

## Maturation of Vaginal Epithelium and Dyspareunia Symptoms in Equol Producing and Non-Producing Menopausal Women

Tita Husnitawati Madjid<sup>1</sup>, Nurlina Juniar,<sup>2</sup> Dian Tjahyadi,<sup>1</sup> Birgitta M. Dewayani,<sup>3</sup> Wiryawan Permadi,<sup>1</sup> Benny H. Purwara,<sup>1</sup> Hadi Susiarno,<sup>1</sup>

<sup>1</sup>Department of Obstetric and Gynecology Faculty of Medicine Padjadjaran University, Hasan Sadikin General Hospital, <sup>2</sup>RSIA Sentul Cikampek, <sup>3</sup>Departement of Pathology Anatomy

Korespondensi: Tita Husniati Madjid, Email: [thusnitawati@gmail.com](mailto:thusnitawati@gmail.com)

### Abstract

**Introduction:** Equol is a metabolite of soy isoflavon called daidzein which is produced by gastrointestinal tract bacteria. This research aims to analyze the maturation of vaginal epithelium and dyspareunia symptoms in producing and non-producing equol menopausal women.

**Methods:** This is a cross sectional research. Subject was a community of menopausal women who fulfilled inclusion criteria. Subjects were asked to sign a written informed consent. Subjects underwent vaginal epithelium maturation assessment and were asked whether she experienced dyspareunia. Research was conducted in January 2017.

**Results:** There was a significant difference on the maturation of vaginal epithelium and dyspareunia symptoms between equol producing and non-producing women ( $p < 0.05$ ). This research found that in equol producing menopausal women, there was a shift-to-the-right vaginal epithelium maturation with more superficial cells compared to parabasal cells produced and less dyspareunia. Meanwhile, in women who did not produce equol, there was a shift-to-the-left vaginal epithelium maturation with more parabasal cells compared to superficial cells produced and more dyspareunia.

**Conclusion:** In equol producing menopausal women, vaginal epithelium will undergo a shift-to-the-right maturation, with more superficial cells produced compared to women who did not produce equol.

**Key words:** dyspareunia symptoms; equol producing; non-equol producing; vaginal epithelium maturation

## Maturasi Epitel Vagina dan Gejala Dispareunia pada Wanita Menopause yang Menghasilkan Equol dan Wanita yang Tidak Menghasilkan Equol

### Abstrak

**Pendahuluan:** Equol adalah metabolit isoflavon kedelai yang disebut daidzein yang diproduksi oleh bakteri saluran pencernaan. Penelitian ini bertujuan untuk menganalisis maturasi epitel vagina dan gejala dispareunia pada wanita menopause yang memproduksi dan tidak memproduksi equol.

**Metode:** Penelitian ini merupakan penelitian *cross sectional*. Subjek penelitian adalah sekelompok wanita menopause yang memenuhi kriteria inklusi. Subjek diminta untuk menandatangani persetujuan tertulis dan menjalani penilaian maturasi epitel vagina dan ditanya apakah mengalami dispareunia. Penelitian dilakukan pada Januari 2017.

**Hasil:** Terdapat perbedaan yang signifikan pada maturasi epitel vagina dan gejala dispareunia antara wanita yang memproduksi equol dan yang tidak memproduksi ( $p < 0,05$ ). Pada wanita menopause yang memproduksi equol, terjadi pematangan epitel vagina *shift-to-the-right* disertai produksi sel superfisial yang lebih banyak dibandingkan sel parabasal dan lebih sedikit gejala dispareunia. Sementara itu, pada wanita yang tidak menghasilkan equol, terjadi pergeseran *shift-to-the-left* maturasi epitel vagina dengan produksi sel parabasal lebih banyak dibandingkan sel superfisial dan lebih sering gejala dispareunia.

**Kesimpulan:** Wanita menopause yang memproduksi equol mengalami maturasi epitel vagina *shift-to-the-right* disertai produksi sel superfisial yang lebih banyak dibandingkan dengan wanita menopause yang tidak memproduksi equol.

**Kata kunci:** dyspareunia; equol; maturasi epitel vagina

## Introduction

Menopause is defined as permanent cessation of menstruation. According to the World Health Organization (WHO), menopause is defined as the time point of permanent menstrual cessation due to loss of ovarian function. Life expectancy in Indonesia in 2012 is about 69 years old, thus every Indonesian woman will experience menopause. The age of female menopause in developed countries such as the United States and Britain is 51.4 years, while in Southeast Asian countries it is 51.09 years. The age of menopause for Indonesian women is 50 years. Upon entering the menopause, associated with decreased estrogen levels, women have an increased risk of chronic abnormalities in the long term, such as coronary heart disease, osteoporosis, decreased cognitive function and malignancy.<sup>1-3</sup>

In menopausal women there will be an anatomical and physiological changes from the vagina, associated directly with reduced estrogen levels. Anatomic changes include reduced collagen, hyalinization, elastin deficiency, epithelial depletion, altered smooth muscle cell function, increased connective tissue density, and less blood vessels. Physiological changes result in reduced vaginal blood flow, vaginal discharge, flexibility, elasticity of the vaginal dome, and increased vaginal pH. In addition, decreased vaginal tissue strength and increased fragility of the vaginal tissues may predispose to vaginal epithelial damage so that during sexual activity vaginal penetration, causing vaginal pain, burning, irritation, and bleeding after sexual intercourse.<sup>4</sup>

Equol is a metabolite of soy isoflavones called daidzein produced by bacterial gastrointestinal tract and not every human being is able to produce equol. Equol is not derived from plants, but is specially produced by bacteria that convert daidzein into the metabolite. The similarity of the structure also

contributes to the prevention of symptoms that occur in menopausal women. Equol is the active metabolite of daidzein which is secreted through urine.<sup>5-7</sup>

Not everyone has intestinal microflora that can turn daidzein into equol. Based on previous research, it was known that only 30 - 60% of human beings were able to produce equol from daidzein; thus, the terminology "equol producer" and "non equol producer" were created.<sup>8,9</sup>

Significant evidence has been found regarding equol: equol can reduce complaints related to menopause. Recent research has been conducted on a group of postmenopausal women in Bandung in 2014. This study is a demographic survey to determine equol producers and not equol producers and their association with menopausal symptoms. The study intended to show that only female equol producers benefit from equol. This study was conducted with the knowledge of the presence of equol in women urine post menopause and knowing of postmenopausal symptoms through a questionnaire of Menopause Rating Scale (MRS). Postmenopausal women selected in this study were women who had similar characteristics, aged 40-60 years, had a homogeneous daily diet, had menopause between 1-5 years, and not being on hormone therapy. The result of the study was that there was a significant positive correlation between postmenopausal women's urine equol levels with menopausal symptoms that arise and the quality of life.<sup>5,10,11</sup>

In this study, the pap smear examination results will be examined in correspondence to the index of vaginal epithelial maturation. The Maturation Index (MI) is a tool that can to evaluate female hormones. MI can be collected from the vagina, conjunctiva, urethra, and buccal mucosal surfaces. The index is read left to right and refers to percent and parabolic, intermediate, and superficial squamous cells appearing in a smear, with

total and all three values equal to 100%. A leftward shift indicates an increase in parabasal or intermediate cells in menopausal women, when a rightward shift indicates an increase in superficial cells or intermediates at the age of reproduction. Dyspareunia complaints in postmenopausal women with right shift of dyspareunia complaints will be less than in menopausal women with a shift to the left.<sup>12</sup>

## **Method**

This research was an observational study with cross sectional approach to analyze vaginal epithelial maturation and dyspareunia symptoms on equal producing and non equal producing phenotypes. This research activity was conducted at Outpatient Installation of Dr. Hasan Sadikin General Hospital, Bandung; Posbindu Teratai group of Cimindi Raya Hill Complex, Pasirkaliki; and Posyandu and Posbindu groups of Cileunyi and Anatomy Pathology Laboratory of Faculty of Medicine Universitas Padjadjaran. The study was conducted from January 2017.

The subjects of the study was community of menopausal women consisting equal producers and non equal producers, who had done equal assessment in previous studies, who were healthy and met the inclusion criteria, were not included in the exclusion criteria and were willing to participate in the study by signing written informed consent until the minimum sample size of RSHS employees and Posyandu group of Cimindi-Pasirkaliki and Cileunyi areas was fulfilled. The inclusion criteria in this study were healthy menopausal women who were willing to follow the study. Meanwhile, the exclusion criteria in this study were menopausal women who underwent hormonal treatment. The total number of minimum samples calculated using the formula for testing the difference of two population proportions was 52 patients.

The independent variables in this

research were equal producing and non equal producer. Meanwhile, the dependent variables in this study were vaginal epithelial maturation, shift-to-the-right and shift-to-the-left, as well as dyspareunia symptoms. Descriptive analysis was used to determine the characteristics of research subjects who become the sample research. Numerical data such as patient age and BMI were presented as mean, standard deviation, median and range. The samples' characteristic data considered as categorical data such as gender and the patient's occupation were coded and presented as frequency distribution and percentage.

Prior to statistical tests, numerical data were tested for the normality of data distribution using the Sapiro-Wilk test if the data were less than 50, or alternatively Kolmogorov-Smirnov test if the data were more than 50. The significance test for comparing the characteristics of the two study groups used the paired t-test if the data was normally distributed and the Mann-Whitney test as an alternative if the data was not normally distributed. Meanwhile, statistical analysis for categorical data was using the Chi-square test if Chi-Square requirements were met, or the Exact Fisher test as an alternative for 2 x 2 table and Kolmogorov Smirnov for table size other than 2 x 2. The criterion of significance used was p-value, p-score  $\leq 0,05$  was considered significant or significant statistically, while p-score  $> 0,05$  was not considered significant or not statistically significant. The data obtained is recorded in a special form and then processed through SPSS version 21.0 for Windows.

## **Results**

This study was conducted for 1 month, January 2017. During this period, there were 52 subjects who met the inclusion criteria and excluded the exclusion criteria. Research subjects were healthy menopausal women

**Table 1 Characteristics of research subjects**

Variables	Group		P-score
	Equol Producer	Non Equol Producer	
	N = 26	N = 26	
<b>Age</b>			<b>0.000**</b>
Mean ± STD	51.73 ± 3.945	55.69 ± 3.283	
Median	51.000	55.5000	
Range (min-max)	47.00-60.00	50.00-65.00	
<b>BMI</b>			<b>0.592</b>
Mean ± STD	26.44 ± 4.130	25.43 ± 8.580	
Median	25.400	23.500	
Range (min-max)	19.20-34.20	19.10-65.04	

including RSHS employees, members of the Posbindu group of Cileunyi region, and members of the Jatinangor Posbindu group who were willing to take anamnesis about the symptoms of dyspareunia, and urine equol examination that had been done in previous research.

Table 1 described the age ratio of the equol producer group and the non equol producer. Mean age of the equol producers group was 51.73 ± 3.945, while the average age of the non equol producer was 55.69 ± 3.283. BMI of the equol producers group averaged at 26.44 ± 4.130, while the non equol producers averaged at 25.43 ± 8,580

These numerical data was tested using paired T-test if the data were distributed normally and Mann Whitney test alternative if the data was not normally distributed; as in age and BMI. The result of statistic test in the age group above obtained that the P-score of age was smaller than 0.05 (P value < 0.05) which meant that it was significant or statistically significant. Meanwhile, the result of the P-score of BMI was greater than 0.05 (P value > 0.05), which meant that it was not significant or not statistically. Thus, it could be concluded that there is no statistically significant difference between the BMI of equol producer and non equol

producer group.

Based on analysis results for age, all P values were less than 0,05 (p <0,05), hence it could be concluded from both group that there were differences in characteristic at the beginning of examination. This showed both groups were not equal or not homogeneous. The result of BMI characteristic analysis, in which all P values were more than 0,05 (p> 0,05), can conclude that both group were equal or that there was no difference of characteristic at the beginning of examination. This indicated that both groups are equal or homogeneous, which meant it was feasible to compare and test for further statistical hypotheses.

Table 2 illustrated the proportion of dyspareunia in the groups of equol producers and non equol producers. In this study, the p-score for dyspareunia was <0.05, indicating that the variable was significant or statistically significant; thus it can be concluded that there was a statistically significant difference between the dyspareunia in equol producer and non equol producers groups. The analysis of categorical data in the above table was tested by using Mann-Whitney statistics test, in which the information obtained a P-value of 0.0001, which was smaller than 0.05 (p value <0.05), and meant that it was

**Table 2 Comparison of dyspareunia experiences between equol producer and non equol producer groups**

Group	Dyspareunia		P-score
	No	Yes	
Equol producer	25 (96.2%)	1 (3.8%)	<b>0.0001</b>
Non equol producer	8 (30.8%)	18 (69.2%)	

**Table 3 Comparison of epithelial maturation in equol producer and non equol producer groups**

Group	Epithelial maturation		P-score
	Shift-to-the-right	Shift-to-the-left	
Equol producer	17 (65.4%)	9 (34.6%)	<b>0.012</b>
Non equol producer	8 (7.7%)	18 (69.2%)	

significant or statistically significant. Thus, it can be explained that there was a statistically significant or statistically significant difference between the dyspareunia variables in the two study groups, ie the equol producer and non-equol producer group.

Table 3 describes the comparison of epithelial maturation in the equol-producing group and the non equol producing group. In this study, the epithelial maturation had a p value < 0.05, so, it was significant or statistically significant; thus it can be explained that there was a statistically significant difference between epithelial maturation variables for the equol producer group and non equol producer group. The analysis of categorical data in the above table was tested by using Mann-Whitney statistical test, where the P value was found to be 0.012, which was smaller than 0.05 (p value < 0.05) which meant that it was significant or statistically significant. Thus, it can be explained that there was a statistically significant difference between epithelial maturation variables in the two study groups, ie. equol producer group and non-equol

producer group.

### Discussions

This research was a study to determine whether there was a difference in vaginal epithelial maturation and dyspareunia symptoms in post-menopausal women producing equol and non equol producing. In this study, subjects were healthy menopausal women who fulfilled the inclusion criteria, not included in the exclusion criteria and were willing to participate in the study by signing written informed consent forms until the number of samples fulfilled the minimum sample size. Samples included employees of RSHS as well as members of Posbindu Group at Cileunyi and Jatinangor. Total number of study subjects who met the inclusion criteria and excluded the exclusion criteria was 52 women. The researchers performed history taking regarding the presence of dyspareunia symptoms and papsmear examination to assess vaginal epithelial maturation.

Based on Table 1, the age of menopausal women averaged at 53.7 years with a



standard deviation of 4.1 years, with median age of 48 years. The youngest respondent was 47 years old and the oldest respondent was 65 years. Overall, it was shown that the average age of menopausal women having various menopausal symptoms was similar to those of previous studies, ie, around 49-51 years of age. Average age of Asian women experiencing menopause was 51 years.

The BMI of menopausal women was in average 25.9, with standard deviation of 6.6, with median BMI of 24.1, lowest BMI was 19 and highest BMI was 65. In this study, it was found that women producing equol had in average higher BMI than women who did not produce equol.

In the equol producing group, 25 or 96.2% of women were found to have no dyspareunia while 1 or 3.8% of women were found to be experiencing dyspareunia. Meanwhile, in the non-equol producing group, 8 or 30.8% of women did not experience dyspareunia and as many as 18 or 69.2% of women were found to be experiencing dyspareunia. Based on categorical data analysis with Chi-square, it was found that the P value for dyspareunia was less than 0.05 ( $p$  value  $< 0.05$ ), which meant that it was significant or statistically significant. Thus it can be explained that there was a significant or statistically significant difference in the proportion of dyspareunia in the two study groups, ie equol producer group and non-equol producer group.

The next purpose of this study as mentioned in the introductory section was to analyze the maturation of the vaginal epithelium and the symptoms of dyspareunia in post-menopausal women who produce and did not produce equol. Currently, in addition to pharmacologic drugs of estrogen preparations, phytoestrogen is also developed to reduce the symptoms in menopausal women. About 60-70% of Asian women and only 20-30% of women in Europe and America are equol producers. A diet rich in soya (isoflavones) is believed to have an

effect on lower menopausal symptoms and higher levels of equol in women in Asia. This study distinguished between equol producers and non equol producers. It also assessed the differences in dyspareunia symptoms in post-menopausal women who produce and did not produce equol.<sup>13-15</sup>

The vagina is an elastic fibromuscular tissue that connects the external genitalia with the internal genitalia and consists of a layer of basal cell of the cube, several layers of parabasal cells and several layers of thin intermediate cells (layered epithelial epithelium), whose thickness changes according to age. The change in thickness and maturation of this epithelium, as well as the content of glycogen in it are related to hormonal status. Estrogen activates mitosis and is associated with increased epithelial thickness and an increase in the amount of glycogen. After menopause, the epithelium becomes thin again. The vagina maintains its ecological balance in which hormones, hormone-sensitive epithelium, hormone-sensitive epithelium and various bacteria interact to maintain a healthy environment. In this ecosystem, estrogen plays a role in estrogen receptive cells to stimulate proliferation and maturation. In addition, the glycogen content of the squamous cells increases. This situation allows the development of Doderlin, Corynebacteria and other non-pathogenic bacteria. As a result, these organisms will metabolize glycogen to produce lactic acid, which will cause low pH to have an effect on the inhibition of the growth of pathogenic organisms.<sup>16</sup>

A decrease in the hormone estrogen causes the vaginal lining tissue to become thin and secretions or mucus in the vagina begin to decline so that when sex will arise pain. Vaginal atrophy occurs because vaginal secretion is reduced after menopause. In addition, the vaginal wall becomes thin and its elasticity is reduced and becomes shorter and lower, resulting in discomfort and pain during

sexual activity. Vaginal narrowing occurs 3-6 months after menopause and symptoms are felt within 5 years of menopause. The vulvovaginitis atrophy is a common condition in menopausal women and has a considerable impact on the quality of life of women. The condition of hypoestrogen causes vaginal atrophy and vulvar vestibulum. The tissue becomes thinner, easily irritated and also more prone to secondary infection. Usually women complain of heat sensation in the vulva, dysuria, pruritus, dyspareunia and pain. A watery discharge may occur and is not uncommon for fissures in the vagina. This disorder can usually be diagnosed by a physical examination, although cytology should be confirmed for confirmation and diagnosis. Vaginal epithelium becomes thinner, pale, rugae decreases. At first the capillary blood vessels will appear like reddish spots that will then appear to be more rare and the vagina looks more slick and shiny. For more objective examination, a smear examination is taken from the upper third of the vagina on the lateral side to see the maturity index.<sup>17</sup>

Since estrogen stimulates the maturity of the vaginal epithelial cells from the basal stratum to the upper layer cells, generally in postmenopausal women smear examination will show a decrease in the number of superficial cells to the absence of any such cells. The presence of basal cells in a dominant number with the absence of superficial cells indicates the occurrence of atrophy.<sup>18</sup>

Dyspareunia is a pain in the genitalia region that arises during or after sexual intercourse without the presence of vaginismus. This repetitive pain will cause less stress, anxiety and interpersonal relationships, which in turn will result in a woman's avoidance of sex. Dyspareunia affects almost 2/3 women during their lifetime. Dyspareunia is one of the most common symptoms and atrophic vaginitis that has not been treated well.

The Maturation Index (MI) is a tool that is able to evaluate hormones that affect women. The index is read left to right and refers to percentage as well as the number of parabasal, intermediate, and superficial squamous cells appearing in a smear, with total and all three values equal to 100%. For example, an MI 0/40/60 represents 0% parabasal cells, 40% intermediates, 60% superficial. A leftward shift indicates an increase in parabasal or intermediate cells, when a shift to the right indicates an increase in superficial or intermediate cells. Between post-menopausal women, equol-producing women experienced less dyspareunia symptoms than non equol producing women.<sup>12</sup>

In this study, dyspareunia is less common in post-menopausal equol producing women because dyspareunia is a result of lack of estrogen in menopausal women. In post-menopausal women who produced equol, due to equol as a derivatives, the maturation of the vaginal epithelium will be better than in menopausal women who did not produce equol. Therefore, in the equol producer group, there were more superficial cells, resulting a shift-to-the-right.

In post-menopausal women, the vaginal epithelium becomes thin, pale and the rugae diminishes. At first the capillary blood vessels will appear like reddish spots that will then appear to be more rare and the vagina looks more slick and shiny. So that in menopausal women who did not produce equol, dyspareunia occurred more frequently and vaginal epithelial maturation had a shift-to-the-left, with more parabasal cells in the vaginal epithelium.

The limitation of this research was that it was a cross sectional study, so future research as a cohort is needed to further evaluate vaginal epithelial maturation and degree of dyspareunia in post-menopausal women producing equol and non equol producing. Further research can be developed to assess whether there is a degree of dyspareunia

relationship with equol levels. A previous study found that soy diet had an increased maturation effect from vaginal epithelium. Consuming soy may increase estrogen levels in menopausal women, resulting in increased vaginal epithelial maturation as well as increased maturation of vaginal epithelium and vaginal epithelial changes from parabasal cells into superficial cells using isoflavones gel. Therefore, postmenopausal women menopause may want to maintain a diet that contains isoflavones.<sup>19, 20</sup>

### Conclusions

Based on research data and discussion, there were a few conclusions: (1) maturation of vaginal epithelium in equol producing menopausal women had a shift-to-the-right compared to women who did not produce equol, and (2) dyspareunia symptoms were experienced less often by equol producing menopausal women compared to women who did not produce equol.

### Reference

1. Depkes. Gambaran kesehatan lanjut usia di indonesia. Departemen Kesehatan Republik Indonesia; 2013 [disitasi 2017].
2. Martin TJ, Sims NA. How cells communicate in the bone remodelling process. *Journal of Korean Endocrine Society*. 2010;25(1):1–8.
3. Angkasa D. Profil perempuan meopause di Indonesia dan pengetahuan sulih hormon: laporan pendahuluan. Jakarta: Universitas Indonesia; 2000.
4. Chiechi L, Putignano G, Guerra V, Schiavelli M, Cisternino A, Carriero C. The effect of a soy rich diet on the vaginal epithelium in postmenopause: a randomized double blind trial. *Maturitas*. 2003;45(4):241–6.
5. Tousen Y, Ezaki J, Fujii Y, Ueno T, Nishimuta M, Ishimi Y. Natural S-equol decreases bone resorption in postmenopausal, non-equol-producing Japanese women: a pilot randomized, placebo-controlled trial. *Menopause*. 2011;18(5):563–74.
6. Burke GL, Legault C, Anthony M, Bland DR, Morgan TM, Naughton MJ, et al. Soy protein and isoflavone effects on vasomotor symptoms in peri- and postmenopausal women: the Soy Estrogen Alternative Study. *Menopause*. 2003;10(2):147–53.
7. Tice JA, Ettinger B, Ensrud K, Wallace R, Blackwell T, Cummings SR. Phytoestrogen supplements for the treatment of hot flashes: the Isoflavone Clover Extract (ICE) Study: a randomized controlled trial. *Jama*. 2003;290(2):207–14.
8. Weaver CM, Legette LL. Equol, via dietary sources or intestinal production, may ameliorate estrogen deficiency-induced bone loss. *The Journal of nutrition*. 2010;140(7):1377S–9S.
9. Setchell KD, Clerici C. Equol: history, chemistry, and formation. *The Journal of nutrition*. 2010;140(7):1355S-62S.
10. Effriyanti RY, Madjid TH, Syam HH. Correlation of Equol (7-Hydroxy-3-(4-Hydroxyphenyl) Chroman) in Woman Urine with the Symptoms of Menopause. *Journal of Biosciences and Medicines*. 2015;4(01):132.
11. Aso T. Equol improves menopausal symptoms in Japanese women. *The Journal of nutrition*. 2010;140(7):1386S–9S.
12. McEndree B. Clinical Application of the Vaginal Maturation Index. *The Nurse Practitioner*. 1999;24(9):48–57.
13. Rafii F. The role of colonic bacteria in the metabolism of the natural isoflavone daidzin to equol. *Metabolites*. 2015;5(1):56–73.
14. Utian WH, Jones M, Setchell KD. S-equol: A potential nonhormonal agent for menopause-related symptom relief. *Journal of Women's Health*.



- 2015;24(3):200–8.
15. Kaunitz AM, Manson JE. Management of menopausal symptoms. *Obstetrics & Gynecology*. 2015;126(4):859–76.
  16. Anwar Z. Menentukan Menopause Berdasarkan Indeks Maturasi dan pH Vagina. *Jumaf*. 2010;42(3):3000.
  17. Wines N, Willsteed E. Menopause and the skin. *Australasian journal of dermatology*. 2001;42(3):149–60.
  18. Greaves M. Flushing and flushing syndromes, rosacea and perioral dermatitis. *Textbook of dermatology*. 1998;3:2099–111.
  19. Lima SMRR, Yamada SS, Reis BF, Postigo S, da Silva MALG, Aoki T. Effective treatment of vaginal atrophy with isoflavone vaginal gel. *Maturitas*. 2013;74(3):252–8.
  20. Lima SMRR, Bernardo BFA, Yamada SS, Reis BF, da Silva GMD, Galvão MAL. Effects of Glycine max (L.) Merr. soy isoflavone vaginal gel on epithelium morphology and estrogen receptor expression in postmenopausal women: A 12-week, randomized, double-blind, placebo-controlled trial. *Maturitas*. 2014;78(3):205–11.