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The Effects of Ajwa Dates Consumption (*Phoenix dactylifera L.*), on Ovarian Follicular Features with Transvaginal Ultrasound in Perimenopausal Women

Erlin Syahril^a, Nusratuddin Abdullah^b, Muhammad Ilyas^c, Suryani As'ad^d, Lina Choridah^e, Rosdiana Natzir^f, Liong Boy Kurniawan^g, Burhanuddin Bahar^h, Nasrudin A. Mappaware^{i,*}, Farah Ekawati Mulyadi^j, Susdianman Sudin Yacca^k

^a Department of Radiology, Universitas Muslim Indonesia, Makassar, Indonesia; ^b Department of Obstetric and Gynecology, Hasanuddin University, Makassar, Indonesia; ^c Department of Radiology, Hasanuddin University, Makassar, Indonesia; ^d Department of Clinical Nutrition, University of Hasanuddin, Makassar, Indonesia; ^e Department of Radiology, Gajah Mada University, Yogyakarta, Indonesia; ^f Department of Biochemistry, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia; ^g Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia; ^h Faculty of Medicine, Hasanuddin University, Makassar, Indonesia; ⁱ Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Muslim Indonesia, Makassar, Indonesia; ^j Department of Physiology, Faculty of Medicine, Universitas Muslim Indonesia, Makassar, Indonesia; ^k Faculty of Medicine, Universitas Muslim Indonesia, Makassar, Indonesia

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Objective: Perimenopause is the transition from the reproductive period to menopause, marked by a decline in the number and function of oocytes. Transvaginal ultrasound is a reliable method for assessing ovarian reserve. Ajwa dates (*Phoenix dactylifera L.*) contain macro- and micronutrients, phenolics, and flavonoids, which have antioxidant potential that may improve ovarian condition in perimenopausal women. This study aims to determine the effect of ajwa date consumption on ovarian follicle characteristics assessed by transvaginal ultrasound in perimenopausal women.

Methods: This quasi-experimental pre-post control group study was conducted at RSIA Sitti Khadijah I Muhammadiyah Makassar, Indonesia, from February to August 2023. A total of 44 perimenopausal women aged 42-48 years were randomly assigned to intervention ($n = 28$) or control groups ($n = 16$). Ovarian follicle changes were analyzed using the Mann-Whitney U test.

Results: The intervention group showed a decrease in follicle number with an increase in follicle size, while the control group showed an increase in follicle number and a decrease in size. These differences were statistically significant ($P < 0.05$).

Conclusion: Consumption of ajwa dates as an exogenous antioxidant can influence the number and size of antral follicles, which serve as predictive indicators of menopausal transition.

Key words: Ajwa dates; Antral follicle; Perimenopause; Transvaginal ultrasound

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* Corresponding author: Faculty of Medicine Universitas Muslim Indonesia, Makassar, Indonesia, Urip Sumoharjo Street No.KM. 5, Makassar, South Sulawesi. (Erlin Syahril)
e-mail: nasrudin.nasrudin@umi.ac.id (N.A. M)

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The period of transition from reproduction to menopause is known as perimenopause. In this condition, there is a decrease in the number and function of oocytes as well as noticeable endocrine changes. Endocrine changes are primarily associated with a decrease in negative feedback coming from ovarian hormones on the hypothalamic-pituitary axis due to a decrease in antral follicles that occurs with age. In this perimenopausal phase, there is a gradual increase in FSH levels in response to a decrease in ovarian hormone levels, followed by the next stage of noticeable menstrual cycle irregularities [1,2].

The measurement of the number of antral follicles by transvaginal ultrasound is a promising examination and is one of the highest predictive ovarian reserve tests [3,4]. Measurement of AFC (antral follicle count) by transvaginal ultrasound is a reliable alternative method of detecting ovarian reserve.

Ajwa dates with a high polyphenol content (50.2 mg/g), especially epicatechins and catechins, provide antioxidant effects both in vivo and in vitro. In addition, ajwa dates also contain phytochemical compounds such as phytosterols and phytoestrogens. Phytoestrogens are useful for modulating the body's estrogen and have proapoptotic and antioxidant activity. Ajwa dates, which also contain quercetin and contain phenolic components, and other flavonoids, are considered potential to improve the condition of the ovaries of perimenopausal women which can be assessed through an increase in the number of antral follicles measured by transvaginal ultrasound.

Various studies to examine the effects of dates on health have been widely developed, including the effect of ajwa dates on women's reproductive health. However, research linking the effect of ajwa dates on ovarian reserve and its relation to the prediction of menopause time is still limited. For this reason, researchers are interested in testing the effect of giving ajwa dates on ovarian reserve which is evaluated through observation of ovarian follicle images with transvaginal ultrasound examination.

Methods

Study design

Quasi-experimental research with a case control approach using a pre-posttest control design, where variable measurements were carried out before and after the intervention. This quasi-experimental study with a pre-post control design involved 50 perimenopausal women aged 43-48 years, conducted at Sitti Khadijah 1 Muhammadiyah Hospital, Makassar, Indonesia, from February

to August 2023.

Samples were randomly taken, namely perimenopausal patients undergoing outpatient treatment at RSUD Sitti Khadijah 1 Makassar, Indonesia. Women are eligible to participate if they meet the following criteria: age 43-48 years; perimenopause at the start of the study; married; have children; have a normal BMI; and are willing to be a sample and sign a statement of willingness to be a respondent. A participant is considered perimenopausal if he or she reports menstrual irregularities lasting less than 12 consecutive months. Perimenopausal women who met the inclusion criteria were randomly selected to be grouped into an intervention group (30 people) and a control group (20 people).

Patients are excluded if they undergo screening with fasting blood glucose results ≥ 126 mg/dL and oral glucose tolerance test ≥ 200 mg/dL; have a history of ovarian disorders (PCOS) or a history of ovarian surgery; smoke; while using hormonal contraceptives; have a history of suffering from chronic infectious diseases (eg tuberculosis, malaria, thalassemia); and suffering from degenerative non-infectious diseases (cardiovascular disease, cancer, chronic kidney failure, and DM).

Antral follicle examination was performed on the intervention group after 8 weeks of consuming Ajwa dates to measure the number and assess the diameter of antral follicles in both ovaries. Based on the participant criteria, participants included in the intervention group were given 7 ajwa dates per day to be consumed every morning before breakfast for 8 weeks. The ajwa dates used in this study were fresh, ripe, medium-sized, and soft-fleshed dates weighing 60-80 gr/7 dates. Ajwa dates are cultivated in the city of Al Madina Al-Munawwarah and purchased from the date distributor "Al Ma'aliy". Participants included in the control group did not consume any dates.

The procedure for transvaginal ultrasound examination is that the subject is asked to remove his underwear and then lie on a special bed with both legs bent and placed on special support so that it is wide open (lithotomy position). Insert a previously gel-smeared transducer into the subject's vagina. The scan begins by looking at the uterus longitudinally and cross-sectionally and measuring the endometrium, after which both ovaries in both longitudinal and cross-sectional appearance are scanned to assess the volume of the overall ovary and the antral follicles are counted from 3 to 10 mm in diameter.

Assessments

To calculate BMI, weight measurement is done using a digital scale, while height measurement is done using a microtome. Measurement of fasting blood glucose and

oral glucose tolerance test was carried out by taking blood samples of participants. Abdominal ultrasound is done to see if there are ovary-related abnormalities (PCOS). The study subjects were given a general explanation of perimenopause and specifically explained the benefits of consuming ajwa dates 7 grains every morning for health. Transvaginal ultrasound was performed before and after treatment in the control and intervention groups to assess the number and size of ovarian follicles. Follicle size was measured in both ovaries, and the values were averaged to obtain a single mean size per participant.

Statistical analysis

Data analysis used the Mann-Whitney test to compare the size and number of antral follicles of transvaginal ultrasound between the intervention group and the control group. Statistical analysis using SPSS 26.0.

Results

The study was conducted on 50 research subjects divided into 30 intervention group subjects and 20 control group subjects. Of the 30 subjects in the intervention group, 2 subjects dropped out because they could not be contacted when they were about to take post-intervention blood, while in the control group, 4 subjects were not willing to have post-intervention blood draws. So the total subjects until the end of this study amounted to 44 people consisting of 28 intervention group subjects and 16 control group subjects.

A comparison of ovarian features between the intervention and control groups assessed through transvaginal ultrasound examination included the number of follicles and the size of ovarian follicles as shown in tables 1 and 2 below.

Based on table 1, the number of ovarian follicles imaged through transvaginal ultrasound in the intervention group was reduced by an average decrease of 4.14 ± 2.27 follicles compared to the number of follicles in the control group which increased by 2.12 ± 3.5 follicles with a statistically significant p value of <0.001 .

Table 2 shows that the size of ovarian follicles in the intervention group after treatment increased by 0.21 ± 0.20 cm compared to follicles in the control group which decreased by 0.01 ± 0.46 cm showing a statistically significant difference with a p-value of 0.014.

Discussion

The use of transvaginal ultrasound in assessing ovarian reserve by detecting the number of ovarian follicles is very easy, minimally invasive, inexpensive, and has a good predictive value to detect an increase or decrease in ovarian function AFC has long been used as a marker of ovarian reserve. A follicle count of 8-10 is considered a predictor of normal response. Different diameter sizes are used to determine antral follicles measuring 2-6 and 7-10 mm. There is no stated consensus as to the exact size of the antral follicle that truly represents ovarian reserve. It was found that the number of antral follicles measuring 2-6 mm decreased with age and correlated with other markers such as FSH but antral follicles measuring 7-10 mm in size remained constant and hence, the appearance of follicles became a better marker of ovaries than simply measuring the number of follicles [4].

During perimenopause, there is a small but important increase in FSH. This increase in FSH causes more follicles to be recruited thus leading to an increase in the rate of follicular depletion, because these follicles, usually do not develop in the direction of ovulation, but

Table 1 Comparison number of ovarian follicles in the intervention and control groups

Group	Number of follicles ^a		Average Δ number of follicles ^a	P value ^b
	Before	After		
Intervention	10.11 ± 2.18	5.96 ± 1.99	$\downarrow 4.14 \pm 2.27$	< 0.001
Control	9.31 ± 2.73	11.44 ± 3.46	$\uparrow 2.12 \pm 3.5$	

^a Mean $\pm$ Deviation standard, ^b Mann - whitney U-test

Table 2 Comparison of ovarian follicular size in the intervention dan control groups

Group	Size of follicles (cm) ^a		Average Δ size of follicles (cm) ^a	P value ^b
	Before	After		
Intervention	0.55 ± 0.15	0.76 ± 0.18	$\uparrow 0.21 \pm 0.20$	0.014
Control	0.74 ± 0.39	0.73 ± 0.29	$\downarrow 0.01 \pm 0.46$	

^a Mean $\pm$ Deviation standard, ^b Mann-whitney U-test

instead end up in involution [5]. An imbalance of the hypothalamus-pituitary-ovarian axis feedback loop in perimenopause causes most of the ovarian cycles women experience to be anovulation. There is a disruption in the positive feedback of estradiol to LH, where in the early stages of folliculogenesis there is a rapid increase in estradiol (no evidence is found whether this increase peaks or not) but this increase in estradiol is not followed by a surge in LH and ends in a cycle without ovulation [6]. The results of this study showed that the number of follicles that developed in the intervention group was less than the control showed the benefit of consuming ajwa dates to suppress the rate of follicular depletion. The follicle size in the intervention group was larger than the control group, showing the effect of ajwa date fruit consumption on improving follicle response and function.

The findings in this study are supported by previous studies on the effect of ajwa date consumption on anti-mullerian hormone (AMH) levels in perimenopausal women which showed that ajwa date consumption was able to inhibit the decline in AMH levels in perimenopausal women. AMH can act as a follicle gatekeeper and ensure that each small antral follicle produces a small amount of E2 prior to selection (i.e. up to a follicle diameter of 8 mm) allowing regulation of the development of selected follicles to be ovulating and inhibiting the rate of follicular depletion [7].

Another study examining the effects of quercetin and rosuvastatin in either single or combined administration on cyclophosphamide-induced POF (premature ovarian failure) in albino female rats found that there was a protective effect of quercetin and rosuvastatin on ovarian toxicity and cyclophosphamide-induced POF. This was evaluated by assessing the presence of a dose-dependent increase in the number of primordial follicles, an increase in serum anti-Mullerian hormone levels, and ovarian tissue glutathione levels ($P < 0.05$), as well as a decrease in the number of dose-dependent atretic follicles and also a decrease in ovarian tissue malondialdehyde levels ($P < 0.05$) compared to the cyclophosphamide only group [8].

The protective role of ajwa dates on the ovaries can also be caused by quercetin content ($1,219 \pm 0,071$ mg/100g) [9]. Quercetin is the bioactive form of flavonoids and has extensive biological benefits, including antioxidant, anti-inflammatory, antiapoptotic, and stimulating mitochondrial biogenesis. In laboratory animals, quercetin has been shown to increase ovarian volume and prevent follicular cell degeneration, bleeding, poor flow in blood vessels, and edema while decreasing follicular cell apoptosis [8,10]. Ajwa dates containing

phenolic components and flavonoids are considered potential to improve the condition of the ovaries of perimenopausal women which can be assessed through ovarian images with transvaginal ultrasound.

Other studies have also proven the benefits of exogenous antioxidant supplementation on female reproductive function, where it was found that AMA (advanced maternal aged, with age ≥ 39 years) patients given antioxidant supplementation had promising reproductive results comparable to younger control groups. Research conducted by Dijazi et al. in 2020 documented that supplementation of 200 μ g of selenium and 400 units of vitamin E can increase ovarian reserve. These results support our findings because analysis of the mineral content of ajwa dates also revealed a selenium content of 0.24-0.4 mg/100 g of Ajwa dates [9,10]. Selenium plays a role in forming the structure of the enzyme selenoprotein GPX1 which is one of the important cofactors in antioxidant enzymes. These enzymes in the ovaries accumulate in healthy, large follicular granulosa cells but are not found in small, atretic follicles [11]. Selenium can act as an antioxidant where selenium is mainly converted into selenomethionine and selenocysteine and incorporated into proteins as a substitute for methionine/cysteine which is essential for the synthesis of all selenoproteins such as PGX, TXNRD, SEPHS2, SELENOF, SELENOH, and SELENOM. Selenium mediates NF- κ B inhibition, and inflammatory cytokine expression and increases antioxidant capacity through Nrf2 activation, thereby reducing cytotoxicity caused by ROS. As a component of seleno-proteins such as GPx and thioredoxin reductase, selenium has an important role in redox reactions in the uterus, granulosa cells, and follicles [12,13].

Transvaginal ultrasound has been identified as one of the most reliable procedures for measuring ovarian reserve in perimenopausal women. In comparison to biochemical indicators such as serum FSH, oestradiol, inhibin B, or AMH, antral follicle count (AFC) collected via ultrasonography allows for direct visualisation of follicular development, is minimally intrusive, and provides real-time information about ovarian function. Several studies have established that AFC evaluated by transvaginal ultrasound is closely connected with ovarian ageing and has a greater prognostic value for reproductive potential than hormonal indicators [4,12]. Furthermore, it is an essential tool for assessing the perimenopausal transition due to its reproducibility and capacity to measure the quantity and size of follicles. Therefore, the use of transvaginal ultrasonography in this study emphasises its therapeutic significance for directing patient care, predicting menopausal state, and

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conducting research.

Transvaginal ultrasound also has the significant benefit of being able to offer data on ovarian volume, stromal blood flow, and follicular growth dynamics parameters that serum indicators are unable to measure directly. There is a strong correlation between less ovarian reserve and an earlier onset of menopause and decreased ovarian volume and AFC, according to studies [11,13]. Furthermore, three-dimensional ultrasound techniques and Doppler imaging have greatly enhanced the accuracy of ovarian assessment by allowing examination of vascularization and follicular health [12]. Therefore, transvaginal ultrasonography is a feasible, non-invasive, and clinically beneficial approach in daily gynaecological practice for counselling women in perimenopause.

Conclusion

Consuming Ajwa dates as an exogenous antioxidant can affect the number and size of antral follicles. Importantly, transvaginal ultrasound, as a reliable and clinically significant tool to evaluate these parameters, provides valuable insight into ovarian reserve assessment, supports menopausal prediction, and has broader implications for reproductive health management in perimenopausal women.

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Authors' Contributions

ES and NA conceived of and designed the study; ES, MI and SA drafted the manuscript; RN and LBK performed statistical design and analysis; BB and NAM performed the research; FEM and SSY acquired and interpreted of the data for the work. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Data Availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethics Commission of Health Research of the Faculty of Medicine in Universitas Hasanuddin acceded to this study with approval number 340/UN4.6.4.5.31/PP36/2023 with protocol number UH23030176. The authors confirmed that all methods were carried out following the relevant guidelines and regulations (Helsinki Declaration).

Conflict of interest

We, the authors, declare that we have no competing interests.

Consent for publication

Not applicable.

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