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Evaluation of DHEA level in serum of fertile and infertile women

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***Abstract*---**The sulfation of the adrenal steroid dehydroepiandrosterone (DHEA) is a critical step in the provision of substrates for estrogen biosynthesis by the placenta during pregnancy. Dehydroepinadrosterone (DHEA) supplementation improves pregnancy chances in women with diminished ovarian reserve (DOR), by possibly reducing aneuploidy. Defects in the synthesis of DHEA cause deficiencies in the synthesis of androgens and estrogens. In the first stage, mutations in the StAR gene cause potentially lethal lipoid CAH. Defects in the cholesterol side chain cleavage enzyme, the second step, are rare. Defects in the third stage, 17-hydroxylation lead to hypertension and female sexual infantilism. Defects in the final stage of DHEA synthesis, 17, 20 lyase activity, are due to disorders of electron transfer to P450c17, including mutations in POR.

***Keywords*---**DHEA synthesis, serum of fertile, infertile women.

Introduction

Infertility is a disease of the reproductive system characterized by inability to achieve pregnancy after 12 or more months of regular unprotected sexual intercourse (Venkatesh *et al.*, 2014). Female infertility, as a complex disorder, may be caused by medical conditions including pelvic inflammatory disease, endometriosis related infertility, ovulatory disorders, tubal factor infertility, and unexplained infertility (Gupta *et al.*, 2014). Infertility affect approximately nine to fifteen percent of the childbearing population, and 55% of these influenced will seek medical help to achieve their desire to have children (Boivin et al., 2011).

Dehydroepiandrosterone (DHEA) is not a hormone but it is a very important prohormone secreted in large amounts by the adrenals in humans and other primates (Labrie, *et al*,. 2005). DHEA secretion is primarily adrenal in origin (85

%), and ovarian to a lesser extent (15 %), and as the major source of androgen synthesis in women is converted via androstenedione to testosterone (Panjar and Davis., 2007).

The sulfation of the adrenal steroid dehydroepiandrosterone (DHEA) is a critical step in the provision of substrates for estrogen biosynthesis by the placenta during pregnancy. DHEA sulfation appears to play an even more important role in the fetus than in the adult, as it acts as the major precursor for placental estrogen biosynthesis, thereby supplying estrogens during pregnancy **(**Zdrojewicz and Ciszko., 2001)**.**

DHEA-S can enter the ovarian follicle and is a key source of ovarian testosterone, which plays an important role in promoting follicular development (Astapova *et al.*, 2019). Because DHEA effects peak at four to five months, a time period similar to the complete follicular recruitment cycle, that have speculated about a DHEA effect on follicular recruitment, possibly mediated via suppressive effects on apoptosis (Barad *et al.*, 2007).

Dehydroepinadrosterone (DHEA) supplementation improves pregnancy chances in women with diminished ovarian reserve (DOR), by possibly reducing aneuploidy. Since a large majority of spontaneous miscarriages are associated with aneuploidy, one can speculate that DHEA supplementation may also reduce miscarriage rates (Gleicher, *et al*,. 2009)**.**

Objective: Evaluate the DHEA level in infertile women.

Material and Methods

The DEHA levels detected by ELISA sandwich method. The study included 125 subjects is divided into two groups: The first group is the patient group, which consists of 80 patients with infertility, which excludede 35 sample according exclusion criteria, And remained 45 patients with infertility was identified by a specialized gynaecologists of the "Fertility Center in AL-Sadder Teaching Hospital in Najaf Governorate/ Ministry of Health/Iraq, during the period from the 1st Nov. 2021 to 1st March. 2022, which requires the presence of at least two of the following characteristics: polycystic ovaries on ultrasound scan, menstrual irregularities, Endometriosis and hyperandrogenism. The second group is the control group, which consists of 45 fertile women. The age group of the patients ranged from (17-44) years. The inclusion criteria for the patients are all women with infertility. The second group is the control group (fertile women), which consists of 45 fertile women, normal testosterone, a regular menstruation period, and normal ovulation are eligible, with normal ovaries as they were observed by the gynecologists which no take contraceptive, with no past of somewhat malady. The age group ranged from (17-42) years.

Exclusion criteria:
Thirty fivet samples were excluded from the initial sample of Eighty due to these pathologies such as Diabetes mellitus, disorder of ovary, and ovarian tumor.

Blood Collection

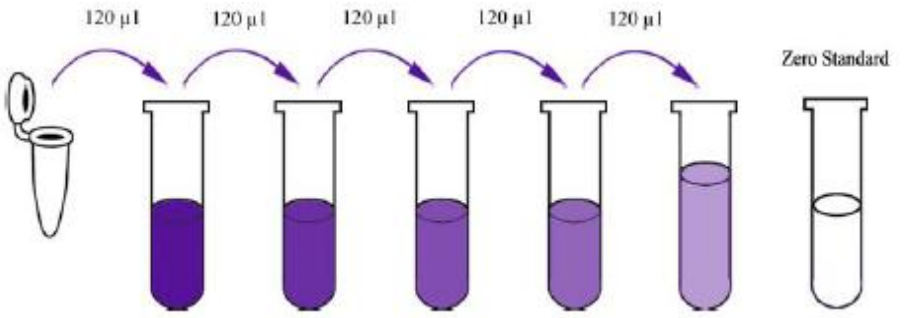
The blood samples of this study were obtained from female in 2nd of menstrual cycle through drag 5 ml of blood by using of medical sterile syringes from brachial vein, and placed in a gel tube. Then the gel tube were placed at room temperature for 30 minutes to coagulate the blood, and then samples were centrifuged (6000 rpm/min) for 5 minutes to separate the serum from other components of the blood. The serum was withdrawn by micro pipette and then placed in the Eppendorf tubes in two repeaters and kept frozen at -20 ◦C for the determination of Dehydroepiandrosterone (Lesser *et al.*, 2020).

Dehydroepiandrosterone measurment method:
Assay Principle:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human DHEA antibody. DHEA present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human DHEA Antibody is added and binds to DHEA in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated DHEA antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human DHEA. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Assay Procedure:

1. Prepared all reagents, standard solutions and samples as instructed. Brought all reagents to room temperature before use. The assay is performed at room temperature.



2. Determined the number of strips required for the assay. Inserted the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Added 50µl standard to standard well. Note: Didn't added antibody to standard well because the standard solution contains biotinylated antibody.
4. Added 40µl sample to sample wells and then added 10µl anti-DHEA antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. Covered the plate with a sealer. Incubated 60 minutes at 37°C.

5. Removed the sealer and wash the plate 5 times with wash buffer. aspirated wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated all wells and washed 5 times with wash buffer, overfilling wells with wash buffer. Blotted the plate onto paper towels or other absorbent material.
6. Added 50µl substrate solution A to each well and then added 50µl substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Added 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

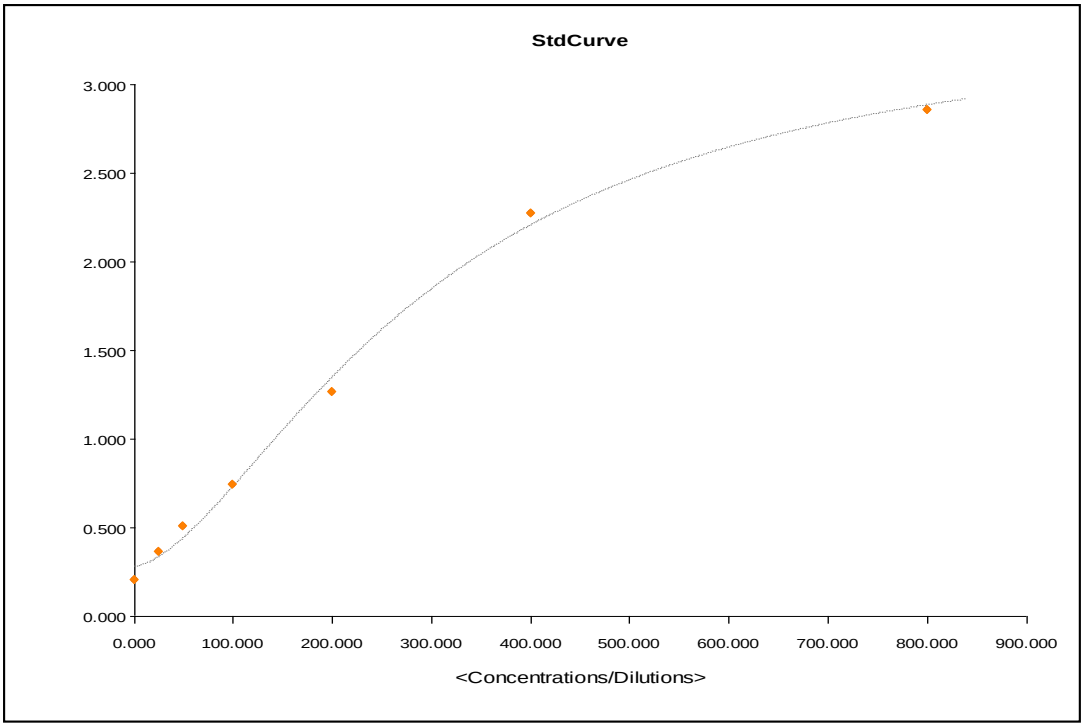


Figure (1): Standard curve of DHEA

Result and Discussion

As shown in the table (1) and figure (2), the mean level of Dehydroepiandrosterone (DHEA) in infertile women group was (119.04 pg/ml) and it was high significantly lower than in fertile women group that mean level was (129.73 pg/ml)

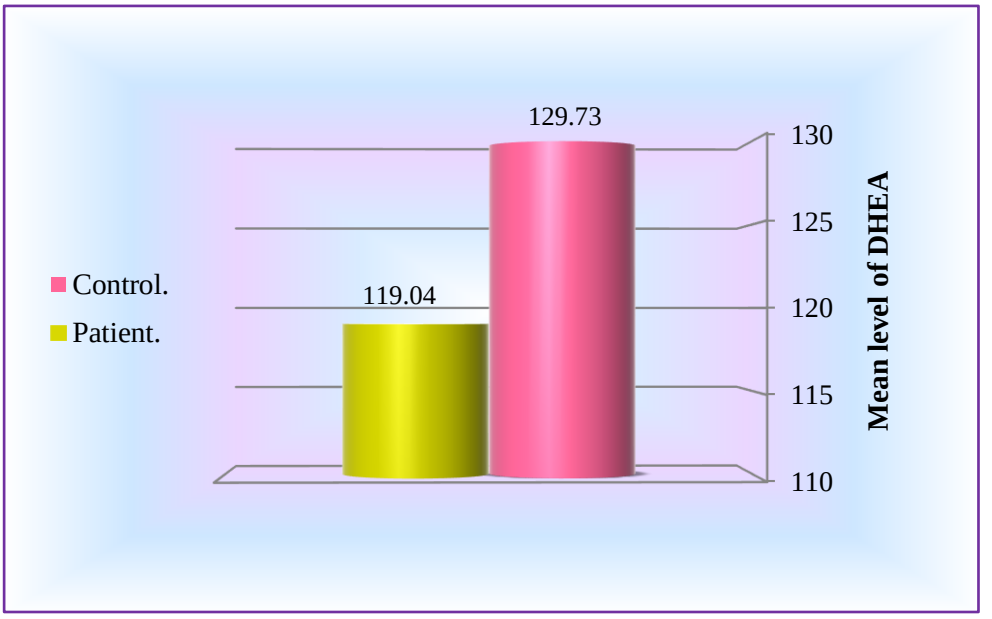


Figure (2): Comparison the mean levels of Dehydroepiandrosterone in infertile women group and fertile women (control) groups

Table (1)
Comparison the mean, standard error and p-value of Dehydroepiandrosterone between infertile women group and fertile women (control) groups.

Parameter		N	Mean	Std. E	P-value
DHEA	Fertile women (Control)	45	129.7	6.03	0.0001**
	Infertile women	45	119	10.48	

P > 0.05 = Non-Significant (NS)
P < 0.05 = significant (*)
P < 0.01 = Highly significant (**)

Barad & Gleicher, (2005) these case report is evidence that an older woman with documented decreased ovarian reserve may dramatically improve her response to ovulation induction over successive cycles. This patient's response may be due to an interaction of DHEA, gonadotropin stimulation, and acupuncture treatments and not an effect of DHEA alone. However, only DHEA, a weak androgen, offers considerable theoretical foundation for a beneficial effect on the ovary.

DHEA is secreted by the adrenal cortex, central nervous system, and the ovarian theca cells and is converted in peripheral tissue to more active forms of androgen or estrogen (Burger, 2002). At physiologic doses, DHEA increases serum levels of the insulin-like growth factor and serves as a prohormone for estrogens. DHEA-exposed rats develop cystic changes similar to polycystic ovary syndrome (PCOS) and have a higher percentage of meiotically active oocytes and less evidence of atresia. Women chronically exposed to androgens can also develop PCOS-like ovaries (Casson *et al.,* 1998).

In general, older ovaries have few antral follicles, high rates of atresia (Faddy, 2000), and increased “resistance” to ovulation induction (Chuang *et al.,* 2003). A “delay” of the atretic process has been noted among anovulatory women with PCOS, where increased follicular DHEA concentration was associated with increased aromatase activity (Franks et al., 2000). During IVF cycles day three, T levels 0.694 nmol/L are associated with poor IVF success rates.

Casson *et al.,* (2000) previously noted only a small increase in follicle number and E2 response to ovulation induction after DHEA administration. They treated five women for only two months with DHEA (80 mg/day) administration. In contrast, our patient’s dramatic increase in oocyte production began after four months of treatment. This treatment duration is in keeping with the interval required for normal follicular initiation of recruitment and growth and raises the possibility that the previous study did not treat its subjects long enough to achieve maximum effect.

Kotdawala *et al.,* (2019) illustrate that an aneuploidy is a known consequence of reproductive aging; however, the average woman of advanced reproductive age will produce too few viable embryos for preimplantation diagnosis. By increasing the number of embryos available for analysis, one may be able to increase the availability of at least a few normal embryos for transfer. That have since used DHEA pretreatment for three additional patients undergoing IVF who had a history of poor ovarian function and elevated baseline FSH. One of these three patients was 45 years old and had a baseline FHS of 25 IU/mL. Not surprisingly, she demonstrated little improvement after DHEA treatment. The other two patients are 42 and 33 years old. They each had a previous unsuccessful IVF cycle with few healthy embryos. Both of these patients were able to establish continuing clinical pregnancies after IVF and are in their first trimester at the time of this submission. The “aging ovary” represents the last frontier of human infertility treatment and is generally considered untreatable with current medical resources. The possibility that any intervention may significantly improve the response of the aging ovary is therefore potentially revolutionary.

Defects in the synthesis of DHEA cause deficiencies in the synthesis of androgens and estrogens. In the first stage, mutations in the StAR gene cause potentially lethal lipoid CAH. Defects in the cholesterol side chain cleavage enzyme, the second step, are rare. Defects in the third stage, 17-hydroxylation lead to hypertension and female sexual infantilism. Defects in the final stage of DHEA synthesis, 17, 20 lyase activity, are due to disorders of electron transfer to P450c17, including mutations in POR. Of these, the defect in StAR is common among Palestinian Arabs and in Eastern Arabia (Miller, 2005).

Labrie *et al.,* (2005) showed that the almost exclusive focus on the role of ovarian estrogens in women’s reproductive physiology has removed attention from the dramatic 70% fall in circulating DHEA which already occurs between the ages of 20 to 30 and 40 to 50 years. In fact, since DHEA is transformed to both androgens and estrogens in peripheral tissues, such a fall in serum DHEA and DHEA-S explains why women at menopause are not only lacking estrogens but are also likely to have been deprived of androgens for a few years, as illustrated by the 50–60% decrease in serum ADT-G.

References

Astapova, O., Minor, B. M., & Hammes, S. R. (2019). Physiological and pathological androgen actions in the ovary. *Endocrinology*, *160*(5), 1166-1174.

Burger, H. G. (2002). Androgen production in women. *Fertility and sterility*, *77*, 3-5.

Casson, P. R., Lindsay, M. S., Pisarska, M. D., Carson, S. A., & Buster, J. E. (2000). Dehydroepiandrosterone supplementation augments ovarian stimulation in poor responders: a case series. *Human reproduction*, *15*(10), 2129-2132.

Casson, P. R., Santoro, N., Elkind-Hirsch, K., Carson, S. A., Hornsby, P. J., Abraham, G., & Buster, J. E. (1998). Postmenopausal dehydroepiandrosterone administration increases free insulin-like growth factor-I and decreases high-density lipoprotein: a six-month trial. *Fertility and Sterility*, *70*(1), 107-110.

Chuang, C. C., Chen, C. D., Chao, K. H., Chen, S. U., Ho, H. N., & Yang, Y. S. (2003). Age is a better predictor of pregnancy potential than basal follicle-stimulating hormone levels in women undergoing in vitro fertilization. *Fertility and sterility*, *79*(1), 63-68.

Faddy, M. J. (2000). Follicle dynamics during ovarian ageing. *Molecular and cellular endocrinology*, *163*(1-2), 43-48.

Gleicher, N., Ryan, E., Weghofer, A., Blanco-Mejia, S., & Barad, D. H. (2009). Miscarriage rates after dehydroepiandrosterone (DHEA) supplementation in women with diminished ovarian reserve: a case control study. *Reproductive Biology and Endocrinology*, *7*(1), 1-7.

Gupta S, Ghulmiyyah J, Sharma R, Halabi J, Agarwal A. Power of Proteomics in Linking Oxidative Stress and Female Infertility. BioMed Research International. 2014;2014:916212. doi:10.1155/2014/916212.

Guyton and Hall. (2011):Textbook of Medical Physiology.12ed, ch81,pp988- 1003.

Kotdawala, A. P., Mir, P., Herrero, J., Khajuria, R., Lalitkumar, P., & Banker, M. R. (2019). Embryonic Aneuploidy after Preimplantation Genetic Screening: Age- and Indication-Matched Comparative Study between Indian and Spanish Population. *Journal of human reproductive sciences*, *12*(2), 141–149. https://doi.org/10.4103/jhrs.JHRS_109_18.

Labrie, F. (2010). DHEA, important source of sex steroids in men and even more in women. In *Progress in brain research* (Vol. 182, pp. 97-148). Elsevier.

Miller, W. L. (2005). Disorders of androgen synthesis–from cholesterol to dehydroepiandrosterone. *Medical Principles and Practice*, *14*(Suppl. 1), 58-68.

Panjari, M., & Davis, S. R. (2007). DHEA therapy for women: effect on sexual function and wellbeing. *Human reproduction update*, *13*(3), 239-248.

Venkatesh T, Suresh PS, Tsutsumi R. New insights into the genetic basis of infertility. Appl Clin Genet, 2014. 7: p. 235-43.

Zdrojewicz, Z., & Ciszko, B. (2001). Dehydroepiandrosterone (DHEA)--structure, clinical importance and the role in human body. *Postepy higieny i medycyny doswiadczalnej*, *55*(6), 835-854.