Clinical Utility of Serum Dehydroepiandrosterone Sulfate (DHEA-S) and Serum Anti-Mullerian Hormone (AMH) Levels in Infertile Females

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ABSTRACT

Objective: To determine the correlation of serum dehydroepiandrosterone sulphate and anti-mullerian hormone levels with infertility in females.

Study Design: Cross-sectional study.

Place and Duration of the Study: Department of Chemical Pathology, the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from July 2022 to August 2023.

Methodology: About 110 infertile females with infertility were enrolled in this study. Serum thyroid-stimulating hormone, luteinising hormone, testosterone, follicle-stimulating hormone (FSH), estradiol, serum Anti-Mullerian Hormone (AMH), and Dehydroepiandrosterone Sulphate (DHEA-S) were measured at 3rd menstruation day. Females with primary ovarian insufficiency, using androgen supplementation, and on hormonal therapy were excluded.

Results: Among the participants, 63.6% had primary infertility and 36.4% had secondary infertility. Both primary and secondary infertility had a positive correlation with serum AMH and DHEA-S levels ($r = 0.685$; $p < 0.001$) and ($r = 0.807$; $p < 0.001$), respectively. After ultrasound, 54 (49.1%) females were normal, 12 (10.9%) had PCOS, 12 (10.9%) had fibroids, 8 (7.3%) had fallopian tube defects, 10 (9.1%) had endometriosis, and 14 (12.7%) had low antral follicular count. The correlation between serum AMH and DHEA-S with different subgroups is as follows: Normal females ($r = 0.731$; $p < 0.001$), PCOs ($r = -0.232$; $p = 0.468$), fibroids ($r = 0.941$, $p < 0.001$), fallopian tube defects ($r = -0.800$; $p = 0.017$), endometriosis ($r = -0.684$, $p = 0.013$), and low antral follicular count ($r = 0.643$, $p = 0.0130$).

Conclusion: This study demonstrated a positive correlation between serum AMH and DHEA-S levels in infertile females.

Key Words: *Infertile women*, *Ovarian reserve*, *Anti-Mullerian Hormone, Dehydroepiandrosterone sulphate*.

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INTRODUCTION

Infertility is defined as the inability to conceive subsequent to one year or more of continuous unprotected sexual activity.[1] Primary infertility delineates a scenario where an individual has never attained pregnancy, while secondary infertility denotes instances where a minimum of one antecedent pregnancy has been achieved.[2] About 8-12% of couples in their reproductive periods have been reported to be affected by infertility all over the world.[3]

Approximately eight weeks after conception, the developing human foetus possesses a pair of ducts known as the Wolffian and Mullerian ducts.[4]

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When the foetus carries male genetic material (XY chromosomes), the embryonic testes produce a substance called Anti-Mullerian Hormone (AMH).[5] AMH inhibits the initial follicle recruitment and is primarily synthesised by the granulosa cells of early follicles. Its secretion starts with follicle differentiation, declines during the antral stage, and diminishes further during dominant follicle selection stimulated by follicle-stimulating hormone (FSH). AMH prevents early follicular development; its absence accelerates primordial follicle recruitment, depleting the pool prematurely.[6] AMH correlates positively with growing follicles, acting as a surrogate marker for ovarian reserve, crucial in predicting infertility treatment success.[7] On the other hand, age and serum AMH levels are negatively correlated in females. Serum AMH levels start to progressively decline beyond the age of 25, reaching undetectable levels after menopause. Given that serum AMH levels decline annually with age, it is likely that these levels might be used to estimate a woman's natural fecundability.[8]

Dehydroepiandrosterone (DHEA), an endogenous steroid hormone having 19 carbons, is spontaneously produced in the body *via* the the cholesterol to pregnenolone pathway. The

adrenal gland zona reticularis is the primary source of DHEA and is converted by the enzyme sulphotransferase into dehydroepiandrosterone sulphate (DHEA-S), sulfate conjugate of DHEA. Both DHEA and DHEA-S are the most prevalent steroid hormones in the human body, and both decrease gradually with age. Production of DHEA-S rises during adrenarche (ages 6-10), peaking at 20, starts declining after 25, and is minimal by 70.[9] DHEA-S functions as a precursor to androgens and estrogens and is readily hydrolyzed back to DHEA in peripheral tissue targets by an enzyme, sulphatase. In ovaries, it boosts intrafollicular androgens, elevating AMH expression, and possibly enhancing ovarian reserve and follicular development. Elevated levels of AMH and serum testosterone have been found to be linked with oral DHEA administration.[10]

With the aforementioned data in consideration, it was hypothesised that serum AMH and DHEA-S levels have a key role in infertility in females. The correlation between serum DHEA-S and serum AMH levels and their effect on fertility remained controversial because of the lack of strong evidence to substantiate it. Hence, the present study was carried out on infertile women to determine the association between serum AMH and DHEA-S levels and infertility.

METHODOLOGY

This observational cross-sectional research was carried out at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from July 2022 to Aug 2023. This study was approved by the Institutional Review Board (IRB), at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan (Number: FC-CHP22-14/5 READ-IRB/23/1629, Dated: 28-Feb-2023).

One hundred and ten females of age 19-48 years were employed in this research. The sample size was calculated using the WHO calculator by keeping a confidence interval of 95%, a margin of error of 5, 7% prevalence of infertility in the Pakistani female population,[11] and it came out to be 101. After providing a detailed explanation of the procedure, written and verbal informed consent was taken from every individual for their inclusion in the research. Record forms for each patient were prepared in advance and contained details such as the patient's age, biochemical details, and ultrasound findings. A non-probability and convenient sampling technique was used for data collection.

This investigation employed females, aged 19-48 years who were unable to conceive and referred to the endocrinology clinic at the Armed Forces Institute of Pathology for infertility work-up. All the females with primary ovarian insufficiency, the ones who were receiving hormonal therapy for the past six months, and those who were taking androgen supplementation were excluded. Patients with incomplete or inconsistent data in the patient database were also excluded.

After the informed consent was taken, 3mL blood samples were collected in serum separator tubes by phlebotomist from all the participants. These samples were then centrifuged at 3500 RPM for three minutes at room temperature. Serum was separated by centrifugation and samples were kept in aliquots at -20°C with proper tagging. After collection of all the samples, they were thawed to room temperature and were run in a batch with controls to minimise intralab variability and to maintain quality assurance. Serum levels of AMH and DHEA-S were analysed using the Chemiluminescence on Maglumi 800 and Immulite 1000 instruments, respectively.

Serum levels including thyroid-stimulating hormone (TSH), FSH, estradiol, luteinising hormone (LH), and testosterone and ultrasound were already available in clinical record. Detailed history and radiological findings were obtained from all the participants. Blood samples were specifically obtained from all subjects on the third menstruation day between 08: 00 and 09: 00 in the morning. In the follicular stage, the reference values were: TSH, 0.35–4.94 µIU/mL; FSH, 4–13 mIU/mL; Estradiol, 39–189 pg/mL; DHEA-S, 35.0–430.0 µg/dL, and LH, 1–18 mIU/mL. Maglumi 1800 was used to test AMH levels. The lowest detected limit of AMH was 0.02 ng/mL. The normal ranges for AMH in the mentioned age groups are as follows: 0-19 years: 0.53-12.86 ng/ml, 19-29 years: 1.23-11.10, 29-39 years: 0.66-8.34, 39-54 years: 0.26-5.81, >54 years: <0.81.

Serum AMH levels <1.2 ng/mL, as determined by the POSEIDON criteria, were termed diminished ovarian reserve (DOR) in this investigation.[12] AMH values 5.0 ng/mL, which were derived from the updated Rotterdam criteria were classified as excess ovarian reserve (EOR).[13] Immulite 1,000 was used to test serum DHEA-S, and the reference ranges for different age groups are as follows: 18–30 years: 45–380 ug/dl, 31–50 years: 12–379 ug/dl, and 12–17 years: 20–535 ug/dl.

The Statistical Package for Social Sciences (SPSS) version 22.00 was used to analyse the data. The Shapiro-Wilk's test was used to determine the normality of the data. Quantitative data (age) were represented using mean with standard deviation while the median (IQR) was calculated for non-normally distributed variable serum AHM and DHEA-S. Frequency and percentages were calculated for categorical variables. Spearman's correlation coefficient was determined for statistical significance at p-value of ≤0.05.

RESULTS

A total of 110 females were employed in the current investigation. The age of subjects was between 19-48 years with a mean age of 32.27 ± 7.15 years. Of the total, 40 (36.4%) and 70 (63.6%) of the females developed primary and secondary infertility, respectively. The median of serum DHEA-S was 86.20 (17.00 – 349.00) and serum AMH was 1.40 (0.30 – 6.90) and the correlation was r = 0.712 with significant p <0.001, Figure 1. Females with primary infertility had a positive correlation between serum DHEA-S and serum AMH (r = 0.685; p <0.001) and females with secondary infertility also had a positive association between serum DHEA-S and serum AMH (r = 0.807; p <0.001), as shown in Figure 2.

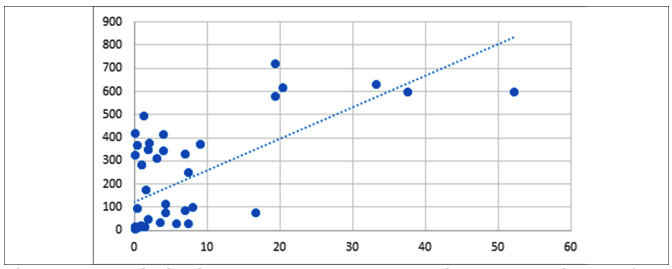

Figure 1: Correlation between serum DHEA-S and serum AMH (n = 110).

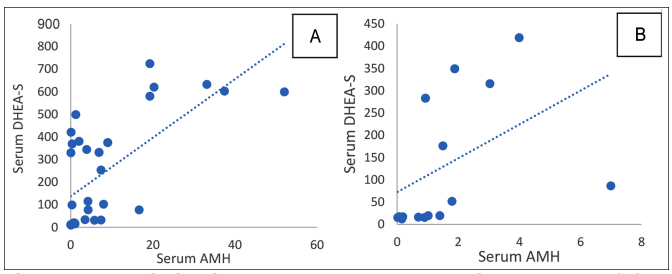

Figure 2: Correlation between serum DHEA-S and serum AMH. (A) In primary infertility (n = 70). (B) In secondary infertility (n = 40).

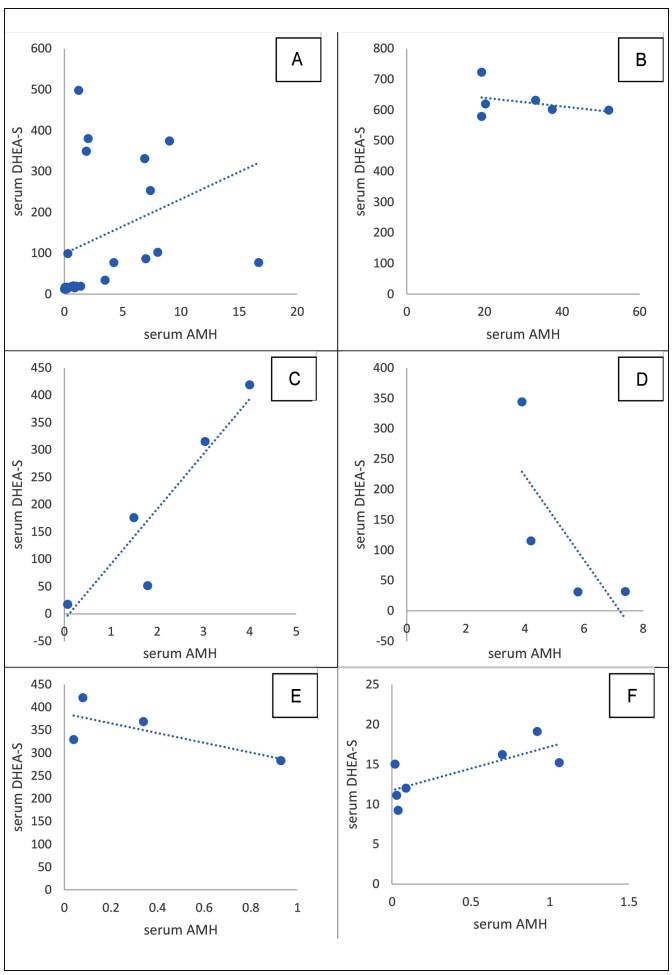

Figure 3: Correlation between serum DHEA-S and serum. (A) In normal females (n = 54). (B) In patients with PCOS (n = 12). (C) In patients with fibroids (n = 12). (D) In patients with fallopian tube defects (n = 08). (E) In patients with endometriosis (n = 10). (F) In patients with low antral follicular count (n = 14).

Table I: Relationship between the mean and standard deviation of blood serum DHEA-S and AMH in different variables.

Variables	Serum DHEA-S	Serum AMH	p-value (spearman's Rho test)
Normal ultrasound	144.87 ± 159.817	3.43 ± 4.0635	<0.001
PCOS	625.62 ± 48.669	30.28 ± 12.631	
Fibroid	165.92 ± 161.673	1.75 ± 1.503	
Fallopian tube defect	130.40 ± 136.804	5.33 ± 1.493	
Endometriosis	336.82 ± 55.516	0.46 ± 0.4155	
Low antral follicular count	13.97 ± 3.247	0.41 ± 0.4476	

However, after the ultrasound, 54 (49.1%) females were normal, 12 (10.9%) had PCOS, 12 (10.9%) had fibroids, 8 (7.3%) had fallopian tube defects, 10 (9.1%) had endometriosis, and 14 (12.7%) had low antral follicular count. The correlation between serum AMH and DHEA-S of normal females was ($r = 0.731$; $p < 0.001$), PCOS was ($r = -0.232$; $p = 0.468$), fibroid ($r = 0.941$, $p < 0.001$), fallopian tube defects ($r = -0.800$; $p = 0.017$), endometriosis ($r = -0.684$, $p = 0.013$), and low antral follicular count ($r = 0.643$, $p = 0.0130$) (Figure 3).

Table I shows the correlation between the mean and standard deviation of blood serum DHEA-S and AMH in different subjects.

DISCUSSION

Conducted under controlled conditions with rigorous methodology and strict exclusion criteria, this study enhances the reliability and validity of the results. It provides valuable insights into reproductive endocrinology by elucidating the interplay between serum DEHA-S and AMH in female infertility. The findings suggested a significant positive correlation between higher serum AMH and DHEA-S levels in infertile women, even after accounting for potential confounding variables. These results contribute to the understanding of hormonal dynamics and may have implications for diagnosing and managing female infertility.

DHEA acts as an intermediary in synthesising androgens and estrogens. Recent research indicated that the serum testosterone levels influence fertility by aiding follicle formation, which is crucial for ovulation.[14] A meta-analysis by Li *et al*. showed that the administration of DHEA was found to significantly increase testosterone levels, especially in normal young women who received high levels of DHEA dosages (>50 mg/day) and had an intervention time of fewer than 12 weeks. This investigation continually demonstrated a positive relationship among serum DHEA-S and T-levels quartile group.[15]

A study by Lin *et al*. showed comparable results to the current investigation.[16] Reported investigation showed a positive association between blood AMH and serum testosterone amounts, suggesting that the lack of androgens may be an indicator of risk for DOR and that excess androgens may induce EOR in females with infertility. Individuals were divided into 4 quartile classes (Q1 to Q4) based on blood testosterone levels. Patients categorised in the low class T quartile (Q1) had substantially lower serum AMH amounts than the ones in the upper T quartile categories (Q4). AMH levels were shown to be rising in the higher T quartile groups following adjustments -

for body mass index, body weight, age, and FSH. Binary logistic regression analysis revealed that the chance of the risk of DOR was 11.44 times more in Q1 than in Q4, and the probability of the potential for EOR was 10.41 times larger in Q4 than in Q1.[16] Similarly, Dewailly *et al*. also postulated that androgens promote the synthesis of AMH by enhancing the effect of FSH.[17]

Moreover, Hu *et al*. presented another investigation showing comparable results to the current study.[18] According to the study, before ovulation, androgen receptor (AR) function in granulosa cells can be elevated by DHEA supplementation. Androgens enhance the activity of the FSH receptor by the AR, which in turn causes FSH-mediated follicle development and stimulates the growth of granulosa cells. After FSH-mediated granulosa cell development, there was a surge in AMH release. Similar to the current study, this one also revealed a positive relationship between serum DHEA and serum AMH.[18]

Xie *et al*. published an analysis of 24 RCTs (randomised control trials) with 1,429 individuals.[19] This study suggested that increasing the amount of DHEA in supplements may increase the synthesis of AMH, most likely by raising levels of IGF-1 and serum testosterone. The findings of the current study were somewhat supported by this research. It showed that administering DHEA significantly raised blood levels of insulin-like growth factor-1 (IGF-1). According to this study, IGF-1 may also promote follicle development triggered by FSH and granulosa cell differentiation. Moreover, it has been shown that IGF-1 can stimulate the activation of primordial follicles. The ovarian reserve had also been found to be correlated with the levels of IGF-I in follicular fluid, which shows a significant association with serum IGF-I levels. To validate the association between serum AMH and DHEA-S levels and to investigate the potential pathophysiological processes behind this association, further investigation was still required.[19]

Gleicher *et al*. included 120 women with DOR in a retrospective longitudinal and cross-sectional investigation and observed that AMH levels showed a substantial rise over time with the administration of DHEA.[20] This effect was especially noticeable in females under a certain age of 38 years, and these results were in coherence with the present study.[20] Yilmaz *et al*. conducted a similar prospective research that demonstrated a significant increase in serum AMH levels (0.32 ± 0.29 *vs*. 0.75 ± 0.70 ng/mL) prior to and following the DHEA treatment among 41 females with DOR.[21]

Moreover, Zhang *et al*.[22] in their meta analysis reported results that were concurrent with the current study. Pooled analysis of all study types demonstrated a significant increase in AMH levels with Weighted Mean Difference 0.34 (95% CI: 0.17 to 0.51, P<0.001) in the DHEA treatment groups compared to the control groups. However, when analyzing data from five RCTs separately, the pooled results did not show a statistically significant increase in AMH levels Weighted Mean Difference 0.1 (95% CI: -0.14 to 0.34, P=0.416).[22]

However, some studies had also shown that serum AMH levels are not affected following DHEA administration. Yeung *et al*. carried out a pilot randomised controlled investigation with 32 PORs and concluded that there had been no statistically substantial variations in ovarian reserve markers (AFC, AMH, or between the DHEA and placebo groups of patients.[23]

This study has major limitations due to its cross-sectional design which did not allow for the determination of a causal association; longitudinal studies would be a more appropriate method for this kind of research. Thus, further long-term research is required to validate the correlation.

Because only infertile females were employed in the current investigation, the findings might not apply to the wider community. As all of the participants in the present research were Pakistani, the relationship between ethnicity and serum AMH level was not examined. The usual infertility survey in this research did not analyse serum sex hormone-binding globulin (SHBG) and free serum testosterone levels.

CONCLUSION

This study showed that serum AMH and DHEA-S levels of infertile females were positively correlated. DHEA-S supplementation may be considered in treatment options before opting for expensive and invasive procedures of assisted-reproductive technologies (e.g., IVF). Further long-term longitudinal researches are still required to validate the current study's findings.

ETHICAL APPROVAL:

This study was approved by the Institutional Review Board (IRB), at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan (Number: FC-CHP22-14/READ-IRB/23/1629, Dated: 28-Feb-2023).

PATIENTS' CONSENT:

After providing a detailed explanation of the procedure, written and verbal informed consent was taken from every individual for their inclusion in the research.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

RS, MY: Worked on literature review, selected the study design, collected data, analysed data, and wrote the manuscript.
ZHH: Worked on results analysis and interpretation of data.
MA, MUM: Provided critical review and approval of the version to be published.
All authors approved the final version of the manuscript to be published.

REFERENCES

1. Thoma M, Fledderjohann J, Cox CM, Adageba RK. Biological and social aspects of human infertility: A global perspective. *Oxford Res Encyclop Glob Pub Health* 2021; doi: 10.1093/acrefore/9780190632366.013.184.

2. Thomas SC. From diagnosis to parenthood: Event history analysis of infertility treatment trajectories in kerala, india (Doctoral dissertation, SCTIMST). 2021. Available from: https://dspace.sctimst.ac.in/items/0b8bfb88-3600-4a3c-8b28-4e72697eec4c.
3. Borght MV, Wyns C. Fertility and infertility: Definition and epidemiology. *Clin Biochem* 2018; **62**:2-10. doi: 10.1016/j.clinbiochem.2018.03.012.
4. Habiba M, Heyn R, Bianchi P, Brosens I, Benagiano G. The development of the human uterus: Morphogenesis to menarche. *Hum Reprod Update* 2021; **27(1)**:1-26. doi: 10.1093/humupd/dmaa036.
5. Kubiak JZ, Kloc M, Piprek R. History of the research on sex determination. *Biomed J Sci Technol Res* 2020; **25(3)**. doi: 10.26717/BJSTR.2020.25.004194.
6. Sivanandy MS, Ha SK. The role of serum anti-mullerian hormone measurement in the diagnosis of polycystic ovary syndrome. *Diagnostics* 2023; **13(5)**:907. doi: 10.3390/diagnostics13050907.
7. Salemi F, Jambarsang S, Kheirkhah A, Abargouei AS, Ahmadnia Z, Lotfi M, *et al*. The best ovarian reserve marker to predict ovarian response following controlled ovarian hyperstimulation: A systematic review and meta-analysis. *Syst Rev* 2024; **13(1)**:1-21. doi: 10.1186/s13643-024-02684-0.
8. Russell N, Gilmore A, Roudebush WE. Clinical utilities of anti-mullerian hormone. *J Clin Med* 2022; **11(23)**:7209. doi: 10.3390/jcm11237209.
9. Schiffer L, Barnard L, Baranowski ES, Gilligan LC, Taylor AE, Arlt W, *et al*. Human steroid biosynthesis, metabolism and excretion are differentially reflected by serum and urine steroid metabolomes: A comprehensive review. *J Steroid Biochem Mol Biol* 2019; **194**:105439. doi: 10.1016/j.jsbmb.2019.105439.
10. Lin LT, Tsui KH. The relationships between serum DHEA-S and AMH levels in infertile women: A retrospective cross-sectional study. *J Clin Med* 2021; **10(6)**:1211. doi: 10.3390/jcm10061211.
11. Shaheen R, Subhan F, Sultan S, Subhan K, Tahir F. Prevalence of infertility in a cross section of Pakistani population. *Pak J Zool* 2010; **42(4)**:389-93. Available from: https://api.semanticscholar.org/CorpusID:42126019.
12. Grisendi V, Mastellari E, La Marca A. Ovarian reserve markers to identify poor responders in the context of poseidon classification. *Front Endocrinol* 2019; **10**:281. doi: 10.3389/fendo.2019.00281.
13. Brouwer J, Laven JS, Hazes JM, Schipper I, Dolhain RJ. Levels of serum anti–mullerian hormone, a marker for ovarian reserve, in women with rheumatoid arthritis. *Arthritis Care Res* 2013; **65(9)**:1534-8. doi: 10.1002/acr.22013.
14. Al-hadrawi KK, ALGarawy RT. The role of some of the level Antioxidant enzymes and Obesity in development infertility women's infertility in Najaf Province Patients, IRAQ. *BIO Web of Conferences* 2023; **65**:05050. doi: 10.1051/ bioconf/20236505050.
15. Li Y, Ren J, Li N, Liu J, Tan SC, Low TY, *et al*. A dose-response and meta-analysis of dehydroepiandrosterone (DHEA) supplementation on testosterone levels: Perinatal prediction of randomized clinical trials. *Exp Gerontol* 2020; **141**:111110. doi: 10.1016/j.exger.2020.111110.
16. Lin LT, Li CJ, Tsui KH. Serum testosterone levels are positively associated with serum anti-mullerian hormone levels in infertile women. *Sci Rep* 2021; **11(1)**:6336. doi: 10.1038/s41598-021-85915-x.
17. Dewailly D, Robin G, Peigne M, Decanter C, Pigny P, Jonard SC. Interactions between androgens, FSH, anti-mullerian hormone and estradiol during folliculogenesis in the human normal and polycystic ovary. *Hum Reprod Update* 2016; **22(6)**:709-24. doi: 10.1093/humupd/dmw027.
18. Hu Q, Hong L, Nie M, Wang Q, Fang Y, Dai Y, *et al*. The effect of dehydroepiandrosterone supplementation on ovarian response is associated with androgen receptor in diminished ovarian reserve women. *J Ovarian Res* 2017; **10(1)**:1-0. doi: 10.1186/s13048-017-0326-3.
19. Xie M, Zhong Y, Xue Q, Wu M, Deng X, Santos HO, *et al*. Impact of dehydroepianrosterone (DHEA) supplementation on serum levels of insulin-like growth factor 1 (IGF-1): A dose-response meta-analysis of randomized controlled trials. *Exp Gerontol* 2020; **136**:110949. doi: 10.1016/j.exger.2020.110949.
20. Gleicher N, Weghofer A, Barad DH. Improvement in diminished ovarian reserve after dehydroepiandrosterone supplementation. *Reprod Biomed Online* 2010; **21(3)**:360-5. doi: 10.1016/j.rbmo.2010.04.006.
21. Yilmaz N, Uygur D, Inal H, Gorkem U, Cicek N, Mollamahmutoglu L. Dehydroepiandrosterone supplementation improves predictive markers for diminished ovarian reserve: Serum AMH, inhibin B and antral follicle count. *Eur J Obstet Gynecol Reprod Biol* 2013; **169(2)**:257-60. doi: 10.1016/j.ejogrb.2013.04.003.
22. Zhang M, Niu W, Wang Y, Xu J, Bao X, Wang L, *et al*. Dehydroepiandrosterone treatment in women with poor ovarian response undergoing IVF or ICSI: A systematic review and meta-analysis. *J Assist Reprod Genet* 2016; **33**:981-91. doi: 10.1007/s10815-016-0713-5.
23. Yeung TW, Chai J, Li RH, Lee VC, Ho PC, Ng EH. A randomized, controlled, pilot trial on the effect of dehydroepiandrosterone on ovarian response markers, ovarian response, and *in vitro* fertilization outcomes in poor responders. *Fertil Steril* 2014; **102(1)**:108-15. doi: 10.1016/j.fertnstert.2014.03.044.