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Original Article

Anatomy Section

Ultrasonographic Assessment of Ovarian Volume: A Cross-sectional Study to Evaluate Ovarian Reserve and its Impact on Reproductive Potential

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ABSTRACT

Introduction: Ovarian Reserve (OR) serves as an important indicator of a female's reproductive potential and remaining reproductive lifespan. With the societal shift towards delayed marriages and increased career focus among women, there is a growing need to understand the impact of age on fertility and the role of assisted reproductive techniques.

Aim: To evaluate the volume of both left and right ovaries in asymptomatic females using ultrasonographic measurement of various morphometric parameters.

Materials and Methods: This cross-sectional observational study was conducted in the Department of Anatomy, in association with the Department of Obstetrics and Gynaecology and Department of Radiodiagnosis, King George's Medical University, Uttar Pradesh, Lucknow, India, from September 2016 to June 2017. Total 100 non pregnant females aged 19-49 years were included in the study. Ultrasonography was used to measure the Longitudinal Length (LL), Transverse Diameter (TD), and Anteroposterior Diameter (APD) of both ovaries (right and left). The volume of each ovary was calculated using the formula: 0.523×LL×TD×APD. Statistical Package for the Social Sciences (SPSS) software version 24.0 was used to assess the maximum, minimum, and mean±Standard Deviation (SD) for all the morphometric parameters of the right and left ovaries. A paired t-test was used to compare these morphometric parameters, and a p-value <0.05 was considered statistically significant.

Results: The mean±SD age of the study participants was 34.06±9.35 years. The mean±SD of LL, TD, APD, and volume for the right ovary were 3.12±0.29 cm, 2.37±0.24 cm, 1.76±0.19 cm, and 6.78±1.08 cm^3, respectively. The mean±SD of LL, TD, APD, and volume for the left ovary were 3.08±0.45 cm, 2.32±0.25 cm, 1.76±0.18 cm, and 6.61±1.06 cm^3, respectively. A comparison between these morphometric parameters of the right and left ovaries did not reveal any statistically significant differences.

Conclusion: The use of sonography is rapidly increasing nowadays in the field of gynaecology and reproductive medicine, and ovarian parameters are of great importance in diagnosing ovarian abnormalities in adolescents, PCOS, and ovarian cancer. In the field of reproductive medicine, ultrasound is helpful in serial follicular monitoring of females on ovulogens to assess their ovarian reserve.

Keywords: Anteroposterior diameter, Infertility, Longitudinal length, Ultrasound

INTRODUCTION

Ovarian reserve acts as a marker that can give the idea of reproductive potential of a female and her residual reproductive life span. It gives an estimate of number of primordial follicles/oocytes remaining in the ovaries [1,2]. Our society is undergoing great socio-economic reforms. Females of the current era are more career-oriented, therefore they are getting married late. Late marriages result in delayed childbirth. The fertility of these females is significantly decreased compared to females who marry at a young age. This leads to an increased frequency of assisted reproductive techniques for pregnancy [3,4]. Extensive researches suggest that a reduction in ovarian volume is suggestive of a poor prognosis for assisted reproduction [5-7].

Female infants have approximately one to two million oocytes at the time of birth. Over time, the number of oocytes reduces due to ovulation, and it is approximately 300,000-400,000 at the age of menarche [8]. A slow depletion of the number of oocytes continues as age advances, and this number reaches approximately 25,000 at the age of 37-38 years [9]. The depletion of follicles continues until menopause, where a few hundred follicles are left in the ovaries [10]. It is very difficult to count the Non Growing Follicles (NGF), so OR can be tested by biochemical tests and ultrasound imaging of the ovaries. Biochemical tests include measurements of Follicle Stimulating Hormone (FSH), Estradiol (E2), Inhibin B, Anti-mullerian Hormone (AMH), and Clomiphene Citrate Challenge Test (CCCT). Sonographically, OR can be tested by measuring the ovarian volume. Ovarian functions and OR can be well predicted by sonographic estimation of AFCs [11].

Endocrine and metabolic disorders are affecting women of reproductive age globally. For the past few years, Polycystic Ovary Syndrome (PCOS) has become very common. It is negatively associated with the pregnancy rate because of elevated androgen levels. A few constraints were highlighted with the estimation of androgen levels, such as which androgen to be measured and also variations in laboratory methods for measuring androgen levels. To minimise this challenge, scientists advised having a non invasive measure that can accurately identify females with PCOS and androgen excess. Hence, sonography of ovaries has been included in the diagnostic work-up, as ovarian volume constitutes the best sonographic indicator for PCOS [12].

Ovarian cancer is diagnosed annually in nearly 250,000 females worldwide and leads to the death of approximately 140,000 females per year. Ovarian cancer has a high mortality rate because of non specific early symptoms. Sometimes, it is asymptomatic and diagnosed in the last stage. Hence, there is a need for a population-based screening program for females, of which sonography is most popularly used along with Cancer Antigen 125 (CA125) [13].

Ultrasound is an inexpensive, uncomplicated, and easily accessible method, and the quality of images produced by ultrasound is very much equivalent to that of other imaging techniques. Though Computerised Tomography (CT) and Magnetic Resonance Imaging (MRI) techniques are available for the location and morphometry of ovaries, they are high-priced and associated with more complications of ionising radiation (CT), in comparison to ultrasonography. In ultrasonography, there is no need for any contrast medium, and there is no risk of ionising radiations; therefore, the procedure can be repeated multiple times [14].

To the best of authors knowledge, very limited research studies are freely available in the online published literature of the English language on the morphometric parameters of ovaries in normal females of North India [15]. As the authors did not find any research study on the morphometry of ovaries in normal females from the Eastern Uttar Pradesh region of India, the authors have measured this organ of utmost reproductive importance using the ultrasonographic method. The findings of the present study will supplement the normative data on ovarian parameters covering a wide age range (from 19-49 years) of North Indian females. The nomogram of ovarian morphometric parameters furnished by the present study will be immensely useful for gynecologists and infertility specialists.

MATERIALS AND METHODS

The present cross-sectional observational study was conducted in the Department of Anatomy, in association with the Department of Obstetrics and Gynecology and Department of Radiodiagnosis, King George's Medical University, Uttar Pradesh, Lucknow, India, from September 2016 to June 2017. Study was conducted after obtaining approval from the Institutional Ethical Committee (Ref. no: 0046/Ethics/R.Cell-16). One hundred non pregnant females aged 19-49 years were included in the study.

Sample size calculation: Formula used to calculate sample size was, $N=\{Z_{\alpha/2}^2 \times p \times (1-p)\}/d^2$

Where, $Z_{\alpha/2}$=critical value of the normal distribution at $\alpha/2=1.96\sim2$ for a 95% confidence level, power 80%, and α is 0.05; p=sample proportion=0.50, i.e., 50% (considered for normative data amongst non pregnant females viz., an infinite population); d=margin of error=0.10, i.e., 10%. Hence, sample size $(N)=22\times0.5\times0.5/(0.1)^2=4\times0.25/0.01=1.00/0.01=100$.

Inclusion criteria: The females who were not pregnant at the time of the study and had a normal regular menstrual cycle were included in the study.

Exclusion criteria: Females taking Oral Contraceptive Pills (OCP) or using any intrauterine device, using oral or injectable ovulation induction drugs, taking Hormonal Replacement Therapy (HRT), suffering from carcinoma of the breast, ovary, or uterus, diagnosed with a pelvic mass, or operated for gynaecological/ obstetrical pathology were excluded from the study.

Study Procedure

In the Obstetrics and Gynecology Department, the GE Logiq 200 PRO Series ultrasound system with a 3.5 MHz frequency probe was used for sonography. In the Radiodiagnosis Department, the Philips Affiniti 70 ultrasound and colour Doppler system with a 1-5 MHz frequency C5-1 probe was used for sonography of females. The following parameters of both ovaries (right and left) were measured:

(i) Longitudinal Length (LL);
(ii) Transverse Diameter (TD);
(iii) Anteroposterior Diameter (APD).

The volume of the ovary was calculated using the following formula [16]: 0.523×LL×TD×APD.

The ultrasound scanning was carried out in the dorsal decubitus position. The urinary bladder was kept full for proper visualisation of the ovaries. Ultrasound conductive gel was applied to the skin and transducer so that the transducer can move smoothly on the skin. The ultrasound probe was placed in the suprapubic area along the midline with the probe indicator facing upward. The bladder was identified as an acoustic shadow. Now the probe was rotated 90° anticlockwise so that the indicator of the probe lies on the subject's right side. By rocking the tail of the probe to the right and left side of the subject, the authors visualised the right and left ovaries, respectively. Usually, the ovaries are situated posterolateral to the uterus and anterior to the internal iliac artery and vein. The long axis of the ovary was directed downward and forward. The maximum LL and APD were assessed in the longitudinal plane, while the transverse plane was used for measuring TD [17].

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS software version 24.0 (IBM, Chicago, Illinois, USA). A descriptive statistic showing the maximum, minimum, and mean±SD for all the morphometric parameters of the right and left ovaries was used. Also, a comparison between these morphometric parameters of the right and left ovaries was made using a paired t-test, and statistical inferences were drawn. The p-value <0.05 was regarded as statistically significant.

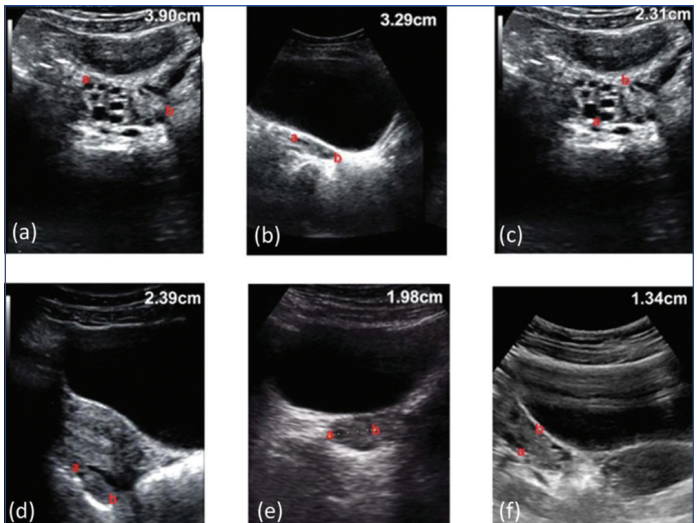
RESULTS

The study involved ultrasonographic assessment of various morphometric parameters of the right and left ovaries among 100 non pregnant females aged 19-49 years (mean age: 34.06±9.35 years) [Table/Fig-1].

Age (years)	Number (n)
19-23	19
24-28	14
29-33	14
34-38	13
39-43	19
44-49	21
N	100

[Table/Fig-1]: Age-wise distribution of females.

It was observed that the maximum and minimum LL of the right ovary were in females aged 32 years and 45 years, respectively. The maximum and minimum TD were found in females aged 45 years and 43 years, respectively. The maximum and minimum APD were seen in females aged 32 years and 45 years, respectively. The maximum LL and maximum AP diameter were observed in the same subject aged 32 years [Table/Fig-2].

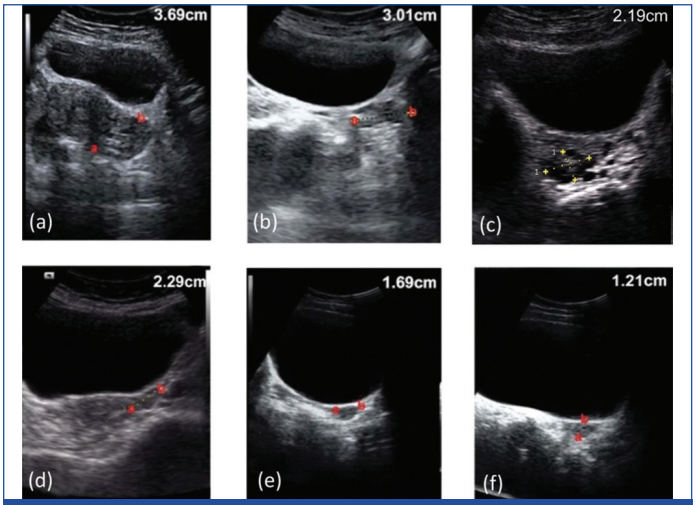


[Table/Fig-2]: Ultrasonographic images showing morphometric parameters of right ovary. (a) Maximum LL (32-year-old female); (b) Maximum TD (45-year-old female); (c) Maximum APD (32-year-old female); (d) Minimum LL (45-year-old female); (e) Minimum TD (43-year-old female); (f) Minimum APD (45-year-old female).

Subsequently, the ovarian volume was calculated using the formula. The mean volume of the right ovary was 6.78±1.08 cm^3 [Table/Fig-3]. It was also observed that the maximum and minimum LL of the left ovary were in females aged 41 years and 45 years, respectively. The maximum and minimum TD were found in females aged 26 years and 45 years, respectively. The maximum and minimum APD were seen in females aged 32 years and 45 years, respectively [Table/Fig-4].

Parameters	Maximum	Minimum	Mean±SD	95% CI	
				Upper	Lower
Longitudinal Length (LL) (cm)	3.90	2.39	3.12±0.29	3.17	3.06
Transverse Diameter (TD) (cm)	3.29	1.98	2.37±0.24	2.42	2.32
Anteroposterior Diameter (APD) (cm)	2.31	1.34	1.76±0.19	1.80	1.72
Volume (cm^3)	9.71	3.78	6.78±1.08	7.00	6.57

[Table/Fig-3]: Morphometric parameters of right ovary.



[Table/Fig-4]: Ultrasonographic images showing morphometric parameters of left ovary. (a) Maximum LL (41-year-old female); (b) Maximum TD (26-year-old female); (c) Maximum APD (32-year-old female); (d) Minimum LL (45-year-old female); (e) Minimum TD (45-year-old female); (f) Minimum APD (45-year-old female).

Subsequently, the ovarian volume was calculated using the formula. The mean volume of the left ovary was 6.61±1.06 cm^3 [Table/Fig-5], and it was slightly lower than the mean volume of the right ovary as already mentioned above.

Parameters	Maximum	Minimum	Mean±SD	95% CI	
				Upper	Lower
Longitudinal Length (LL) (cm)	3.69	2.29	3.08±0.45	3.13	3.03
Transverse Diameter (TD) (cm)	3.01	1.69	2.32±0.25	2.37	2.28
Anteroposterior Diameter (APD) (cm)	2.19	1.21	1.76±0.18	1.80	1.73
Volume (cm^3)	9.13	2.45	6.61±1.06	6.82	6.40

[Table/Fig-5]: Morphometric parameters of left ovary.

When the morphometric parameters of ovaries of right and left-side were compared, there was no statistically significant difference [Table/Fig-6].

Parameters	Mean df.	SD	t-value	p-value
Longitudinal Length (LL) (cm)	0.04	0.34	1.074	0.286
Transverse Diameter (TD) (cm)	0.04	0.31	1.398	0.165
Anteroposterior Diameter (APD) (cm)	0.00	0.22	0.218	0.828
Volume (cm^3)	0.17	1.11	1.551	0.124

[Table/Fig-6]: Comparison between morphometric parameters of right and left ovaries (using paired t-test).

DISCUSSION

The ovary provides oocytes and sex hormones, estrogen and progesterone; therefore plays a crucial role in the establishment of pregnancy and the development of secondary sexual characteristics in females [18]. Kelsey TW and Wallace WHB (2012) stated that growing and Non Growing Follicles (NGFs) and the stromal tissues constitute the important components of the ovary. They found a very strong and positive correlation between the mean ovarian volume and the mean NGFs population in females aged 25-51 years. They hypothesised that there is a lesser number of non growing follicles in small ovaries. According to their calculation, a population of 1000 NGFs corresponds to 3.01 cm^3 of ovarian volume at any age [3]. Lass A et al., suggested that there is a >50% risk of abandonment of IVF cycle prior to the recovery of oocytes in females having small ovaries of <3 cm^3 [19]. Sharma N et al., also supported this statement by saying that the ovarian volume at a cut-off of 3 cm^3 is a highly specific predictor for cycle cancellation and non pregnancy in 92-93% of cases of assisted reproduction [20]. In the present study, the minimum value for the volume of the right ovary is 3.78 cm^3, which is higher than the cut-off value of 3 cm^3. Therefore, it can be infered that the females in the present study have good reproductive potential.

Primordial follicles or NGFs constitute the functional unit of reproduction, and their aggregate forms the Ovarian Reserve (OR) [21]. Wallace WH and Kelsey TW hypothesised that ovarian volume helps in the determination of reproductive age and OR in females. They said that the age of menopause can be predicted using ovarian volume, but this prediction will only be applicable to women who have no history of ovarian pathology and who are not utilising hormonal contraceptive methods [22].

Ovarian volume constitutes a useful indirect criterion of OR. According to some studies, poor outcomes in assisted conception can be fairly predicted by reduced ovarian volume [3]. It has been observed by various authors that ovarian volume is a good indicator for predicting OR and clinical pregnancy rate in combination with Antral Follicle Count (AFC) [23,24].

Ovarian volume is the principal factor in diagnosing various pathological entities of the ovaries [16]. By adding the volumes of both ovaries, the Total Basal Ovarian Volume (TBOV) can be derived [20]. In female neonates, the ovarian volume is around 1 cm^3. Prepubertal ovarian volume is less than 2 cm^3, and both the size and morphology remain relatively stable between the ages of two and nine years. Premenarchal ovarian volume ranges from 2-4 cm^3. Postmenarchal ovarian volume is more than 4 cm^3 [25]. Ovarian volume does not change much during the reproductive years of a female until menopause. A decrease in ovarian size is seen in menstruating women around the age of 40 years [19]. According to the first normative model of ovarian volume, which was generated and robustly validated by Kelsey TW et al., age is the only factor that causes 69% of the variations in ovarian volume. They observed a peak of ovarian volume at the age of 20 years [26]. In the present study, we also found that the maximum right ovarian volume occurred at the age of 20 years. According to a three-parameter cumulative Lorentzian normative model of ovarian volume, the volume starts to decline rapidly around the age of 33 years, and the rate of decline slows around the age of 47 years. It is worth noting that the rate of decline of ovarian volume changes substantially with ethnicity [20].

The findings of Korsholm AS et al., are in concordance with the report of Kelsey TW and Wallace WHB, which demonstrates a positive correlation between ovarian volume and the count of NGFs (antral follicles). They found that the right ovary had 8.1% more antral follicles and a 10.7% larger volume compared to the left ovary [3,27]. In the present study as well, we observed that the mean volume of the right ovary was greater than the mean volume of the left ovary. This association may be explained by the establishment of a larger pool of primordial follicles in the right ovary during foetal life. Different

studies have reported conflicting results regarding the volume of the right and left ovaries. Some authors reported a higher volume of the right ovary [28,29]. While others reported a larger volume of the left ovary [30-32]. Upadhyaya RP et al., also found larger volume in the left ovaries, and no significant variation was observed in the volumes of the right and left ovaries [16]. Additionally, Veena M and Banerjee C found equal volumes of both ovaries [33]. In our study, we did not find any statistically significant difference between the volumes of the right and left ovaries. The ovarian volumes reported in different studies have been tabulated in [Table/Fig-7] [16,28-33].

Author and year of the study	Study place and age range (years)	Sample size	Method	Mean ovarian volume±SD (cm^3)	
				Right ovary	Left ovary
Wehba S et al., (1996) [28]	Brazil, 20-40	40	Transvaginal Ultrasonography (USG)	6.5±2.2	6.3±2.0
Joseph E et al., (2009) [32]	Nigeria, 16-45	141	Transabdominal USG	9.5	10.0
Nwankwo NC and Madufuro CO (2011) [30]	Nigeria, 18-43	50	Transvaginal USG	9.70±2.1	10.35±1.8
Qasim R et al., (2012) [29]	Bangladesh, 13-45	60	Cadaveric Dissection	8.64±0.89	8.61±0.89
Mohammad H et al., (2013) [31]	North-central Nigeria, ≤20-≥50	207	Transvaginal USG	6.4±3.8	6.5±3.3
Upadhyaya RP et al., (2020) [16]	Nepal, 16-60	305	Transabdominal USG	5.94±2.70	6.05±2.79
Veena M and Banerjee C (2020) [33]	India, 21-30	15	Transvaginal and Transabdominal USG	6.12	6.12
Present study	North India, 19-49	100	Transabdominal USG	6.78±1.08	6.61±1.06

[Table/Fig-7]: Ovarian volume in various studies [16,28-33].

Ovarian volume is a helpful predictor of the success of In Vitro Fertilisation (IVF) along with the total antral follicular count. Lass A et al., in their study on infertile females with a mean age of 35.8 years, observed that females with an ovarian volume of less than 3 cm^3 were poor responders to Human Menopausal Gonadotropins (HMG), which is a combination of FSH and Luteinising Hormone (LH). These individuals required a higher dose of HMG for oocyte retrieval. Therefore, measuring ovarian volume has clinical importance as a decrease in ovarian volume indicates a reduction in ovarian follicles and aging of the ovaries. Furthermore, they observed that the risk of Ovarian Hyper Stimulation Syndrome (OHSS) is very high in females with an ovarian volume exceeding 7.25 cm^3. Females with ovarian volume less than 7.25 cm^3 required a lesser quantity of FSH and fewer days of stimulation, and they expressed a higher ovulation rate [19]. In the present study, the females had an ovarian volume less than 7.25 cm^3. Therefore, if ovulation induction occurs in the current studied population group, OHSS will not be evident in the North Indian population.

Limitation(s)

In the present study, the authors could not take into consideration the menstrual cycle history of the studied female population. Moreover, the ultrasonography method used in the present study was transabdominal rather than a transvaginal scan, which could have been a better approach. Additionally, various anthropometric parameters were not considered in the current study, which could have provided a more specific nomogram for our region.

CONCLUSION(S)

This study establishes normative data for the morphometric parameters of ovaries in asymptomatic females ranging from 19-49 years. The mean volume of the left ovary was slightly lower than the mean volume of the right ovary, but the results revealed no statistically significant differences between the morphometric parameters of the right and left ovaries. These findings provide essential insights for gynaecologists and infertility specialists in understanding ovarian reserve and its implications for assisted reproductive techniques.

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