrieval System Online (MEDLINE/PubMed) databases.

Selection of Studies For sample selection, articles that met the proposed objectives were included, published in English, Spanish and Portuguese, the use of Health Sciences Descriptors (DeCS): *autistic* OR *autism* AND *autism spectrum disorder* AND *folic acid*, AND, with the use of the Medical Subject Headings (MeSH): autistic OR *autism* AND *autistic spectrum disorder* AND *folic acid*.

Data Collection Data extraction was performed by the reviewers with a preestablished data collection formulary.

Data Synthesis The Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) was used based on a checklist with 27 items and a 4-step flowchart.

Keywords
- autistic spectrum disorder
- folic acid
- pregnancy
- supplementation

received
October 17, 2020
accepted
July 7, 2021

DOI https://doi.org/10.1055/s-0041-1736298.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Results A total of 384 articles was found by the search strategies, of which 17 were eligible following the pre-established criteria. The main findings of the present review point to maternal FA supplementation in the pre-conception period and beginning of pregnancy as a protective effect in relation to ASD, which should be indicated in this period as prevention to the problem.

Conclusion According to the research analyzed, more studies are necessary to know its effects on pregnancy, since the consumption of excessive FA may not be innocuous.

Resumo

Objetivo Analisar a produção científica a respeito da suplementação de ácido fólico (AF) materno e sua relação com o transtorno do espectro autista (TEA).

Fontes de Dados Realizamos buscas eletrônicas irrestritas nas bases de dados do banco virtual BIREME, Biblioteca Virtual em Saúde (VHL) e Medical Literature Analysis and Retrieval System Online (MEDLINE / PubMed).

Seleção dos Estudos Incluímos os artigos publicados em inglês, espanhol e português, com o uso dos DeCS: *autistic* OR *autism* AND *autism spectrum disorder* AND *folic acid*, e com o uso dos Medical Subject Headings (MeSH, na sigla em inglês): autistic OR autism AND Autistic Spectrum Disorder AND folic acid ".

Coleta de Dados A extração de dados foi realizada pelos revisores com um formulário de coleta de dados pré-estabelecido.

Síntese dos Dados Foram usados os itens de relatório preferidos para protocolos de revisão sistemática e meta-análise (PRISMA-P) com base em uma lista de verificação com 27 itens e um fluxograma de 4 etapas.

Resultados Foram encontrados 384 artigos pelas estratégias de busca, dos quais 17 eram elegíveis segundo os critérios pré-estabelecidos. Os principais achados da presente revisão apontam para a suplementação de AF materno no período de preconcepção e início da gravidez como efeito protetor em relação ao TEA, que deve ser indicada neste período como prevenção do problema.

Conclusão De acordo com as pesquisas analisadas, mais estudos são necessários para conhecer seus efeitos sobre a gravidez, uma vez que o consumo excessivo de AF pode não ser inócuo.

Palavras-chave
- transtorno do espectro autista
- ácido fólico
- gravidez
- suplementação

Introduction

Autism Spectrum Disorder (ASD) is characterized by persistent deficits in social interaction and communication, with the presence of repetitive interests and activities. Considered as a neurodevelopmental disorder, it can manifest with extremely variable phenotypes, from severely compromised individuals to independent individuals.[1,2]

The World Health Organization estimates that 1 in 160 children has ASD. The use of preconception folic acid (FA) should be indicated at least 2 months before conception and in the 1st 2 months of pregnancy, as it has a protective effect against open defects of the neural tube.[3]

Since 2004, in Brazil, the Ministry of Health, through the National Health Surveillance Agency (ANVISA, in the Portuguese acronym), adopted the Collegiate Directorate Resolution (RDC, in the Portuguese acronym) No. 344 of December 13, 2002, establishing mandatory FA fortification in wheat flour and corn to reduce the prevalence of maternal anemia and defects of the neural tube. This fortification of synthetic FA may have generated a population group with high serum levels of nonmetabolized FA. This finding occurs when $>$ 200mg/day is ingested.[4]

The theme has great importance in the social context because it involves the patient, their family, the state, and the multiprofessional action, which makes its study fundamental to guarantee the practice of evidence-based health care. The objective of the present review is to describe the relationship between maternal FA supplementation and ASD, according to scientific publications.

Methods

This is a qualitative exploratory study, of the metasynthesis type. The search for data was performed between February 2018 and February 2020, based on the BIREME virtual bank, the Virtual Health Library (VHL), and the Medical Literature Analysis and Retrieval System Online (MEDLINE/PubMed) databases.

The inclusion criteria were: original articles, systematic reviews, available in full, free of charge, studies with human beings, published in English, Spanish, and Portuguese,

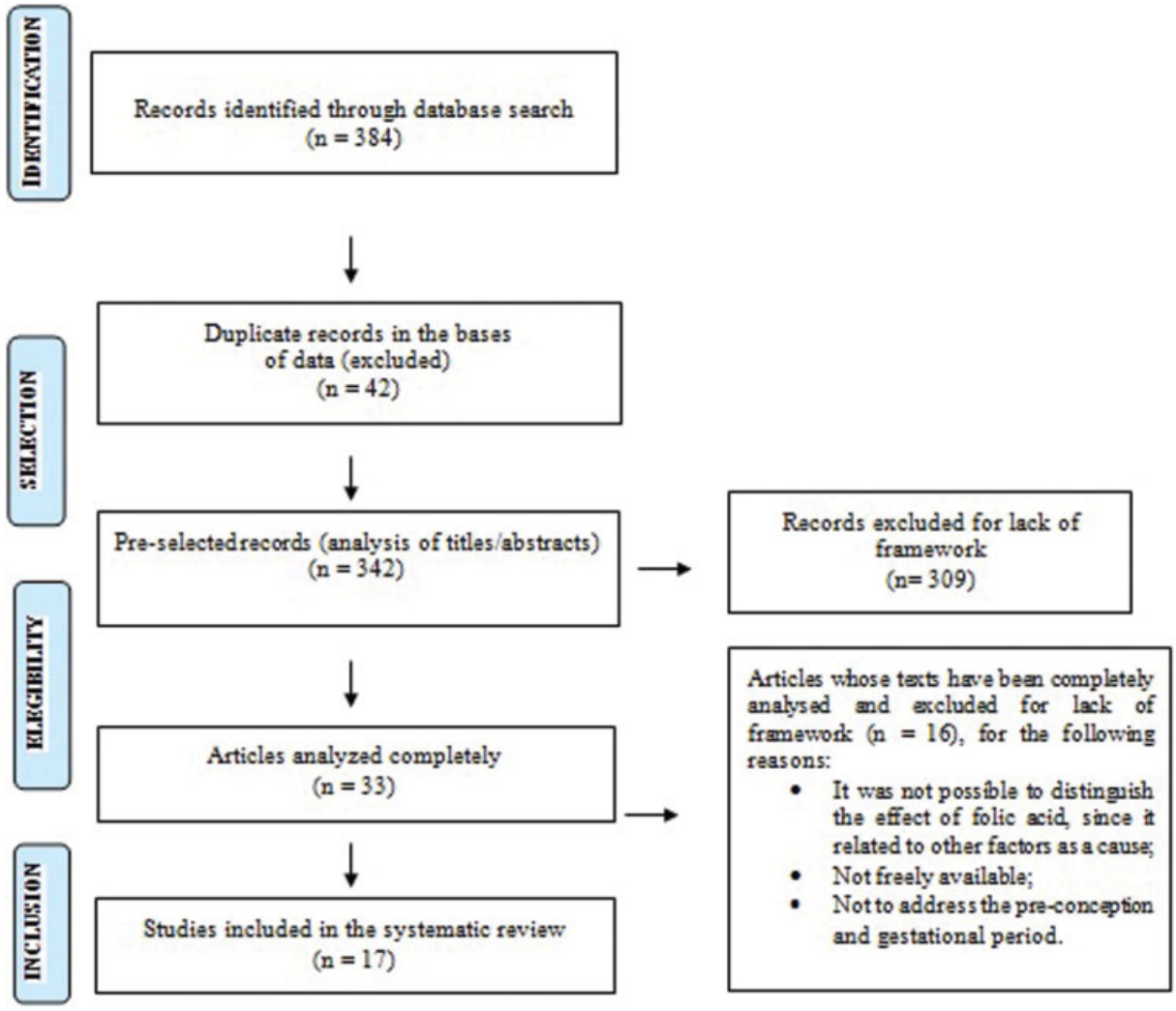

Fig. 1 Flowchart of eligible items. Source: Flowchart prepared according to PRISMA recommendations.

which addressed the use of FA related to the occurrence of ASD.

The exclusion criteria used in this study start with free availability, that is, studies published on non-free platforms were excluded from this research. The year of publication was also one of the criteria used in this review, these were strictly maintained between the years 2013 to 2020, another criterion used was the thematic, to exclude all works in which their title and later their summary dealt with a different theme of the association between maternal FA supplementation and ASD.

Studies that depict ASD associated with other drugs or other factors that do not correspond to consumption of supplemental FA were excluded from the study, or those that deal with the consumption of FA without associating it with ASD. The period in which FA is used was also one of the criteria used in the present review, and all those dealing with the consumption of FA at different periods of the periconceptional and gestational period were excluded. Studies with animals and those that portrayed other subjects outside the area of interest of the present research were excluded.

The operationalization of the search for data collection was performed in the BIREME database using Health Science Descriptors (DeCS) linked to Boolean operators and using quotation marks in compound words, (*autistic* OR *autism*) AND ("*autism spectrum disorder*") AND ("*folic acid*"). In the PubMed database, the search was performed in English using Medical Subject Headings (MeSH) linked to Boolean operators and quotes in compound words, (*autistic* OR *autism*) AND ("*autistic spectrum disorder*") AND ("*folic acid*").

The Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) was used based on a checklist with 27 items and a 4-step flowchart.[5] In the present review, the acronym PICO (Patients; Intervention; Comparison; Outcome) was also used to construct the guiding question of the study and to perform the bibliographic search. For the presentation of eligibility criteria, the PRISMA and the table containing information such as authors, year of publication, place and results were used (►**Fig. 1**).

Results

According to the search strategy adopted, 220 articles were found at BIREME and 164 at PubMed, totaling 384 selected

Table 1 Characteristics of the articles that composed the body of analysis of the study according to authors, year, place and main results

Authors	Year	Place	Main results
Steenweg-de-Graaff et al.[6]	2015	Rotterdam, Netherlands.	The concentration of folic acid at the beginning of pregnancy does not show a direct relationship with autism in the offspring. However, it shows a decrease in traces of childhood autism, related to early folic acid supplementation.
Neggers[14]	2014	Alabama, USA.	Autism may be related to the potential association between DNA methylation by high folic acid intake, food fortification with folic acid, and it may also be pointed out as a protective factor for autism. Therefore, the cause of autism could not be affirmed, only possible relations with the problem, being necessary further studies in this respect.
Braun et al.[7]	2014	Cincinnati, Ohio, USA	Among the mother-son pairs who used multivitamins with folic acid in the 2nd trimester of pregnancy, it was shown to have a beneficial effect in reducing autistic characteristics. When the concentrations of folate in maternal whole blood alone were seen, a protective relationship against the disorder was not observed.
Berry et al.[15]	2013	Atlanta, Georgia, USA.	The increasing number of ASD cases, together with the fortification of food folic acid, leads to some aspects of ASD. It may be protective in relation to folic acid supplementation and ASD or it may have an adverse effect of this excessive supplementation, increasing the risk for the problem. As its role is not yet clear regarding its relationship with ASD, future studies are needed.
Schmidt[8]	2013	California, USA.	The prevalence of ASD decreases as preconception folic acid is used and in early pregnancy. A 40% decrease in the risk of developing the disorder is observed.
Gao et al.[21]	2016	Columbia, USA.	Although studies have been observed that indicate a relationship between folic acid supplementation and autism, most of the articles studied in this review suggest that folic acid supplementation in the pregnancy has a protective effect against ASD.
Raghavan et al.[18]	2018	Boston, USA.	Based on folate measured in the maternal blood plasma, there is a relationship between increased and decreased folic acid consumption with an increased risk of ASD. However, moderate intake of multivitamins indicates a decreased risk for ASD.
Strøm et al.[22]	2018	Denmark	The supplementary consumption of preconception folic acid and at the beginning of pregnancy did not present ASD as an outcome. A detailed examination of genetic factors and biomarkers of exposure is necessary.
Wang et al.[9]	2017	China	Maternal folic acid supplementation demonstrates protective action against ASD. The consumption of folic acid could allow a significant reduction in the risk of the disorder when compared with those who did not perform the supplementation.
Rangel-Rivera et al.[16]	2015	Colombia	Folic acid remains a major ally against structural defects of the central nervous system. There are aspects that affirm its protective effect in relation to ASD, but this study is inconclusive to affirm its protection.
Wiens et al.[13]	2017	Iowa, USA.	Excessive consumption of folic acid proves not to be innocuous, high levels of nonmetabolized folic acid denote a negative effect on neurological development, being consequently related to disorders such as ASD.
Moussa et al.[10]	2016	Houston, Texas, USA.	Preconception and early pregnancy folic acid consumption show a positive effect in reducing the risk of ASD, but its effects are not known if used during the entire pregnancy.
Neggers[17]	2014	Alabama, USA.	Fortification of folic acid associated with preconception folic acid supplementation has shown a close relationship with the increase in the number of ASD cases. Further studies in this area are needed to reach a conclusion.
DeVilbiss et al.[19]	2015	Philadephia, USA.	Folic acid consumption has shown a relationship with ASD, and this relationship is both with risk, related to high consumption, and with insufficient intake, given its necessity in neurodevelopment. The dosage of folic acid, vitamin B12 and homocysteine is necessary for a more complete and conclusive evaluation of this relationship.
Surén et al.[11]	2013	Norway	Maternal folic acid supplementation between the 4th week and before the 8th week after the beginning of pregnancy is associated with a lower risk of ASD. However, the inverse association observed represents a causal relationship, indicating that folate deficiency around conception and early pregnancy, or reduced capacity to use available folate, are important causes of ASD.

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Table 1 (*Continued*)

Authors	Year	Place	Main results
Tan et al.[12]	2019	China	The study demonstrates that maternal use of folic acid and/or micronutrients during pregnancy offer reduced the risk of autistic characteristics in the offspring. Observing characteristic signs regarding social cognition, media, autism behavioral mannerisms, adaptive behavior, coarse motor behavior and problems in gastrointestinal tract of children with ASD. There is also a relationship with the consumption of the micronutrient in pregnancy and vitamin status in children with ASD.
Altamimi[20]	2018	Palestine	Through a systematic review, the relationship of ASD with maternal use of micronutrients was evaluated, and it was observed that folic acid can be related to ASD due to its relationship with glutathione, a product of the methionine cycle that depends on folic acid and B12. This element is involved in neuroprotection against oxidative stress and neuroinflammation in the brain. According to studies, glutathione is deficient in children with autism compared with typically developed children.

Abbreviation: ASD, autism spectrum disorder.
Source: Own elaboration, 2020.

articles, as seen in ►**Fig. 1**, and the studies are presented in tabular form, as seen in ►**Table 1**.

The main result of the present review is that the use of FA in the preconception period and in the beginning of pregnancy is effective in preventing ASD. Therefore, early supplementation with FA is beneficial for neurodevelopment and, consequently, acts on the prevention of ASD. The consumption of additional FA at an early stage should be indicated, because in addition to preventing ~ 70% of neural tube defects cases, it is associated with a protective effect in relation to ASD.

It was also found that excess FA consumption and the gestational period in which it is used can have a negative influence on the occurrence of ASD. Too much intake can be observed, since FA, in addition to being consumed in the usual diet, can also be added in the form of oral supplementation, which, associated with the extended time of use, demonstrates a causative effect for the disorder.

Many studies addressed in the present review bring inconclusive results regarding the effect of FA in relation to ASD, indicating the need for future investigations of maternal FA serum levels and also a detailed investigation regarding its consumption from the 2nd trimester of pregnancy, a period considered a risk for its use, in relation to the disorder. All this is relevant, so that optimal doses are indicated according to individual needs and maternal nutritional deficiency.

Of the 15 studies evaluated, 8 were unable to safely determine the effects caused by FA. The majority found that there is an effect on neurodevelopment and even observed a relationship with ASD, but in a contradictory way, which may be protective or even cause the problem. They also point out the need for further studies and that an evaluation of FA in the blood plasma of the mother should be performed to analyze its possible indication, in optimal dose and time.

Discussion

After the analysis of the eligible articles and following the already defined method, three thematic categories emerged: FA as a protection to ASD, increased risk of ASD in relation to excessive use of FA, and inconclusive association between FA consumption and ASD.

Folic Acid as Protection against Autism Spectrum Disorder

Studies carried with children diagnosed with ASD whose mothers used FA in the 6 weeks before and after conception pointed to the protective effect of preconception FA and the occurrence of ASD.[6–8] Folic acid consumption is related to a decrease in the occurrence of ASD when compared with other mothers who did not have the supplementation.[9,10] Corroborating in the study, the nonuse or deficiency in the folate metabolism in this same period can be pointed out as a cause for the development of autism disorder.[11]

A study was conducted in a Chinese population with a total of 416 children with ASD and 201 children with typical

development (TD). It concluded that children born to mothers without FA and micronutrient supplementation during pregnancy exhibited more severe social cognitive impairment, such as social communication, behavioral mannerisms, delays in developing raw and adaptive motor behavior, and gastrointestinal problems than children born to mothers who used FA and micronutrient supplements, demonstrating the need for micronutrient supplementation during pregnancy and periconception.[12]

In this category, it is clear that FA can be considered a protector for autism, especially when used in the preconception period and at the beginning of pregnancy, since research has shown that it reduces the risk of autism in the offspring. This beneficial effect cannot be confirmed if consumed during the rest of the course of pregnancy. It should be noted that many studies point to FA supplementation as having a beneficial effect in relation to ASD, since 8 of the 15 analyzed studies point to this effect.

Increased Risk of ASD in Relation to Excessive Use of Folic Acid

Studies show that maternal FA supplementation, together with food fortification, has resulted in a population with superior parameters of AF than expected, and with an excess of nonmetabolized folate in the body. This may be associated with an increasing number of ASD cases.[13]

Inconclusive Association between Folic Acid Consumption and Autism Spectrum Disorder

It is still too early to reach a conclusion on the association between FA consumption and ASD. Studies show its protective effect against ASD, but others intrigue us with their investigations of alterations in the metabolic pathways of maternal folate, its potential role in DNA methylation through high maternal FA intake and its relationship with autistic traits. Finding the optimal level of maternal FA intake is difficult, but it may be the answer to these hypotheses.[14]

The beneficial or harmful effect of FA on ASD cannot be stated. This requires intensified studies to discover the relationship of nutrition in different populations and the association with food fortification and additional FA supplementation.[15,16] The increase in the number of ASD cases may be linked to several factors, such as changing diagnostic criteria, increased information, and excess FA consumed by women of childbearing age.[17]

Supplementation with periconceptional FA reduced neural tube defects by up to 70% and resulted in fortification of cereal products in several countries. The recommendation was that all women of childbearing age should consume 400µg/day of FA. It was also observed that the metabolism of maternal folate can vary between women, which may be involved with the increase in the number of cases of ASD.

Based on postpartum maternal blood samples, folate and vitamin B12 were analyzed in mothers who reported multivitamin supplement consumption at least in the 3rd trimester of pregnancy. The diagnosis of ASD was observed through electronic medical records. The results can be understood as a "U" format, where the increased risk for ASD is at both extremes, when the supplement was consumed in excess and when it was consumed in small amounts. In contrast, moderate supplementation showed a protective effect in relation to ASD.[18]

The maternal folate status may be related to ASD. Insufficient FA consumption may lead to hypomethylation of DNA, which is associated with neurodevelopment. It has also been found in some studies that FA consumption can be considered protective for autism.

The author indicates that repeated biological measurements of folate, vitamin B12 and homocysteine during the 1st trimester of pregnancy are necessary, as well as genetic variants relevant to folate involved in carbon metabolism, and the epigenetic mechanisms.[19]

The relationship of ASD with maternal use of micronutrients such as iron, zinc, vitamin D and AF were evaluated, and it was observed that the AF shows contradictory effects in relation to the TEA, which, in some studies, is presented as a protective effect and, in others, as causal effects. Thus, it was not possible to demonstrate whether or not there is a relationship between PA supplementation and ASD. Another finding in relation to ASD involves its relationship with glutathione, a product of the methionine cycle that depends on FA and B12, this element is involved in neuroprotection against oxidative stress and neuroinflammation in the brain. Research shows that glutathione is deficient in children with autism when compared with children with typical development.[20]

Conclusion

The use of FA in the preconception period and in the beginning of pregnancy is effective in preventing ASD. Therefore, early supplementation with FA is beneficial for neurodevelopment and, consequently, acts on the prevention of ASD. The present study deals strictly with the consumption of FA with the development of ASD, and the action of FA in the treatment of other neurological problems has not been reviewed. The study also did not aim to associate the use of FA with other drugs. The present study recommends that new investigations be performed to identify optimal doses and identify whether FA consumption from the 2nd trimester can really be associated with the increase in the number of new cases of ASD as mentioned by some studies in the present review.

Contributors
All the authors participated in the concept and design of the study; in the analysis and interpretation of data; in the draft or revision of the manuscript; and they have approved the manuscript as submitted. All authors are responsible for the reported research.

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

Acknowledgments
We thank all the collaborators in the present study for their commitment and dedication.

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Oral Iron Supplementation in Pregnancy: Current Recommendations and Evidence-Based Medicine

Suplementação oral de ferro na gravidez: recomendações atuais e medicina baseada na evidência

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Rev Bras Ginecol Obstet 2021;43(10):782–788.

Abstract

Objective To review the evidence about universal iron supplementation in pregnancy to prevent maternal anemia.

Methods Bibliographic research of randomized and controlled clinical trials, meta-analyses, systematic reviews, and clinical guidelines, published between August 2009 and August 2019, using the MeSH terms: *iron; therapeutic use*; *pregnancy*; *anemia*, *prevention and control*.

Results We included six clinical guidelines, three meta-analyses and one randomized controlled clinical trial.

Discussion Most articles point to the improvement of hematological parameters and reduction of maternal anemia risk, with supplementary iron. However, they do not correlate this improvement in pregnant women without previous anemia with the eventual improvement of clinical parameters.

Conclusion Universal iron supplementation in pregnancy is controversial, so we attribute a SORT C recommendation strength.

Keywords
- iron
- dietary supplements
- pregnancy
- anemia
- prenatal care

Resumo

Objetivo Rever a evidência sobre a necessidade de suplementação universal de ferro na gravidez para prevenção de anemia materna.

Métodos Pesquisa bibliográfica de ensaios clínicos aleatorizados e controlados, metanálises, revisões sistemáticas e normas de orientação clínica, publicados entre agosto de 2009 e agosto de 2019, utilizando os termos MeSH: *iron, terapêuticas use; pregnancy; anemia, preventivos and control.*

Resultados Incluímos seis normas de orientação clínica, três metanálises e um ensaio clínico randomizado e controlado.

Discussão A maioria dos artigos aponta para a melhoria dos parâmetros hematológicos e redução do risco de anemia materna por meio da suplementação com ferro.

Palavras-chave
- ferro
- suplementação nutricional
- gravidez
- anemia
- cuidado prey-natal

received
October 20, 2020
accepted
August 5, 2021

DOI https://doi.org/10.1055/s-0041-1736144.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Todavia, eles não correlacionam a melhoria destes parâmetros em grávidas sem anemia prévia com a eventual melhoria de parâmetros clínicos.
Conclusões A suplementação universal com ferro na gravidez é controversa, pelo que atribuímos uma força de recomendação SORT C.

Introduction

Gestational anemia is the most common health problem in pregnancy. Its prevalence varies with geographic region, and, according to data form the World Health Organization (WHO), the global prevalence is 38.2%, and ~ 26% in Europe. In developing countries, the prevalence is higher, reaching 48.7% in Southeast Asia and 46.3% in Africa.[1] On the other hand, the estimated prevalence of iron deficit in pregnant women in the USA is 18.6%, and 16.2% of these have anemia.[2] Taking into account the World Development Indicators of the Global Health Observatory, The World Bank, the prevalence of gestational anemia in 2016 in Brazil was 37.3%, and 25% in Portugal.[3] In Portugal, the EMPIRE study described a prevalence of anemia of 54.2% in pregnant women, with regional variability, with ferropenia being the most frequent cause.[4,5]

Ferropenic anemia is associated with an increase in maternal morbidity, such as greater severity or susceptibility to infection, increased risk of peripartum transfusion, preeclampsia, premature detachment of normally inserted placenta, and may culminate in maternal death.[5,6] Maternal anemia may also increase the risk of postpartum hemorrhage.[7]

Currently, there is an inconsistency between the recommendations of the clinical guidelines (CG) of the Portuguese Directorate-General of Health (Direção-Geral da Saúde [DGS, in the Portuguese acronym)[8] because the National Low-Risk Pregnancy Surveillance Program of recommends iron supplementation in all pregnant women from 14 weeks of gestation on, but the CG of the DGS on the approach, diagnosis and treatment of ferropenia suggests serum ferritin determination before starting iron at the first consultation and at 28 weeks.[9] Furthermore, universal supplementation with iron during pregnancy is not widely practiced by obstetricians.

The aim of the present review is to study the evidence on the need for oral iron supplementation in all pregnant women to prevent maternal anemia.

Methods

We conducted a bibliographic survey of CGs, systematic reviews (SRs), meta-analyses (MAs) and randomized controlled clinical trials (RCTs) in August 2019 in the PubMed, National Guideline Clearinghouse, Guidelines Finder, Canadian Medical Association Practice Guidelines Infobase, The Cochrane Library and Evidence Based Medicine online, SciELO, and DGS databases, considering the articles published in the past 10 years in English and in Portuguese. We used the MeSH search terms *iron*; *therapeutic use*; *pregnancy*; *anemia*; and *prevention and control*, at PubMed and The Cochrane Library. We used the keywords *anemia* and *pregnancy* in the other databases, since these do not integrate search by MeSH terms. As inclusion criteria, we considered the studies whose target population consisted of asymptomatic pregnant women; that is, without established anemia, where we evaluated the prevention of maternal anemia as a result, through supplementation with oral iron, comparing it with nonsupplement. We excluded articles divergent from the objective of the work, repeated articles or included in selected MA/RS, nonrandomized clinical trials, classic reviews, articles not accessible online, articles whose intervention was in pregnant women with already known anemia or ferropenia, evaluations only in twin pregnancies, evaluations in pregnant women with major comorbidities, trials in which the iron intervention group was associated with another compound (for example, folic acid) and was not compared with the control group comprising the same compound, or where the intervention was supplementation with iron in nonoral formulations.

We determined the level of evidence and strength of recommendation using the American Family Physician Strength of Recommendation Taxonomy (SORT) scale.[10] This scale classifies articles according to 3 levels of evidence (level of evidence 1: studies with patient-oriented evidence of good quality; level of evidence 2: studies with patient-oriented evidence of limited quality; evidence level 3: studies with disease-oriented evidence) and 3 degrees of recommendation strength (recommendation strength A: patient-oriented evidence consistently; recommendation strength B: patient-oriented evidence of inconsistent or limited quality; recommendation strength C: disease-oriented and consensus-based evidence).

Results

In the initial research, we identified a total of 704 articles, of which we excluded 639 by the title, that is, because they did not fit the objective of the present review. Of the total of 65 articles, we excluded 28 by reading the abstract and 11 after full reading, because they did not meet the inclusion criteria. We also excluded duplicate 13 articles and 2 because there are more up-to-date reviews by the same author. Thus, we included 11 articles, of which 6 were CGs, 3 MAs, 1 SR and 1 original article, as shown in ►**Fig. 1**.

The MA of Imdad et al,[11] published in 2012 (►**Table 1**), aimed to evaluate the impact of universal iron supplementation on maternal anemia and on perinatal results.

A total of 14 of the 18 studies that evaluated the parameter "maternal anemia at term" considered the intervention to be

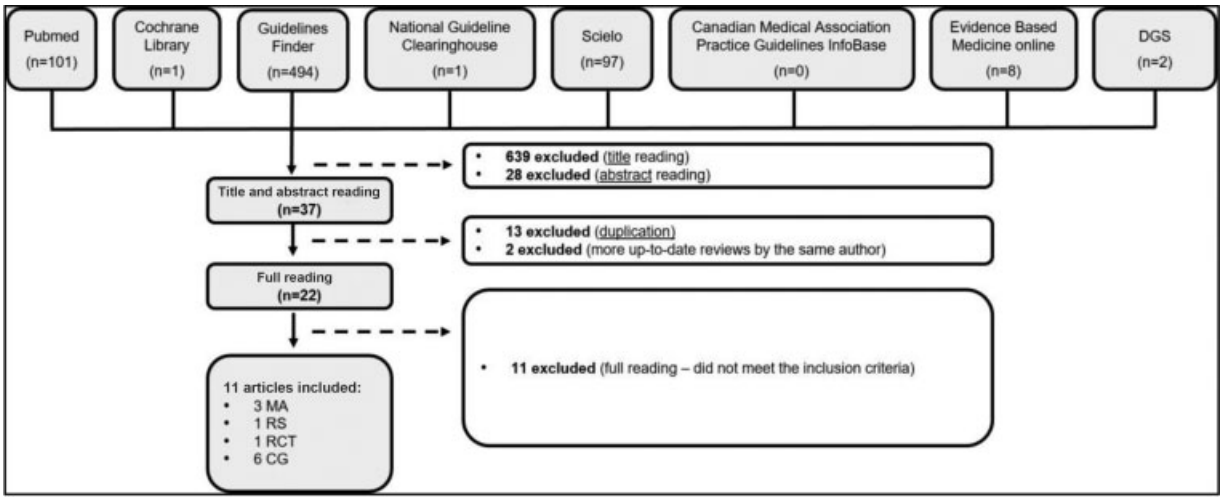

Fig. 1 Illustrative scheme of article selection. CG – Clinical Guideline; DGS – Directorate-General of Health; MA - Meta-analysis; MA - Meta-analysis; RCT - Randomized and Controlled Clinical Trial; SR - Systematic Review.

oral iron alone. A subgroup analysis was performed comparing studies with iron and iron in combination with folic acid, demonstrating similar results. Thus, from the combined analysis of the 18 studies, they concluded that there was a significant reduction (69%) in the intervention group compared with the control group. There was no statistically significant difference between intermittent administration of iron/iron-folic acid and daily administration, based on 3 studies (data not shown). As a limitation of this MA, significant heterogeneity was identified, especially due to the prevalence of anemia in the various places of the included studies being different. The authors conclude that prophylactic iron supplementation during pregnancy has a significant benefit in reducing the incidence of maternal anemia, classifying the quality of the results as "moderate."

The MA of Haider et al.,[12] published in 2013 (►**Table 1**), aimed to summarize the evidence on the association of maternal anemia and iron supplementation in pregnancy with maternal hematological effects and adverse outcomes in pregnancy; it also evaluated the potential relationship between iron exposure (regarding dose, duration of use and hemoglobin [Hb] concentration) and maternal outcomes. A total of 48 randomized clinical trials ($n = 17{,}793$) and 44 cohorts ($n = 1{,}851{,}682$) were included. Regarding maternal Hb concentration, 36 trials evaluated this parameter in the 3rd trimester and at the time of delivery and concluded that it was significantly higher in the iron supplemented group, with or without folic acid; heterogeneity was not found in this analysis. The effect of the intervention on the prevention of maternal anemia was evaluated in 19 trials. The use of iron, with or without folic, acid led to a 50% reduction in the risk of anemia in the 3rd trimester or at delivery, with significant heterogeneity. The effects on Hb concentration in the 2nd trimester were not evaluated because it was a small number of trials. The analysis also showed significant reductions in iron deficit in 8 trials and in iron deficiency anemia in 6 trials. Haider et al.[12] concluded that iron supplementation in women during pregnancy can be used as a preventive strategy to improve hematological status, advocating that prenatal anemia and iron deficiency were identified as preventable risk factors for developing disease.

Peña-Rosas et al.[13] conducted a systematic review published in the Cochrane Library in 2015 (►**Table 1**) whose objective was to evaluate the effect of iron supplementation in pregnant women as a public health intervention in antenatal care. A total of 44 studies were included ($n = 43{,}274$ women), which compared the effects of daily iron supplementation versus no iron or placebo. The results regarding maternal outcomes showed that preventive iron supplementation reduced maternal anemia at term by 70% (relative risk [RR] 0.30; 95% confidence interval [CI]: 0.19–0.46; 14 trials, 2,199 women; low quality evidence), iron-deficiency anemia at term (RR 0.33; 95%CI: 0.16–0.69; 6 trials; 1,088 women), and iron deficiency at term by 57% (RR 0.43; 95%CI: 0.27–0.66; 7 trials; 1,256 women; low quality evidence). The authors concluded that iron supplementation reduces the risk of maternal anemia and iron deficiency during pregnancy. The implementation of iron supplementation in all pregnant women can lead to heterogeneous results depending on the risk of anemia in the population in question, as well as the level of adherence to the measure.

The MA of Abraha et al.,[14] published in 2018 (►**Table 1**), aimed to evaluate and summarize the evidence of systematic reviews regarding oral iron administration to prevent critical pregnancy outcomes, to facilitate the formulation of health recommendations and policies.[14] Regarding the prevention of maternal anemia at term, the results showed that any supplement containing iron reduced maternal anemia by 67%, with significant heterogeneity; an analysis of the subgroup of studies using doses $\geq$ 200 mg/day was then performed, since they presented lower heterogeneity. In this analysis, account was taken of the fact that not all studies considered the control group to have the same micronutrients or other vitamins that

Table 1 Included articles

Reference/Type of study	Findings	LE
Imdad et al. (2012)[11] *MA*	• Daily supplementation of iron: ◦ 69% reduction in the incidence of anemia at term in the intervention group compared with control (Relative Risk (RR) 0.31; 95% confidence Interval [CI]: 0.22–0.44]); ◦ 66% reduction in ferropenic anemia at term (RR 0.44 [95% CI: 0.28–0.68]) compared with no intervention/placebo; • There was no statistically significant difference between intermittent administration of iron/iron-folic acid and daily administration, based on 3 studies. (RR 1.61 [95%CI: 0.82–3.14])	1
Haider et al. (2013)[12] *MA*	• Daily iron supplementation (effects in the 3rd trimester and delivery): ◦ increased the mean maternal Hb concentration by 4.59 g/L (95%CI: 3.72–5.46) compared with the control group; ◦ significantly reduced the risk of anemia (RR 0.50; 95%CI: 0.42–0.59); ◦ significantly reduced iron deficiency (RR 0.59, 95%CI: 0.46–0.79) and ferropenic anemia (RR 0.40, 95%CI: 0.26–0.60). • The effects on Hb concentration in the 2nd trimester were not evaluated by a small number of trials.	2
Peña-Rosas et al. (2015)[13] *SR*	• Preventive iron supplementation reduces: ◦ maternal anemia at term in 70% (RR 0.30; 95%CI: 0.19–0.46); ◦ ferropenic anemia at term (RR 0.33; 95%CI: 0.16–0.69); ◦ iron deficit at term by 57% (RR 0.43; 95%CI: 0.27–0.66); ◦ Women who received added iron supplement increased risk of hemoconcentration at the end of pregnancy (average RR 3.07; 95%CI: 1.18–8.02) • The implementation of iron supplementation in all pregnant women can lead to heterogeneous results, since the risk of anemia is different between the various populations studied, as well as the level of adherence.	2
Abraha et al. (2019)[14] *MA*	• Any supplement containing iron reduced maternal anemia by 67% (RR 0.32; 95%CI: 0.20–0.49) with significant heterogeneity; ◦ the subgroup analysis of studies using doses $\geq$ 200 mg/day (lower heterogeneity) obtained results consistent with the main analysis (RR 0.15; 95%CI: 0.08–0.28; participants = 360; studies = 5). • Iron-containing supplements reduced the incidence of ferropenic anemia, with no statistically significant difference (RR 0.33; 95%CI: 0.16–0.69); • The supplementation with iron resulted in a higher frequency of side effects compared with the control group (22 versus 18%), but with no statistical difference (RR 1.42; 95% CI: 0.91–2.21).	2
Parisi et al. (2017)[15] *RCT*	• **Levels of Hb:** ◦ Concentration decreased in all groups; less pronounced decrease in liposomal iron groups (positive effect of interaction between time and groups – LI14 $p < 0.03$ and LI28 $p < 0.001$); ◦ There were no statistically significant differences in Hb values between the control group and the ferrous iron group. • **Ferritin levels:** ◦ statistically significant differences between ferritin concentration between liposomal iron and control groups (LI14 $p < 0.02$ and LI28 $p < 0.001$); • High incidence of iron deficit/iron anemia in the control group and with ferrous iron (30%).	2

Abbreviations: CI, Confidence Interval; Hb, Hemoglobin; LE, Level of Evidence; MA, Meta-analysis; RCT, Randomized and Controlled Clinical Trial; RR, Relative Risk; SR, Systematic Review.

were associated with iron in the intervention group. Thus, based on these studies with better quality, they concluded that the results are consistent with the main analysis. Iron supplements also reduced the incidence of ferropenic anemia by 67%, with no statistically significant difference. Iron supplementation resulted in a higher frequency of side effects compared with the control group (22 versus 18%), with no statistical difference. In conclusion, evidence of moderate quality was found to support the use of iron to prevent the incidence of anemia in pregnant women. This meta-analysis has limitations, especially regarding the variability of the type of iron supplementation – while most reviews considered the comparison of iron supplementation with placebo or non-treatment, there is a review whose objective was to evaluate the impact of supplementation with various micronutrients on pregnancy outcomes, in comparison with supplementation with iron alone or with folic acid, not fulfilling the objectives of our review, which may cause interpretation bias.

The RCT of Parisi et al.,[15] published in 2017 (►**Table 1**), aimed to evaluate the effect of conventional ferrous iron and liposomal iron formulations, compared with a placebo control group, on maternal reserves during pregnancy. The

exclusion criteria were: pregnant women with known pathology, use of chronic medication or any micronutrient supplementation in the first trimester of pregnancy, except for folic acid, extreme body mass index (BMI) (BMI $< 18 kg/m^2$ or BMI $> 30 kg/m^2$), serum Hb < 10.5 g/dl and/or ferritin < 15 mg/l in the selection phase, known fetal pathologies, and pregnancy complications. In addition, women with a vegetarian or vegan diet or any food restriction (allergies or food intolerance) were excluded. The total sample consisted of 80 pregnant women, randomized into 4 groups (supplementation into 3 groups and 1 control group with placebo, 20 women in each: group 1 with 30mg/ferrous iron day; group 2 with 14-mg/day liposomal iron (LI14); group 3 with 28mg/day liposomal iron (LI28); group 4, control group, without supplementation). The authors concluded that iron supplementation during pregnancy reduces the presence of iron deficit and maternal ferropenic anemia, compared with no supplementation in healthy women and without anemia. This RCT has some limitations, such as the fact that it is not double blind, consists of a small sample and there has been a large number of abandonments. The CG found in the performed research, regarding the recommendations on iron supplementation in pregnancy, are not unanimous (►**Table 2**).

The U.S. Preventive Services Task Force (USPSTF),[2] in its CG published in 2015 (►**Table 2**), states that there is consistent evidence of increased maternal levels of Hb and ferritin, establishing that the evidence is inconsistent regarding whether this increase leads to improved maternal clinical outcomes in non-anemic pregnant women. The USPSTF concludes that the evidence on the effect of universal iron supplementation on asymptomatic pregnant women on maternal outcomes is insufficient and that more evidence is needed for the balance between benefits and risks to be determined.

The CG of the DGS "Approach, diagnosis and treatment of ferropenia in adult" published in 2013 (►**Table 2**), refers to the early diagnosis of ferropenia in the prenatal period, as it reduces transfusion needs.[9] To this end, it suggests that serum ferritin should be determined before starting iron, and that it should only be prescribed if ferritin < 70ng/mL. They also state that iron supplementation should be individualized, based on clinical-laboratory parameters such as blood count, ferritin, and C-reactive protein (CRP) at the 1st consultation and at 28 weeks.

The CG of the DGS – National Low-Risk Pregnancy Surveillance Program – published in 2015 (►**Table 2**) was elaborated with the main objective of defining a set of recommendations and appropriate interventions in the preconception, pregnancy and puerperium.[8] Regarding the

Table 2 Clinical Guidelines included

Reference	Recommendations	SR
U.S. Preventive Services Task Force (2015)[2]	Evidence on the effect of universal iron supplementation on asymptomatic pregnant women on maternal clinical parameters is insufficient. More evidence is needed, and the balance between benefits and risks cannot be determined.	C
Direção-Geral da Saúde (2013)[9]	Supplementation with iron should be individualized, based on clinical-laboratory parameters such as blood count, ferritin and CRP in the first consultation and at 28 weeks. Make the determination of serum ferritin before starting iron, and only prescribe if ferritin $<$ 70 ng/mL.	C
Direção-Geral da Saúde (2015)[8]	Start supplementation with 30–60 mg/day of elementary iron between 14 and 16 weeks and 6 days, in the absence of contraindications.	C
National Institute for Health and Care Excellence (2019)[16]	Iron supplementation should not be offered in a universal way to all pregnant women, since it has no benefits on maternal health and may have unpleasant side effects.	C
Areia et al.[17] (2019)	Perform universal screening of anemia in pregnancy, with blood count and ferritin in the preconception and/or 1st trimester, between 24 and 28 weeks of pregnancy, and in the 3rd trimester. In women without anemia, supplementation should be initiated in pregnant women with ferritin $<$ 30 ng/mL - daily oral administration of at least 60 mg elemental iron (consider intermittent administration on nonconsecutive days to decrease side effects and increase absorption and adherence).	B
Pavord et al. (2011)[7]	Iron deficiency anemia in pregnancy is common and associated with increased risk of maternal morbidity and mortality. Routine screening with serum ferritin, outside the context of research, to diagnose anemia in pregnancy is not currently recommended. Individual approach, based on the results of anemia screening, as well as identification of women at increased risk. Nonanemic women at risk of iron deficiency should be identified and either started on prophylactic iron empirically (40–80 mg of elemental iron once a day) or have serum ferritin checked first (and iron offered if ferritin $<$ 30 ug/l).	C

Abbreviations: CRP, C-reactive protein; SR, strength of recommendation.

supplementation with oral iron concerns, it determines that supplementation with between 30 and 60 mg/day of elemental iron should be started between 14 and 16 weeks and 6 days, in the absence of contraindications.

The National Institute for Health and Care Excellence (NICE)[16] presents a CG published initially in 2008, but updated in February 2019 (►**Table 2**), which aims to provide information on best practices for the clinical follow-up of healthy pregnant women with a single fetus low-risk pregnancy. Among other recommendations, it argues that iron supplementation should not be offered universally to all pregnant women, since if does not benefit maternal health and may have unpleasant side effects.

The Portuguese Society of Obstetrics and Maternal-Fetal Medicine (SPOMMF, in the Portuguese acronym) issued a CG in July 2019 (►**Table 2**) on the approach to anemia during pregnancy and postpartum, which explains that there is no consensus on the universal and systematic supplementation with iron in pregnant women to improve maternal and neonatal outcomes.[17] It also says that recent studies even indicate that unnecessary iron supplementation is associated with an increased risk of adverse outcomes such as preterm birth, low birthweight, and gestational diabetes. In this way, it recommends the universal screening of anemia in pregnancy, with blood count and ferritin in the preconception and/or 1st trimester, between 24th and 28th weeks of pregnancy, and in the 3rd trimester of pregnancy. For iron supplementation, in women without anemia, supplementation with ferritin < 30 ng/mL should be initiated in pregnant women by daily oral administration of at least 60 mg elemental iron. It also considers intermittent administration, on nonconsecutive days, to decrease side effects and increase absorption and adherence.

The British Committee for Standards in Hematology (►**Table 2**) issued a recommendation in 2011 to address iron deficiency in pregnancy as a strategy to prevent ferropenia. At the date of the article review process for submission, the authors verified that there is an update to this guideline published in October 2019, so it was decided to include the updated version, whose purpose was to provide health professionals with recommendations on prevention, diagnosis, and treatment of iron deficiency in pregnancy and in the postpartum period.[7] They state that iron deficiency anemia in pregnancy is common and is associated with increased risk of maternal morbidity and mortality. Regarding the diagnosis of anemia in pregnancy, they report that the optimal strategy is unknown, but routine screening with serum ferritin outside the context of research is not currently recommended. They present a list of indications for empirical iron supplementation and/or serum ferritin evaluation: anemic women in whom testing serum ferritin is necessary prior to iron supplementation (known hemoglobinopathy, before the parenteral replacement of iron); nonanemic women with high risk of iron depletion for empirical iron treatment with/without serum ferritin testing (previous anemia, 3 or more deliveries, twin or higher order multiple pregnancy, consecutive pregnancy < 1 year after delivery, vegetarians/vegans, pregnant teenagers, recent history of clinically significant bleeding); nonanemic women in whom serum ferritin may be necessary (high risk of bleeding during pregnancy or at birth, women declining blood products, such as Jehovah's Witnesses, women for whom providing compatible blood is challenging).

Although it was proposed by some authors, routine screening using serum ferritin is not recommended due to the costs, delays, and limitations of the parameter, and a detailed clinical history is preferred to identify which pregnant women meet the criteria. A serum ferritin level < 30 ug/l in pregnancy is indicative of iron deficiency, but higher levels do not rule out iron deficiency or depletion. In short, as a general recommendation, the authors argue that nonanemic women at risk of iron deficiency should be identified and either started on prophylactic iron empirically (between 40 and 80 mg of elemental iron once a day) or have serum ferritin checked first (and iron offered if ferritin < 30ug/l). Thus, they conclude that there is insufficient evidence to assess the benefits and potential hazards of routine iron supplementation for all women in pregnancy.

Discussion

Prophylactic iron supplementation leads to an improvement in serum iron and hematological parameters in pregnancy. However, its correlation with maternal health benefits and risks is not well documented, with inconsistent results.

Of the scientific articles included in our analysis, the fact that they are directed to the disease stands out, having no proper correlation between the prevention of maternal anemia and the health benefits and quality of life for pregnant women.

Regarding the CG of the DGS existing in Portugal, for which most family doctors are governed, we consider that they need updating. The guidelines of the National Program for Low Risk Pregnancy Surveillance are based on the publication of the "Guideline: Daily Iron and Folic Acid supplementation in Pregnant Women" of the WHO, from 2012,[18] which in turn is based on exclusively laboratory maternal parameters, namely, reduction of the risk of maternal anemia at term and iron deficit at term.[8,18] Regarding clinical results, only the prevention of low weight at birth is mentioned, but this is not the objective of our review. A note also for the CG on the diagnosis and treatment of ferropenia in adults that, in our opinion, presents inconsistent data. This CG considers a ferritin value of 70 ng/ml as indicative for prescription of oral iron, using as bibliographic reference the British Committee for Standards in Haematology (2012 version), where the serum ferritin value taken as the lower limit for starting oral supplementation is 30 ng/ml and not 70 ng/ml.[9,19] Even in the updated version of the guideline on which they are based, the cutoff value of ferritin for supplementation remains 30 ng/ml.

We consider that there are some limitations in this review, mainly due to the heterogeneity between the included studies and MA, justified by the variable sample size, the different inclusion criteria, the variability of the daily elemental iron dose and iron type and the variability of micronutrients or of other vitamins associated with iron in the intervention groups, which leads to inconsistent results. High-quality RCTs are

required, with more homogeneous methodologies, involving a greater number of pregnant women, to obtain more robust conclusions on the efficacy and safety of iron supplementation in all pregnant women for the prevention of maternal anemia. It is necessary to correlate the improvement of laboratory parameters in pregnant women without previous anemia with the possible improvement of clinical parameters. The great variability in the prevalence of anemia in pregnancy described in the introduction of this article may justify the difficulty in reaching consensus on the recommendations on universal supplementation, constituting itself a limitation to the present review.

To complement the present review, it would also be useful to evaluate the evidence on the impact of universal prophylactic iron supplementation on fetal outcomes and delivery.

Conclusion

Given the different results between ►**Tables 1** e **2**, it is possible to conclude that prophylactic iron supplementation in pregnancy is controversial and should not be performed universally in asymptomatic pregnant women (SORT C).

Contributions

All authors participated in the concept and design of the present study; analysis and interpretation of data; draft or revision of the manuscript; and they have approved the manuscript as submitted. All authors are responsible for the reported research.

Conflict of Interests

The authors have no conflict of interests to declare.

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Abnormal Uterine Bleeding in Adolescence: When Menarche Reveals other Surprises

Hemorragia uterina anormal na adolescência: Quando a menarca revela outras surpresas

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Rev Bras Ginecol Obstet 2021;43(10):789–792.

Abstract

Introduction Abnormal uterine bleeding is more frequent in adolescence. Although, most commonly, it has a non-structural etiology, it may be due to any cause described.
Clinical case A 12-year-old adolescent, with no relevant personal history, menarche 1 month before, was observed in the emergency department for severe menstrual bleeding with progressive worsening, and hemodynamic repercussion in need of transfusion support. Physiological ovulatory dysfunction associated with possible previously unknown coagulopathy was considered to be the most likely diagnosis and medical treatment was initiated. Without response, the patient was submitted to sedated observation and uterine aspiration, which ultimately led to the diagnosis of a Burkitt Lymphoma.
Discussion Although structural causes, and particularly malignancy, whether gynecological or not, are a rare cause of abnormal uterine bleeding in this age group, they must be considered, thus enhancing the fastest and most appropriate treatment.

Keywords
- abnormal uterine bleeding
- adolescence
- Burkitt lymphoma

Resumo

Introdução A hemorragia uterina anormal é mais frequente na adolescência. Apesar de maioritariamente de etiologia não estrutural, pode dever-se a qualquer causa descrita.
Caso clínico Adolescente de 12 anos, sem antecedentes pessoais relevantes, com menarca há 1 mês, observada no serviço de urgência por hemorragia menstrual grave com agravamento progressivo e repercussão hemodinâmica com necessidade de suporte transfusional. Foi colocada a hipótese de disfunção ovulatória fisiológica associada a eventual coagulopatia desconhecida previamente e foi instituído tratamento médico. Por ausência de resposta a tratamento médico, foi submetida a observação sob sedação e aspiração uterina que evidenciou tratar-se de um Linfoma de Burkitt.
Discussão Apesar de as causas estruturais, e particularmente as neoplasias, do foro ginecológico ou não, serem uma causa rara de hemorragia uterina anormal nesta faixa etária, elas devem ser levadas em consideração potenciando assim um tratamento mais célere e adequado.

Palavras-chave
- hemorragia uterina anômala
- adolescência
- linfoma de Burkitt

received
September 7, 2020
accepted
August 9, 2021

DOI https://doi.org/10.1055/s-0041-1736143.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Introduction

Abnormal uterine bleeding (AUB) and heavy menstrual bleeding (HMA) constitute a few of the main gynecological problems. They are more frequent in adolescence and one of the main reasons for referral to gynecology consultation in this age group.[1–4]

The etiology of AUB can be structural or not. Nonstructural causes are more frequent in adolescence, mainly ovulatory dysfunction, physiological or not, followed by coagulopathies.[2–6] However, any cause described can occur at this age and a multifactorial etiology is common. Therefore, it is imperative to not exclude any cause before a thorough work-up simply because of age, even malignancies, which are rarely present.[5–8]

Clinical Case

We present the case of an adolescent, 12 years old, healthy, who had the menarche in the previous month with adequate menstrual flow for 7 days. She presented to the emergency room for menstruation with increased flow for 9 days (between 10 and 15 patches/day), and persistent vomiting. The patient had no other symptoms, especially those compatible with coagulopathy.

She was referred to gynecology due to progressive worsening of heavy menstrual bleeding and severe hemodynamic repercussion (severe anemia with Hb 7.3 g/dl, 117,000 platelets and a normal summary coagulation study). At the gynecological examination, an intact hymen and abundant vaginal bleeding with clots were observed. A rectal examination was performed, and it was suggestive of a mass on the posterior wall of the vagina, which on palpation worsened the blood loss through the vagina.

Abdominal and rectal ultrasound was suggestive of a bicorporal uterus, with a noncommunicating left hemicavity with echogenic liquid content compatible with hematometra, and a right hemicavity with irregular endometrial thickening (24.8 mm of greater anteroposterior dimension); and an enlarged right ovary (57×41mm), dense stroma, and dispersed vascularization, without cystic formations (►**Fig. 1**).

Abnormal uterine bleeding due to ovulatory dysfunction was admitted, eventually exacerbated by undiagnosed

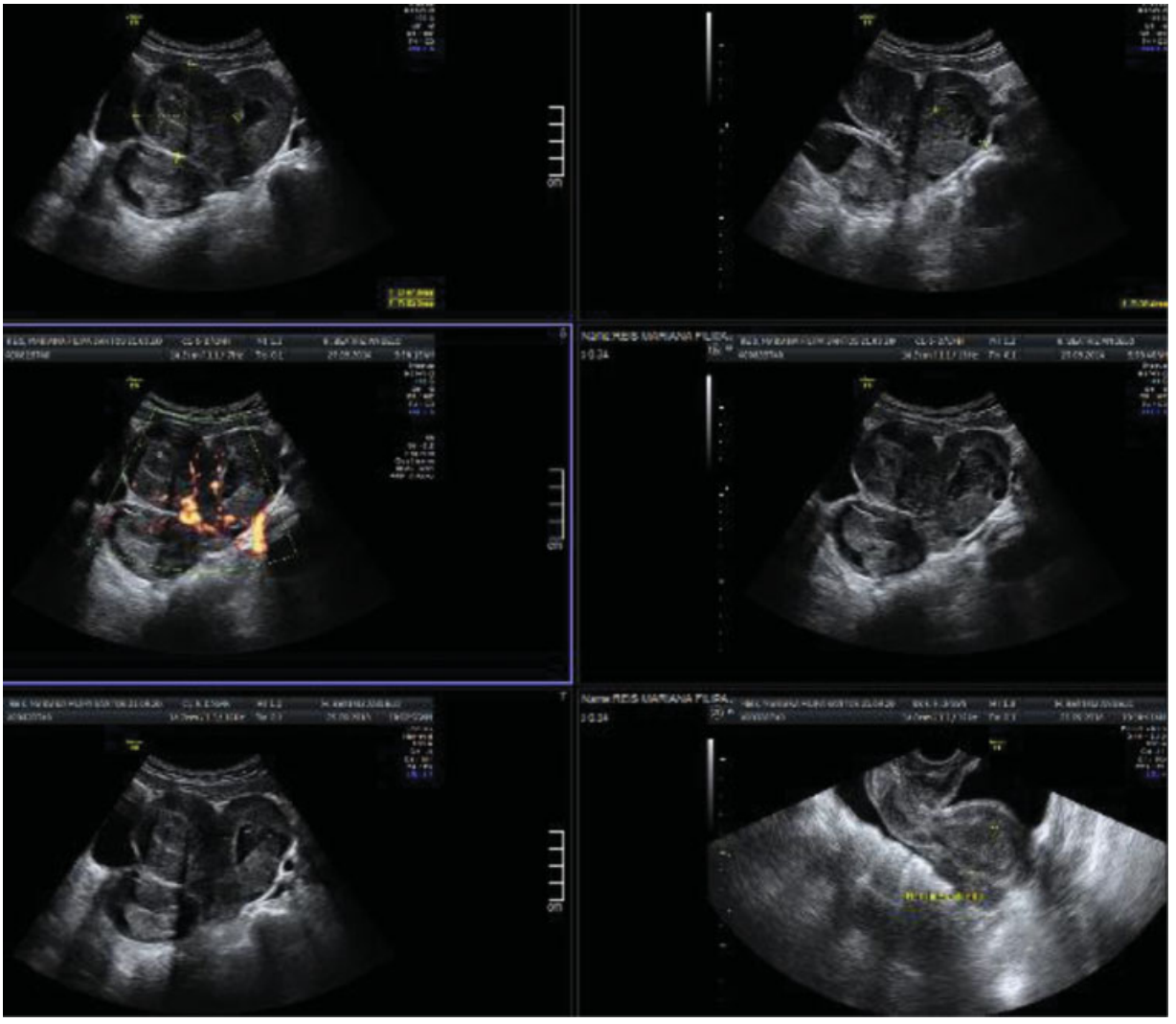

Fig. 1 Abdominal and rectal ultrasound scan showing "bicorporal uterus, with a right hemicavidity with irregular endometrial thickening".

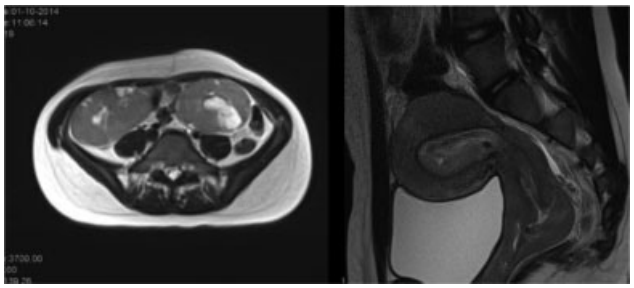

Fig. 2 Computed tomography images with evidence of uterine and ovarian involvement due to previously diagnosed Burkitt lymphoma.

coagulopathy. The patient was started on antifibrinolytics (intravenous tranexamic acid), estroprogestative (high dose combined oral hormonal contraception) and transfusion support. Due to worsening of the hemorrhage, she underwent therapy with a gonadotropin agonist. On the 3rd day of hospitalization, due to severe worsening with hemodynamic instability refractory to medical treatment, a gynecological examination was performed under sedation. A vagina with blood clots and a rough anterior wall, mainly in the upper third and hypertrophic single cervix, was observed. A partial aspiration of the right uterine hemicavity was performed, with significant hemorrhage reduction. The aspirated material was sent for analysis and the histological diagnosis was Burkitt lymphoma. Subsequently, complementary exams showed multiorgan impairment (hepatic, renal, breast, and ovarian). The patient was transferred to the Portuguese Institute of Oncology where the remaining diagnostic work-up also showed infiltration of the central nervous system. She was successfully treated with vincristine, cyclophosphamide, rituximab, methotrexate and arabinosine C, having been in remission for 5 years (►**Fig. 2**).

Discussion

Abnormal uterine bleeding can be acute or chronic: acute requiring immediate intervention, whether episodic or in a chronic context; chronic if present in most of the preceding 6 months.[1,2] It affects between 10 and 20% of women and is more prevalent in adolescence. The evaluation of the menstrual cycle, as an additional vital sign, allows an early identification of an abnormal pubertal progression or, as exemplified in our case, the importance of the menstrual cycle as an initial manifestation of systemic disease.[3]

Contrary to adult age, in adolescence the main causes of AUB are nonstructural, of which ovulatory dysfunction is the most frequent. When physiological, due to the immaturity of the hypothalamus-pituitary-ovary-axis, despite being a diagnosis of exclusion, it appears in more than ⅔ of the cases. When pathological, it occurs due to hyperandrogenism, hyperprolactinemia, hypothalamic or pituitary dysfunction or thyroid pathology.[1–4]

Although rare in the general population (1%), coagulopathies are the second cause of AUB in adolescence: they are present in 20% of adolescents with AUB and in 30% of those in need of hospitalization. Heavy menstrual bleeding in menarche, even without a history of coagulopathy, is a frequent form of presentation, and in 50% of cases the first sign of a coagulation disorder.[5–9] The main associated coagulopathy is von Willebrand disease, and ~ 13% of women with HMA have a variant of this disease.[2,4] Disorders of platelet function, coagulation factors, and thrombocytopenia are also prevalent in adolescence.[8,9]

In this case, HMA in the first menstruation after menarche could lead to the presumptive diagnosis of coagulopathy. However, the absence of other symptoms of hemorrhagic dyscrasia and the analytical evaluation performed showed a low probability for this etiology.

As described, the first approach to a patient with acute AUB is to assess signs of hypovolemia and hemodynamic instability. Subsequently, the etiological investigation is performed according to the acronym PALM-COEIN (classification system approved by the Federation of Gynecology/Obstetrics): **P**olyps, **A**denomyosis, **L**eiomyomas, **M**alignancy and hyperplasia, **C**oagulopathy, **O**vulatory dysfunction, **E**ndometrium, **I**atrogenia and **N**ot classifiable.[2,4]

Medical therapy should always be the first approach, being the only one necessary in 90% of cases of AUB in adolescents: hormonal, with high-dose combined oral contraception (or oral progestatives, if contraindicated for estrogens) or antifibrinolytic. The theoretical thrombotic risk in its association has not been proven and the medications should be combined if monotherapy fails.[4,10,11] Surgical therapy should be reserved for the failure of medical therapy or hemodynamic instability.[2,8]

As recommended, in our case, in the face of AUB refractory to medical therapy with clinical instability, uterine aspiration was performed. This was a difficult decision, but one which allowed an early definitive histological diagnosis.

Intrauterine balloon tamponade (Foley catheter) could be an alternative, but with the disadvantages of not allowing histological diagnosis and having a proven use only in the postpartum period.[8,12]

The use of gonadotropin agonists, even when refractory to medical therapy, is questionable. These play some role in severe chronic AUB but have limited use in acute AUB (due to flare up and response time). In this context, its use was due to the initial suspicion of a nonstructural cause and an attempt to avoid more invasive measures due to the age of the patient.

Burkitt lymphoma is a fast-growing tumor rarely diagnosed in adolescence. It can be classified as endemic, sporadic or associated with immunodeficiency. As a rule, it has a high 5-year survival rate (between 60 and 85%) but may have an adverse prognosis in the rare presence of genital involvement. Given its high response to chemotherapy, timely diagnosis and treatment is essential.[13–15]

With the presentation of this case, we intend to alert to the approach of severe acute AUB in adolescence and to the possible less frequent structural etiologies in this age group. Although rare, the possibility of neoplasia must be considered in the diagnostic work up to enhance the appropriate treatment of our patients.

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

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Urology-Related Conditions Should Not Be Overlooked in Women with Polycystic Ovary Syndrome

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Rev Bras Ginecol Obstet 2021;43(10):793.

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Dear Editor,

It has been documented that women with polycystic ovary syndrome (PCOS) may suffer from sequelae that can surpass the obstetrics and gynecology armamentarium. For example, Shah and Rasool[1] gathered data from the literature showing clear evidence that PCOS is associated with an increased risk of developing type-2 diabetes mellitus, hypertension, dyslipidemia, endometrial cancer, and subclinical atherosclerosis. Therefore, screening for these disorders at the initial contact with these patients, as well as periodically, is recommended.

The findings reflecting the extent of the PCOS sequelae are intriguing. Nevertheless, I would like to highlight some points. We should look further beyond when it comes to PCOS and other associated diseases. The syndrome has been specifically implicated in a few urological conditions as well. In 1988, Fowler et al.[2] first described Fowler syndrome in young women with unexplained complete urinary retention with a unique abnormal pattern of electromyographic (EMG) activity and clinical features of PCOS. They suggested that the abnormal EMG activity was driven by a relative deficiency of progesterone leading to impairment of the urinary sphincter relaxation.[2] Meanwhile, other authors[3] have speculated that hyperoestrogenemia in PCOS might impair the relaxation of the urethral sphincter, leading to urinary retention. It is believed that PCOS in women with Fowler syndrome occurs in $\sim$ 64% of the cases.[4] Furthermore, It has been hypothesized that PCOS may lead to urolithiasis by triggering stone formation in the urinary tract.[5]

Therefore, given that PCOS and these urological issues seem to be correlated, it is advised that women treated for one condition be screened for the other condition and vice versa. I hope that the aforementioned facts can add to the general awareness for women with PCOS and widen the circle of clinical assessment for these patients.

Conflict of Interests

The author has no conflict of interests to declare.

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received
May 30, 2021
accepted
August 31, 2021

DOI https://doi.org/10.1055/s-0041-1736552.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Rescue Vaginal Cerclage to Stop Funneling Following Laparoscopic Cerclage

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Rev Bras Ginecol Obstet 2021;43(10):794–795.

Dear Editor,

Laparoscopic cerclage is an effective treatment option for cervical insufficiency leading to repeated preterm birth. However, surgical intervention with various cerclage techniques, such as vaginal, transabdominal laparoscopic approaches, still remains the ultimate solution, unfortunately, without the guarantee of success.[1–3] There is still no consensus regarding the priority of each technique over the other. However, when laparoscopic cerclage fails to completely treat cervical insufficiency, an additional vaginal cerclage should be considered as a rescue intervention. We suggest considering Shirodkar vaginal cerclage a rescue technique following laparoscopic transabdominal cerclage which is compromised by further funneling. Here, we report, after obtaining written consent, the cases of three patients who needed additional vaginal cerclage to prevent further funneling and membranous bulging despite intact laparoscopic cerclage material.

These three patients had recurrent pregnancy loss despite having undergone vaginal cerclages. Demographic data, as well and the obstetric and surgical histories of the patients, are shown in ►**Table 1**. Considering their history, the first preferred intervention was laparoscopic cerclage. However, we detected funneling and bulging of amniotic membranes below the level of the laparoscopic cerclage during their follow-up visits. Then, we performed an additional Shirodkar vaginal cerclage to prevent further funneling. The images of the patients' cervix immediately after the Shirodkar cerclage are shown in ►**Fig. 1**. The patients were followed-up with frequent ultrasound (US) examinations; images of funneling following vaginal cerclage persisted in two patients, whereas funneling disappeared completely in one patient after vaginal cerclage. All patients had uneventful deliveries at 38 weeks.

Table 1 Demographic data, and obstetric and surgical histories of the patients

Patient	1	2	3
Age	36	33	34
Gravida	10	5	3
Para	1	1	0
Abortus	8	3	2
Previous gynecological operation	Septum resection	None	None
Live birth	1 at 28 weeks	1 at 30 weeks	None
Number of previous elective McDonald vaginal cerclages	3	2	1
L/S cerclage	+	+	+
Issue	Funneling	Funneling	Funneling
Week at performance of vaginal Shirodhar cerclage	13 weeks, 5 days	23 weeks, 2 days	26 weeks, 1 day
Delivery at	38 weeks, 3 days	38 weeks, 1 day	38 weeks, 2 days

Laparoscopic abdominal cerclage is an effective management option for refractory cervical insufficiency. It is reported to improve the rates of second-trimester loss and neonatal survival,[4] and to be superior to low vaginal cerclage, especially for patients with failed previous vaginal cerclage.[5] However, it can be insufficient in conditions such as laparoscopic interventions with loose first knots or medial deviation into the cervical stroma during suturation, or vaginal infections. Further funneling and bulging of amniotic membranes can be warning signs of pregnancy loss even after an uneventful and intact laparoscopic cerclage. This condition can be due to congenital or acquired cervical tissue defects, previous repeated surgeries of the cervix, or a lax

received
August 30, 2021
accepted
September 18, 2021

DOI https://doi.org/10.1055/s-0041-1736553.
ISSN 0100-7203.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

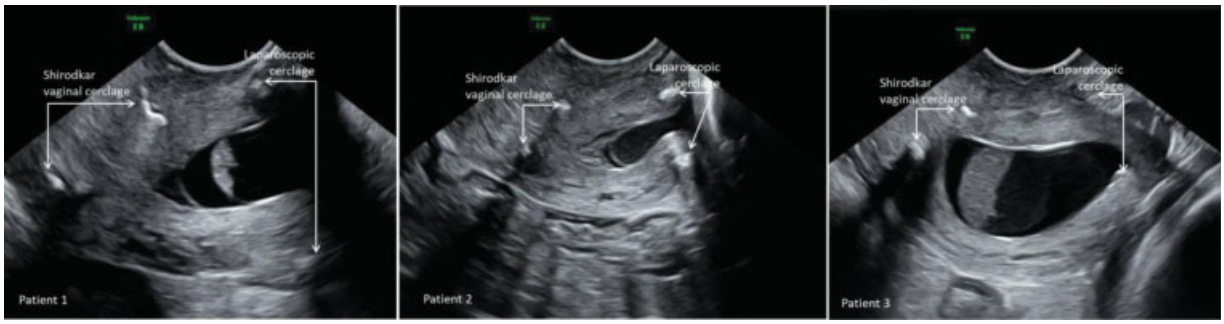

Fig. 1 Ultrasonography images of the patients' cervixes after Shirodkar vaginal cerclage.

laparoscopic cerclage. In these cases, we preferred to supplement the previous laparoscopic cerclage with a subsequent vaginal one through the Shirodkar technique, which is performed at a higher level of the cervix compared with the McDonald technique. This intervention refortified the cervix mechanically for further dilatation. We suggest that the alternative use of this well-known technique may be considered in such difficult cases to provide live births for patients with long history of pregnancy loss.

Conflicts of Interests
The author has no conflict of interests to declare.

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Reply to Letter to the Editor

Comments by the President of the National Commission Specialized in High-Risk Pregnancy (Febrasgo)

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Abdominal cerclage should be restricted to cases in which it is impossible to perform the procedure vaginally, as it leads to greater maternal morbidity: it determines a greater risk of bleeding, infection, rupture of the membranes, and cesarean section. I think that, if Shirodkar cerclage was possible after laparoscopic surgery, it should have been the first treatment option, which would reduce the risks and guarantee success. In addition, the fact that the funnel appeared after surgery shows that the tape was not properly tightened in the suture via the abdominal route, keeping the canal widened, as if the cerclage had not been performed. Thus, cerclage via the abdominal route should be very well indicated and very well performed when necessary.

Conflicts of Interests
The author has no conflict of interests to declare.

febrasgo
Federação Brasileira das Associações de Ginecologia e Obstetrícia

FEBRASGO POSITION STATEMENT

Fertility preservation in women with endometriosis

Number 10 - October 2021
DOI: https://doi.org/10.1055/s-0041-1739234

The National Specialty Commission in Ensometriosis 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:

- Endometriosis is a common benign disease that can compromise female fertility.
- Fertility preservation is a key consideration in the care of girls and women with endometriosis, especially those with ovarian endometriomas.
- Although there is no definitive study on the subject yet, correct information on disease progression, treatment options, and the risks involved should be available for these women.
- It is too early to define fertility preservation as the standard of care for all women with endometriosis.
- Fertility preservation, however, should be considered for women with unoperated bilateral endometriomas and those who have previously removed unilateral endometriomas and need surgery for a contralateral recurrence.
- Since age is the most important prognostic factor, all patients should be aware of its adverse effect on fertility and pregnancy.
- Available strategies include cryopreservation of embryos and oocytes.
- Women should be counseled individually about the risks, benefits and costs involved. In this scenario, the approach of a multidisciplinary team on endometriosis is essential to reach good results.

Recommendations:

- Endometriosis is a benign disease that affects women during menacme and can adversely affect their fertility. The relationship between endometriosis and infertility is quite complex and remains unclear, therefore correct information about disease progression, treatment options and the risks involved should be available for these women.
- Several mechanisms are responsible for infertility in women with endometriosis, including a high production of cytokines and chemokines, an altered hormonal environment, increased oxidative stress, and compromised tubal and sperm function. Furthermore, endometriomas can interfere with folliculogenesis.
- It is too early to define fertility preservation as the standard of care for all women with endometriosis. However, this should be an important point to be considered in the care of girls and women with endometriosis, especially those with ovarian endometriomas.
- Women with unoperated bilateral endometriomas and those who have previously had unilateral endometriomas removed and need surgery for a contralateral recurrence should be advised on fertility preservation and available strategies, which include embryo and oocyte cryopreservation.
- Since age is the most important prognostic factor, all patients should be aware of its effect on their fertility plans.
- Women should be counseled individually about the risks, benefits and costs involved. In this scenario, the approach by a multidisciplinary team of endometriosis is a fundamental step for successful results.

Clinical context

Fertility preservation is a topic that has attracted more attention from physicians and patients in recent years.[1] The increase in life expectancy and the possibility of cure achieved with advances in cancer treatments worldwide have made reproductive care and motherhood an

important issue for young women undergoing cancer treatment. Likewise, women with other benign medical conditions, or for social reasons (postponement of motherhood), have now turned their attention to fertility.[2-4] The development of techniques that allow cryopreservation of oocytes has opened new perspectives to maintain these women's reproductive potential.[1]

The topic is relevant for women with endometriosis, a condition that affects about 10% of women of reproductive age and up to 50% of women with chronic pelvic pain and infertility and who may have their ovarian reserve and future fertility compromised by the disease.[3,4] Several pathophysiological mechanisms can explain the occurrence of infertility in women with endometriosis, namely adnexal adhesions, tubal obstruction, hormonal imbalances, oocyte dysfunction, endometrial changes, inflammations that interfere with sperm-oocyte interaction, worse embryonic quality, lower implantation rate and decreased ovarian reserve.[3] Studies have shown that endometriosis may be associated with decreased ovarian reserve and a smaller number of oocytes in assisted reproduction technique (ART) treatments.[5] Apparently, in some cases, an interaction between the numerous pathophysiological changes may act through mechanisms not fully elucidated yet.[3]

The exact effect of endometriosis on ovarian reserve remains to be established. The presence of ovarian endometriomas appears to adversely affect ovarian reserve markers, such as the anti-Mullerian hormone (AMH) by affecting its production or producing a direct effect not yet known.[6] In addition, large endometriomas can interfere with ovarian vascularization, and the treatment of endometriosis often requires surgery, particularly in patients with ovarian cysts and deep endometriosis. Repeated ovarian surgeries can lead to reduced ovarian reserve and even premature ovarian failure, as the healthy ovarian tissue, in part, ends up being excised along with the disease capsule.[7]

Therefore, fertility preservation has become a relevant issue for women with endometriosis, especially those undergoing surgery for ovarian cysts. In this setting, these women should be appropriately advised about fertility issues prior to the procedure and receive evidence-based information about disease progression, ovarian reserve, available therapeutic options and the risks involved.[7,8]

Cryopreservation of oocytes and embryos is an established fertility preservation technique that requires controlled ovarian hyperstimulation (COH) and follicle puncture for oocyte retrieval. Other techniques such as collection of immature oocytes followed by in vitro maturation for cryopreservation and cryopreservation of ovarian tissue were also studied.[2,8]

Cryopreservation of ovarian tissue during surgery for endometriosis, previously considered an experimental practice, is already being routinely used in several countries. Thus, it is no longer experimental, becoming an interesting option for patients with endometriosis and surgical indication.[1]

Topic discussion

Fertility preservation is a relevant issue for women with endometriosis, and gynecologists should take it into account whenever evaluating these women. Key points to consider include the assessment of ovarian reserve, possible effects of surgery on fertility and the available fertility preservation options.[4,8] Oocyte cryopreservation should be routinely offered to women with endometriosis and infertility, who are at greater risk of needing in vitro fertilization (IVF) in the future. In this context, important issues to be discussed include how to approach young women with endometriosis, the possible effects of surgery on fertility, and the available options for fertility preservation.[9]

How to assess ovarian reserve?

The progressive loss of ovarian follicles is often responsible for subfertility and may also impact negatively on results obtained with the use of ARTs. Such loss is important for women currently not trying to become pregnant, but interested in preserving the chances of future pregnancy.[7] The assessment of ovarian reserve is an essential step in the treatment of women with endometriosis, especially those who will undergo infertility treatment thus, it should support and guide physicians with regard to fertility preservation. The presence of endometriotic lesions and cysts and the surgical procedures to treat these women may put the ovarian reserve at risk and reduce the number of oocytes available for the performance of ARTs.[3,5,6]

The woman's age is the most important predictor of success with ARTs, given the reduction in pregnancy rates with advancing age.[9,10] Therefore, ovarian reserve markers should be evaluated to better inform patients about the expected success rates before starting any fertility-preserving procedure or surgery to treat endometriosis. Available tests include hormonal dosages of follicle-stimulating hormone (FSH) in the follicular phase and AMH, in addition to antral follicle count (AFC) and ovarian volume estimated by transvaginal ultrasound. Such tests can predict the number of oocytes obtained after COH and are related to pregnancy rates.[9,10]

The ideal marker would be able to reflect a significant change throughout a woman's reproductive life, with significant shift from adolescence levels to the late reproductive period, allow the age-independent prediction of a woman's reproductive life span, in addition to spontaneous pregnancy in the general population.[9,10]

Antral follicle count and AMH are the most reliable and used ovarian reserve markers. The AFC consists of

counting the number of follicles with a diameter ranging from 2 to 10 mm and is widely used in ART clinics due to its ready availability and ease of evaluation. It correlates well with the response to ovarian hyperstimulation with gonadotropins.[9,10]

The presence of ovarian endometriosis is associated with lower serum AMH, lower AFC, lower response to COH, and higher doses of gonadotropins used in ART cycles. Reduced ovarian reserve has been reported not only in women with ovarian endometriomas, but also in those with minimal to mild disease.[9]

As excised endometriomas have oocytes firmly attached to the cyst wall, damage to the ovarian reserve is an important concern in endometriotic cyst surgery. There is a 2.4% risk of ovarian failure after bilateral ovarian endometrioma excision. Cystectomy can also have negative effects on ovarian blood supply and spontaneous ovulation rates. The impact of cystectomy on ovarian reserve can be reliably assessed by serum AMH measurements.[11]

Despite the assumption that cyst drainage and wall ablation may be less harmful to ovarian reserve, they are associated with a lower chance of symptom reduction, lower pregnancy rates and higher endometrioma recurrence rates, so they are not recommended as first choice procedure.[11,12]

Thus, patients considering pregnancy should not undergo repetitive ovarian preservation surgeries and minimize damage to the follicular reserve.[12] For those who do not plan to become pregnant immediately, a fertility-preserving approach should be considered before endometrioma surgery, or even during surgeries for advanced endometriosis. Martyn et al.[9] believe that AMH dosage should be offered to all women in their 30s who are not thinking about becoming pregnant, as the clinical evaluation will identify only about 50% of women at risk of reduced ovarian reserve.[2,13]

How should counseling about effects of surgery on future fertility be?

Pain relief and fertility improvement are the main goals of surgical treatment in women with endometriosis. Removing the disease while maintaining reproductive potential with minimal damage to reproductive organs remains a challenge in superficial, ovarian or deep endometriosis.[12]

What is the role of ovarian endometrioma surgery?

In the therapeutic planning of women who wish to maintain their reproductive potential, it is extremely important to take into account that the presence of endometriosis in any of its forms - superficial, ovarian or deep - can interfere with ovarian function and the endometrioma surgery may aggravate this situation.[3,5,7]

Superficial endometriosis is associated with lower fertility rates and reduced ovarian reserve with low levels of AMH.[5] The presence of endometrioma also affects ovarian function, although the relationship between endometriomas and ovarian reserve damage remains controversial. The spontaneous ovulation rate is lower in the ovary with endometrioma. Follicular density is lower and fibrosis is more frequent in the ovarian cortex containing endometriomas.[14] In addition, the presence of deep endometriosis may be associated with reduced ovarian reserve and fewer oocytes retrieved in IVF cycles, probably due to the pelvic inflammatory process found in deep endometriosis.[14]

Endometrioma surgery reduces follicular reserve with compromised ovarian function. This was demonstrated by the significant decrease in serum AMH levels after cystectomy and the decrease in ovulation rates after laparoscopic cystectomy compared to rates before surgery.[13] The decrease in AMH is greater in bilateral cystectomy compared to unilateral cystectomy. In IVF cycles, a lower number of oocytes was obtained with a decrease in the rates of pregnancies and live births after bilateral cystectomy, compared to cycles without endometriomas.[11,12] Muzii et al.[6] published a meta-analysis in which AFC was used to assess the effect of endometrioma surgery on ovarian reserve and reported that it did not decrease after endometrioma removal. However, as repeated surgeries to remove endometriomas seem to be more damaging to the ovarian reserve, indications for surgical treatment of endometrioma recurrence should be carefully evaluated.[11]

Obviously, the larger the ovarian endometriomas and the more extensive and complex the pelvic adhesions, the worse the reproductive prognosis, and it is the surgeon's responsibility not to aggravate this situation. The fundamental principles governing these objectives are the preservation of ovarian follicular reserve and prevention of postoperative pelvic adhesions with a minimum possibility of residual disease.[11,12]

What is the role of deep endometriosis surgery in infertility?

Deep endometriosis is a specific entity arbitrarily defined in histological terms as endometriotic lesions extending more than 5 mm below the peritoneum, usually responsible for painful symptoms. Although deep endometriosis is often associated with infertility, the evidence for a clear connection between the disease and infertility is weak. Studies suggest that infertility in these women is likely due to the strong link between deep endometriosis and adhesions, superficial endometriotic implants, ovarian endometriomas, and adenomyosis.[15]

Despite the evidence of the association between deep endometriosis and infertility, it is still unclear if surgery to treat this form of the disease can act on fertility, since the main indication of surgical approach was for the treatment of pelvic pain.[12,15]

While some advocate complete surgical removal of endometriotic lesions for fertility improvement, others argue that extensive deep endometriosis surgery and intraperitoneal surgery in infertile women do not improve overall fertility prognosis and may be associated with a higher rate of complications.[15,16]

In summary, the effect of surgery on the fertility of women with deep endometriosis remains unanswered given the heterogeneous nature of the disease and the lack of reliable trials with sufficient power and follow-up to study the topic.[16]

What are the fertility preservation options available?

Embryo cryopreservation and mature oocyte cryopreservation are established techniques to preserve fertility in women during the reproductive period.[17] In both cases, COH is required, followed by oocyte recovery with transvaginal ultrasound. The mature oocytes obtained can be cryopreserved or fertilized, and the resulting embryos cryopreserved. Embryo cryopreservation is an effective option, provided there is time to perform ovarian stimulation and an available partner. Oocyte cryopreservation is the best option for preserving fertility in women with endometriosis who wish to postpone pregnancy or will undergo surgical treatment for endometriosis and do not have a partner yet.[17] Vitrification appears to be an efficient method for cryopreservation of oocytes, keeping fertilization and pregnancy rates similar to those of IVF techniques with fresh oocytes.[18]

There is concern about the quality of response in cases of endometriosis, as some studies suggest that women with endometriosis who undergo IVF cycles have lower rates of pregnancy and implantation compared to those with tubal infertility.[3] This would occur as a result of the reduced quality of oocytes, embryonic development and endometrial receptivity. Harb et al.[19] published a meta-analysis showing reduced rates of fertilization in women with grade I/II endometriosis, and of pregnancy and implantation in women with grade III/IV endometriosis. Therefore, more cycles of COH and IVF may be needed to obtain enough good quality oocytes to generate embryos with appropriate development and quality for freezing. Ovarian hyperstimulation does not seem to increase the risk of progression of endometriosis or recurrence of lesions in treated patients.[20] In addition, the presence of endometrioma at the time of ovum collection may increase the risk of pelvic infection and abscess formation.[11,12,21]

Surgical approach should be carefully discussed in infertile patients with ovarian endometrioma. Excision of the endometrioma capsule increases the rate of spontaneous pregnancy in the postoperative period compared to drainage and electrocoagulation of the endometrioma wall.[22] However, these surgical techniques may present a risk of decreased ovarian reserve, either by removal of normal ovarian tissue during excision or by thermal damage to the ovarian cortex during ablation. Published data show a significant reduction in AMH values in the presence of endometriomas compared to the absence of endometriosis.[7] Surgical excision of endometriomas appears to negatively influence ovarian reserve, but only temporarily.[22,23] Other data suggest that the mere presence of an endometrioma adversely affects the ovarian reserve and it may be difficult to measure these effects before surgery.[22,23] Therefore, despite the efforts of laparoscopic surgeons to minimize surgical damage, the ovarian reserve can still suffer in the presence of endometrioma by itself. The size of the endometrioma, the risk of bilaterality of subsequent ovarian failure, the surgical technique, the surgeon's experience, and the patient's age should also be taken into consideration before surgical excision if future fertility is a concern.[22,23]

Currently, cryopreservation of ovarian tissue is used for fertility preservation in women in reproductive years at high risk of losing ovarian function (chemotherapy, radiotherapy, or benign conditions associated with a high risk of premature ovarian failure).[1,17] In prepubescent girls at risk of losing their reproductive potential, this may be the only available alternative. However, note that cryopreservation of ovarian tissue is still considered experimental (Ethics Committee of the American Society for Reproductive Medicine, 2014; Decanter et al., 2018).[1,24] In patients with endometriosis, healthy fragments of the ovarian cortex can be isolated and cryopreserved during surgical removal of endometrioma. The technique should be carefully evaluated, given the risk of transferring small foci of endometriosis in the cryopreserved tissue.[17,24] The advantage of tissue cryopreservation is that there is no need for ovarian hyperstimulation. Many unanswered technical questions remain related to the choice of cryopreservation technique, chances of recovery of ovarian function after transplantation, and pregnancy rates after the procedure.[17,24] Data are still scarce regarding the use of this fertility preservation technique in women with endometriosis and further studies need to be conducted before indicating cryopreservation of ovarian tissue as the first choice for fertility preservation in patients with endometriosis.[24]

Figure 1 summarizes the suggested assessment for fertility preservation in women with endometriosis.

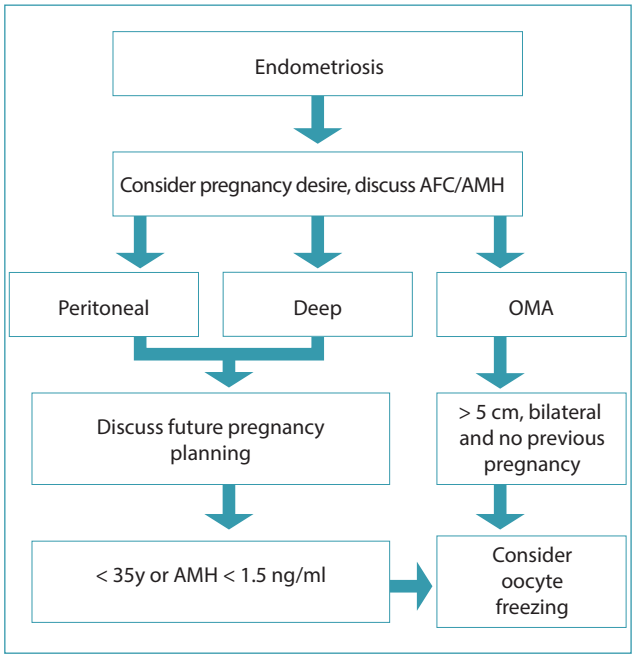

Figure 1. Evaluation flowchart for fertility preservation in women with endometriosis

Final considerations

Endometriosis is a common benign disease that carries significant risks to reproductive organs. Fertility preservation is a key consideration in the care of girls and women with endometriosis, especially those with ovarian endometriomas and advanced age. Although there is no published prospective cohort study on the subject to date, reliable information on disease progression, treatment options, and the risks involved should be available to these women. It is too early to define fertility preservation as the standard of care for all women with endometriosis, as few cases have been reported and the available data do not allow for adequate robust cost-utility analyzes. However, fertility preservation should be considered for those with unoperated bilateral endometriomas and for those who have previously removed unilateral endometriomas and need surgery for a contralateral recurrence. Furthermore, age is currently the most important prognostic factor associated with fertility. Available strategies include cryopreservation of embryos and oocytes, and women should be counseled individually about the risks, benefits and costs involved. In this scenario, the management of endometriosis by a multidisciplinary team is a fundamental step towards the achievement of successful results.

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Conflicts of interest: none to declare.

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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.

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- Title page;
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Title Page

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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

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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?

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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.

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

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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:
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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.

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*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.***

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