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Review Article

Are DHEAS Levels at Initiation of Ovarian Stimulation Different Between Pregnant and Non-pregnant after *In-Vitro*-Fertilization? A Systematic Review and Meta-analysis

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Abstract

The aim of this study was to evaluate whether dehydroepiandrosterone sulphate (DHEAS) levels at initiation of ovarian stimulation are different between pregnant and non-pregnant women after IVF. A systematic review/meta-analysis showed that no significant difference in DHEAS levels at initiation of ovarian stimulation was observed in women without polycystic ovarian syndrome (PCOS) who achieved or not clinical pregnancy [standardised mean difference (SMD): -0.15, 95%CI: -0.47 to +0.16, five studies, 2448 patients]. Similarly, no significant difference in DHEAS levels at initiation of ovarian stimulation was observed in women in the general population (SMD: +0.37, 95%CI: -0.25 to +0.99, single study, 53 patients). In expected poor responders, significantly higher DHEAS levels at initiation of ovarian stimulation were observed in patients who achieved clinical pregnancy compared with those who did not (SMD: +0.87, 95%CI: +0.32 to +1.41, single study, 129 patients). Similar DHEAS levels at initiation of ovarian stimulation are present between patients who achieve or not clinical pregnancy in the general population as well as in women without PCOS. However, there is weak evidence to suggest that higher DHEAS levels at initiation of ovarian stimulation are present in poor responders who achieve clinical pregnancy compared with those who did not.

Keywords: Androgen, Pregnancy, In-vitro fertilization, IVF, Assisted reproductive technology

Abbreviations: DHEAS: Dehydroepiandrosterone Sulphate; PCOS: Polycystic Ovarian Syndrome; SMD: Standardised Mean Difference

Introduction

Androgens are involved in endometrial physiology since their receptors are present in all endometrial cell types [1]. Effective decidualization, a decisive step towards normal uncomplicated pregnancy, is controlled by a proper androgenic environment. This is guided by endogenous signalling that up- or down-regulate androgen receptors (AR) [2]. Androgens are abundant in the granulosa cells of healthy antral and pre-antral follicles. Furthermore, human granulosa cells carry AR and possess sulphatise activity that allows them to use dehydroepiandrosterone sulphate (DHEAS) for testosterone, androstenedione, and oestrogen production [3].

In the primate ovary, androgens, especially DHEAS and testosterone act as critical regulators of follicular development by augmenting the expression of insulin growth factor 1 (IGF1) and enhance follicular recruitment by increasing the expression of follicle stimulating hormone (FSH) receptors on the granulosa cells [4-6]. DHEAS and its inactive form DHEA are produced mostly by the adrenal glands and are the most abundant steroids in the

plasma, providing large amounts of a substrate which is converted to other androgens and oestrogens [3,7,8]. The advantage of DHEAS over other androgens is that DHEAS is produced by DHEA only in target tissues that provide the necessary enzymatic environment, thus eliminating potential side effects from androgen and oestrogen action systematically [8].

Several studies have examined the association between endogenous DHEAS levels and achievement of pregnancy after in-vitro fertilization (IVF), producing conflicting, however, results [9-12]. The aim of this systematic review and meta-analysis was to answer the following research question: are DHEAS levels at initiation of ovarian stimulation different between pregnant and non-pregnant women after IVF?

Materials and Methods

Identification Of Literature

A computerized literature search in MEDLINE, CENTRAL and randomized controlled trials (RCTs) registries covering the period until March 2022 was performed independently by two reviewers (V.E.C and E.M.K), aiming to identify all available studies evaluating the research question of interest: Are DHEAS levels at initiation of ovarian stimulation different between pregnant and non-pregnant women after IVF? For this purpose, several keywords were used, shown in Supplementary Table 1.

Supplementary Table1: Search strategy used for the identification of eligible studies (these terms were search as "free text terms").

		Setting		Outcome
(Dehydroepiandrosterone Sulphate) OR (DHEAS)]	AND	[(IVF) OR (in-vitro fertilization) OR (in vitro fertilization) OR (in-vitro fertilisation) OR (in vitro fertilisation) OR (fertilization) OR (fertilisation) OR (intracytoplasmic sperm injection) OR (intra-cytoplasmic sperm injection) OR (ICSI) OR (microinjection) OR (ovarian response) OR (ovarian stimulation) OR (controlled ovarian hyperstimulation)]	AND	[(live birth) OR (clinical pregnancy) OR (ongoing pregnancy) OR (delivery) OR (conception)]

Additionally, the citation lists of all relevant publications and review articles were hand-searched. No language limitations were applied. Institutional Review Board was not obtained as previously published data were used. This study followed the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-analyses) guidelines [13] (PROSPERO registration number: CRD42022315804) (Supplementary Table 2), (Tables 1-4), (Figures 1-3).

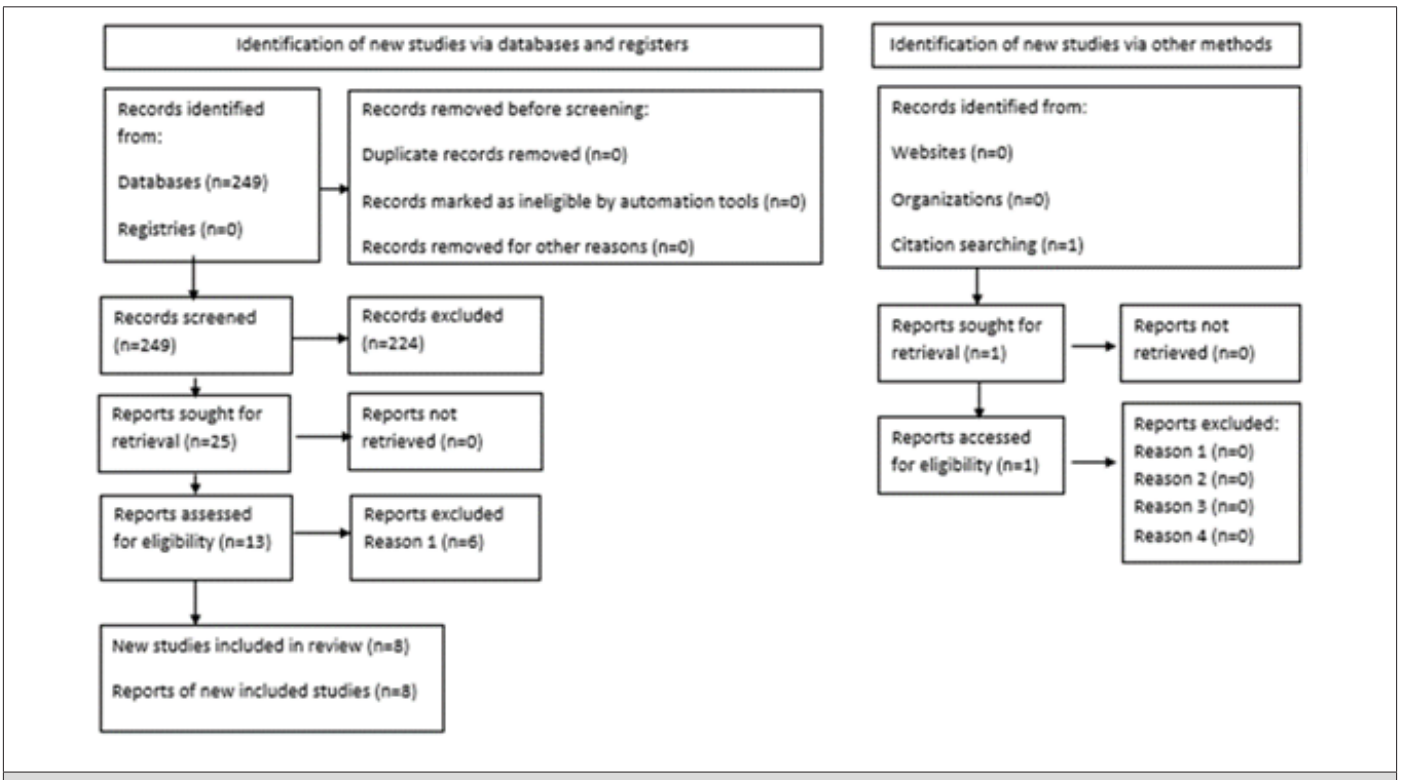

Figure 1: PRISMA flow diagram detailing selection of studies for inclusion in the systematic review/meta-analysis.

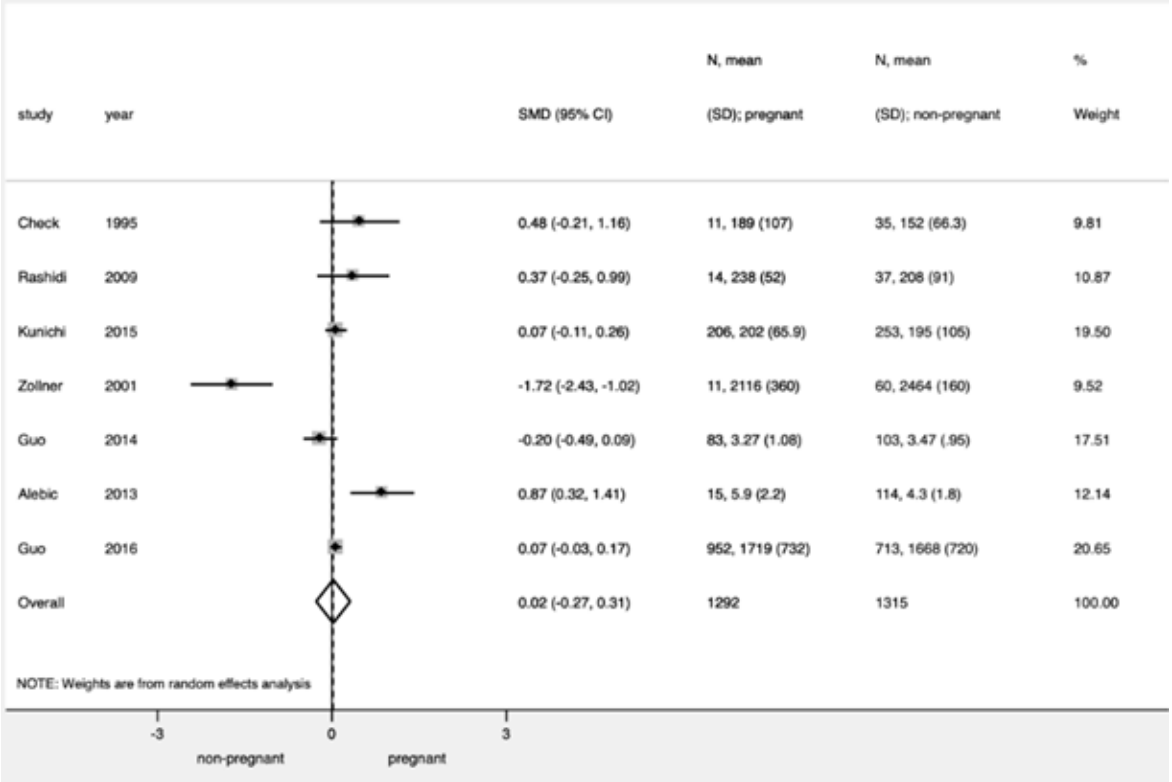

Figure 2: Difference in DHEAS levels at initiation of ovarian stimulation in patients who achieved clinical pregnancy after IVF/ICSI compared with those who did not.

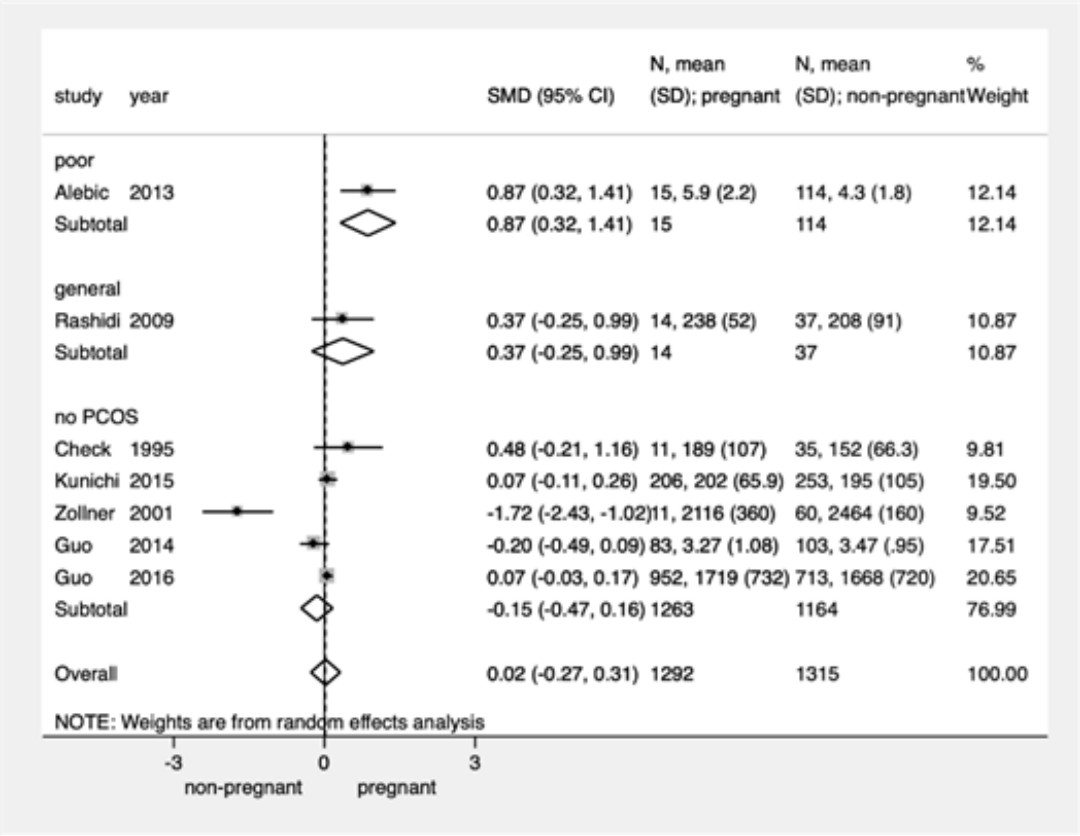

Figure 3: Difference in DHEAS levels at initiation of ovarian stimulation in patients who achieved clinical pregnancy after IVF/ICSI compared with those who did not, performed by subgroup analysis by patient population.

Supplementary Table 2: From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 372: n71.

Section and Topic	Item #	Checklist item	Location where item is reported
Title			
Title	1	Identify the report as a systematic review.	1
Abstract			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
Introduction			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	3,4
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	4
Methods			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	5
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	5
Search strategy	7	Present the full search strategies for all databases, registers, and websites, including any filters and limits used.	Supplementary Table 1
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	5
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	6
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g., for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	6
	10b	List and define all other variables for which data were sought (e.g., participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	6
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	6
Effect measures	12	Specify for each outcome the effect measure(s) (e.g., risk ratio, mean difference) used in the synthesis or presentation of results.	7
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g., tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	7
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	7
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	7
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g., subgroup analysis, meta-regression).	6,7
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A

Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	N/A
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	7
Results			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	8
Study characteristics	17	Cite each included study and present its characteristics.	8, Tables 1, 2
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	8, Supplementary Table 3
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimates and its precision (e.g., confidence/credible interval), ideally using structured tables or plots.	10,11, Figures 2-3
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	8,9
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g., confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	10,11
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	10,11
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	N/A
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	N/A
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	10,11
Discussion			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	12,13
	23b	Discuss any limitations of the evidence included in the review.	13
	23c	Discuss any limitations of the review processes used.	13
	23d	Discuss implications of the results for practice, policy, and future research.	12,13
Other Information			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	5
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	5
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	13
Competing interests	26	Declare any competing interests of review authors.	13
Availability of data, code, and other materials	27	Report which of the following are publicly available and where they can be found template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	14,15,16

Table 1: Characteristics of eligible studies evaluating the probability of pregnancy after IVF/ICSI in patients with high or low DHEAS levels at initiation of stimulation (A).

Study/ country of origin/ Journal or meeting	Study period	Study type/n centres	Number of patients	Pregnancy outcomes evaluated	Inclusion criteria	Population studied/ DHEAS threshold used	Time of androgens assessment	Financial Support by Industry
Frattarelli , et al.,2004/ USA/Fertility and Sterility	1/ 2001-12/2001	Retrospective/ Single centre	43	Clinical pregnancy (undefined)	age: 19-42 years, FSH <12mIU/mL	Women with FSH <12mIU/mL/not reported	Cycle day 3 within three months of initiation of IVF cycle	Not reported
Alebic, et al., 2013/ Croatia/International Journal of Endocrinology	Not reported	Retrospective/ Single centre	129	Clinical pregnancy (cardiac activity ~28 days after OPU)	Null gravidity, normal uterus, and uterine cavity, no hx of pelvic disease or surgery, no hx of use of medications that could interfere with basal hormone status, sperm count of at least 1x106mL, serum AMH concentrations <6.5pmol/L	Expected poor responders/5.7µmol/L	Previous cycle day 3-5	Not reported
Alebic, et al., 2014/Croatia/Reprod Biomed Online	10/2010-01-07-2012	Retrospective/ Single centre	90	Positive HCG, clinical pregnancy (FH at 6weeks), live birth	Age £ 37 years, null gravidity, normal uterus and uterine cavity, no hx of pelvic disease or surgery, no use of medications that could interfere with basal hormonal status, sperm count of at least 1x106/ml, first IVF/ICSI cycle, AMH <6,5pmol/l, HMG: fixed 300IU	Expected poor responders/5.4mmol/l	Previous cycle day 3-5	Not reported

Note*: IVF: *In-vitro* fertilization, ICSI: intra-cytoplasmic sperm injection, SEI: successful embryo implantation, FEI: failed embryo implantation, OPU: oocyte pick-up, Hx: history, FH: fetal heart, PCOS: polycystic cystic ovarian syndrome, HCG: human chorionic gonadotropin, DHEAS: dehydroepiandrosterone sulphate.

Table 2: Characteristics of eligible studies evaluating the probability of pregnancy after IVF/ICSI in patients with high or low DHEAS levels at initiation of stimulation (B).

Study/country of origin, journal, or meeting	Stimulation protocol	GnRH-analogue used/ dose	GnRH-analogue protocol	Gonadotrophin type/ starting dose	Signal for triggering final oocyte maturation/ Dose	Criteria for triggering final oocyte maturation	Timing of OPU	Fertilization	ET policy Amount of embryos/ Stage of development	Luteal phase support
Frattarelli, et al., 2004, USA, Fertility and Sterility	OCP-GnRH Agonist	Not reported/ Not reported	Long luteal	Not reported/Not reported	Not reported/Not reported	Not reported	Not reported	IVF	Not reported/Not reported	Not reported
Alebic, et al., 2013, Croatia, International Journal of Endocrinology	OCP-GnRH Antagonist	Cetrotide/0.25mg s.c. per day	Fixed	hMG/300 IU	HCG/10.000 IU	≥1 follicle of >17mm	36 h after triggering	IVF/ ICSI	≤3/ Day 2–3	Progesterone vaginally. 1500 IU HCG
Alebic, et al., 2014, Reprod Biomed Online	GnRH Antagonist	Cetrotide/0.25mg s.c. per day	Flexible	hMG/300 IU	HCG/10.000 IU	≥1follicle of >17mm	35-36 h after triggering	IVF/ ICSI	01-03-2022/ Day 2-3	Progesterone, vaginally, 1500 IU HCG

Note*: ET: embryo transfer, OCP: oral contraceptives, OPU: oocyte pick-up, GnRH: Gonadotrophin releasing hormone, hMG: human chorionic gonadotrophin, rFSH: recombinant Follicle stimulating Hormone (FSH), IVF: in-vitro fertilization, ICSI: intracytoplasmic sperm injection SC: subcutaneous; IM: intramuscular.

Table 3: Characteristics of eligible studies evaluating the difference in DHEAS levels at initiation of ovarian stimulation in patients who achieved pregnancy or not after IVF/ICSI (A).

Study/ country of origin/ journal or meeting	Study period	Study type/n centres	Number of patients	Pregnancy outcomes evaluated	Inclusion	Population studied	Time of androgen assessment	Financial Support by Industry
Check, et al., 1995/USA/ Gynaecology Endocrinology	Not reported	Unclear/ Single centre	46	Clinical pregnancy (presence of gestational sac)	Women registering for the 1st IVF cycle, no PCOS	Women with no PCOS	Pre-stimulation, 10 days after GnRH agonist treatment, starting on cycle day 21	Not reported
Zollner, et al., 2001/ Germany/ Arch. Gynecol. Obstet	Not reported	Retrospective/ Single centre	71	Clinical pregnancy (presence of gestational sac)	Women without known endocrinopathies (PCOS, amenorrhea, pituitar adenoma)	Women with no PCOS	Previous cycle, days 4-9	Not reported
Frattarelli, et al., 2006/ USA/Fertility and Sterility	5/2002-5/ 2004	Prospective/ Single centre	109	Clinical pregnancy (cardiac activity at 6 weeks)	Age 19-42, FSH<12mIU/mL	General population	Cycle day 3	Not reported
Rashidi, et al., 2009/ Iran/Gynaecology Endocrinology	9/2006-8/2007	Prospective/ Single centre	53	Clinical pregnancy (6 weeks)	Age: 20-40 years, FSH<12mIU/ml, normal prolactin, no hx of chronic and infectious diseases	General population	Pre-stimulation, day 3 of menstruation, after GnRH agonist treatment starting on cycle day 21	Not reported
Alebic, et al., 2013/ Croatia/International Journal of Endocrinology	Not reported	Retrospective/ Single centre	129	Clinical pregnancy (cardiac activity ~28 days after OPU)	Null gravidity, normal uterus and uterine cavity, no hx of pelvic disease or surgery, no hx of use of medications that could interfere with basal hormone status, sperm count of at least 1x106 mL, serum AMH concentrations <6.5pmol/L	Expected poor responders	Previous cycle days 3-5	Not reported
Guo, et al., 2014/ China/Reproductive Biomedicine Online	3/2011 –3/2013	Retrospective/ Single centre	207	Clinical pregnancy (presence of gestational sac)	1st IVF cycle, age £35, no androgen supplementation, no anatomical abnormalities, no endocrine disorders, no PCOS	Women with no PCOS	Previous cycle day 3	No financial support
Guo, et al., 2016/ China/Reproduction, Fertility and Development	3/2010-8/2012	Retrospective/ single centre	1665	Clinical pregnancy (FH at 6 weeks)	1st IVF cycle, age: 20-40 years, FSH: 3-12 IU/L, LH> 1 IU/L, AFC>5 follicles, regular menstrual cycles, no smoking, no endocrine disorders, no PCOS, no anatomical abnormalities	Women with no PCOS	Cycle day 3 within 3 months before the treatment cycle	Not reported

Note*: IVF: *In-Vitro* fertilization, ICSI: intra-cytoplasmic sperm injection Hx: history, OPU: oocyte pick-up, Hx: history, FH: fetal heart, PCOS: polycystic cystic ovarian syndrome, HCG: human chorionic gonadotropin, DHEAS: dehydroepiandrosterone sulphate.

Table 4. Characteristics of eligible studies evaluating the difference in DHEAS levels at initiation of ovarian stimulation in patients who achieved pregnancy or not after IVF/ICSI (B).

Study/country of origin/ journal or meeting	GnRH-analogue used/ dose	GnRH-analogue protocol	Gonadotrophin type/ starting dose	Signal for triggering final oocyte maturation/ Dose	Criteria for triggering final oocyte maturation	OPU	Fertilization	ET policy Number of embryos/ Stage of development	Luteal phase support
Check, et al., 1995/USA/ Gynaecology Endocrinology	Leuprolide acetate/ 1mg per day	GnRH agonist long luteal	hMG/300IU	HCG /10.000 IU	2 follicles ≥20mm and E2>800pg/ ml	36 h after triggering	IVF	Not reported/Day 2	Not reported
Zollner et al., 2001/ Germany/ Arch. Gynecol. Obstet	Triptorelin/3.75mg depot	GnRH agonist long luteal	hMG/Not reported	HCG /10.000IU	≥1 follicle ≥18mm	34-36 h after triggering	IVF	≤3/ Day 2	Progesterone vaginally
Frattarelli et al., 2006/ USA/Fertility and Sterility	Leuprolide acetate/1mg per day	GnRH agonist long luteal, micro-dose flare	hMG/300 IU	HCG/Not reported	2 follicles ≥17 mm or a cohort of follicles 15–16 mm	36 h after triggering	IVF/ICSI	Not reported/Day 2-3	Progesterone i.m. or vaginally
Rashidi et al., 2009/Iran/ Gynaecology Endocrinology	Busereline/500 μg per day	GnRH agonist long luteal	rFSH /150-225 IU	HCG/10.000 IU	≥3 follicles ≥17 mm	36-38 h after triggering	IVF/ICSI	Not reported/Not reported	Progesterone vaginally
Alebic et al., 2013/Croatia/International Journal of Endocrinology	Cetrotide/0.25 mg per day	GnRH antagonist fixed	hMG/300 IU	HCG/10.000 IU	≥1 follicle of >17 mm	36h after triggering	IVF/ICSI	≤3 /Δαψ 2–3	Progesterone vaginally, 1500 IU HCG
Guo et al., 2014/China/ Reproductive Biomedicine Online	Triptorelin/1.25 mg depot or 0.85 mg/ampoule, 0.1 mg or 0.05 mg/ ampoule	GnRH agonist long luteal	rFSH/Not reported	HCG/Not reported	≥1 follicle of >18 mm	36-38h after triggering	IVF/ICSI	02-03-2022/Day 2-3	Progesterone i.m
Kunichi et al., 2015/Poland/ PLOS ONE	Triptorelin/Not reported	GnRH agonist long luteal	hMG/Not reported	HCG/5.000 IU	≥3 follicles ≥17 mm	36 h after triggering	ICSI	/Day 5	Natural micronized progesterone,micronized 17 beta oestradiol
Guo et al., 2016/China/ Reproduction, Fertility and Development	Triptorelin/1.25mg depot or 0.1 mg per day	GnRH agonist long luteal	rFSH or rFSH+hMG/ Not reported	HCG/10.000 IU	≥1 follicle ≥18 mm	36-38 h after triggering	IVF/ICSI	02-03-2022/Day 2-3	Progesterone i.m.

Selection Of Studies

Criteria for inclusion/exclusion of studies were established prior to literature search. Eligible studies for inclusion in the current systematic review and meta-analysis were prospective or retrospective studies that had assessed serum DHEAS levels at initiation of ovarian stimulation in women subjected to IVF by either conventional insemination or intracytoplasmic sperm injection (ICSI). Ovarian stimulation for multi-follicular development should have been performed using gonadotrophin releasing hormone (GnRH) analogues and gonadotrophins. Selection of studies was performed independently by two of the reviewers (V.E.C and E.M.K) and any disagreement was resolved by discussion.

Data Extraction

Data extraction was performed independently by two of the authors (V.E.C and E.M.K). The following data were extracted from each of the eligible studies: demographic (year of publication, country, study period, number of patients included), methodological

(type of study), procedural [whether financial support was declared, type of GnRH analogue and protocol used for luteinizing hormone (LH) surge inhibition, type and starting dose of gonadotrophin administered for ovarian stimulation, type and dose of medication used for triggering final oocyte maturation, criteria used for triggering final oocyte maturation, type of fertilization, day of embryo transfer, type of luteal support, endometrial preparation]. Any disagreement between the two authors responsible for data extraction was resolved by discussion. In case of missing data or ambiguities in study design or trial conduction, the study authors were contacted by e-mail to request additional information. Primary outcome was the difference in DHEAS levels at initiation of ovarian stimulation between pregnant and non-pregnant women after IVF.

Risk Of Bias and Study Quality Assessment

The quality of the eligible studies was assessed with the use of the Newcastle-Ottawa quality assessment scale (NOS) [14] adapted for the specific research questions allowing up to 9 stars (Supplementary Table 3). Two of the authors (V.E.C and E.M.K) independently evaluated the overall quality of the evidence. Any disagreement was resolved by discussion.

Supplementary Table 3: Quality assessment using the Newcastle-Ottawa Scale for non-randomized studies.ssss

Study	Selection	Comparability	Outcome	Quality score
Check, et al., [9]	***	*	***	Good quality
Zollner, et al., [10]	***	*	***	Good quality
Alebic, et al., [11]	***	*	***	Good quality
Guo, et al., [12]	***	*	***	Good quality
Frattarelli, et al.,[24]	****		***	Poor quality
Rashidi, et al.,[25]	****	*	****	Good quality
Kunichi, et al., [26]	***	*	***	Good quality
Guo, et al., [27]	***		***	Poor quality

Subgroup Analysis

The influence of population type (general population, expected poor responders, women without PCOS) was explored by performing subgroup analysis.

Statistical Analysis

Pooling of data was performed using the inverse variance method *Hedges, et al.*, in the case of fixed effects model, and the *DerSimonian* and *Laird method* [15], in the case of the random effects model. When the outcome of interest was of a continuous nature, the differences were pooled across the studies which provided information on this outcome, resulting in a standardized mean difference (SMD) with 95% confidence interval (CI). Study-to-study variation was assessed by using the Chi2 statistic (the hypothesis tested was that the studies are all drawn from the same population, i.e., from a population with the same effect size). In addition, the use of the I2 statistic was employed to indicate heterogeneity between studies that could not be attributed to chance, with I2 ≥40% indicating significant heterogeneity [16]. A fixed effects model was used, where no heterogeneity was present, while in the presence of significant heterogeneity, a random effects model was applied. The presence of publication bias was tested by using the Harbord-Egger's test [17]. Statistical significance was set at a p-level of 0.05. Meta-analysis of weighted average effect sizes was performed using STATA v14.0 (Stata Corp. 2015. Stata Statistical Software: Release 14. College Station, TX: Stata Corp LP).

Results

Identification of Literature

Literature search yielded 249 studies, the screening of which by study title resulted in 25 potentially eligible publications. Twelve out of these 25 studies were excluded after reading their abstracts, while the remaining 13 studies were further evaluated by retrieving their full text. Six out of these 13 studies were excluded since they did not provide data to answer the research question [18-23]. After hand search of the citation lists of all relevant publications and review articles, one additional study was found to provide data that answered the research question [24]. Eight studies were eligible for the systematic review (2747 patients) [9-12,24-27], assessing the difference in DHEAS levels between pregnant and non-pregnant women after IVF. A PRISMA flow diagram detailing selection of studies for inclusion is presented in Figure 1.

Systematic Review

Eight studies (two prospective, five retrospective and one of unclear design) published between 1995 and 2016 were eligible for the systematic review, including a total of 2747 patients. Characteristics of the studies included in the systematic review are presented in Tables 1-2. Most of the studies were of "good quality" as assessed by NOS Supplementary Table 2. Number of included patients ranged from 46 to 1665 (median=120). Three studies were conducted in Asia (n=1925), three studies in Europe (n=659)

and two studies in the USA (n=163). Five studies provided data regarding the research question in women without PCOS (n=2448), two studies in the general population [where normal, high (women with PCOS) and poor responders were included] (n=170) and one study in expected poor responders (n=129).

As shown in Table 1, all studies provided data for clinical pregnancy, defined as the presence of intrauterine sac at 6-8 weeks gestation. Power analysis was performed in one of the two prospective studies.

As shown in Table 2, to inhibit premature LH surge, GnRH agonists were used in seven out of the eight studies, and GnRH antagonists were used in the remaining study. Ovarian stimulation was performed with human menopausal gonadotrophins (hMG) in five studies, with the use of recombinant FSH (rFSH) in two studies and with either rFSH or rFSH and hMG in a single study. Human chorionic gonadotrophin (hCG) was used to trigger final oocyte maturation in all studies Table 2. Oocyte retrieval was performed 34-38h after hCG administration in all studies.

Conventional insemination was performed in two studies, ICSI in one study and either conventional insemination or ICSI in five studies. Embryo transfer was performed either at the cleavage stage (six studies), on days 3-5 of embryo culture (single study), or this information was unclear (single study).

Luteal phase was supported with vaginal progesterone in four studies, with intramuscular progesterone in two studies and either with intramuscular or vaginal progesterone in the remaining study. In addition to progesterone, estrogens or hCG were added in a single study. No information was present regarding luteal phase supplementation in a single study Table 2.

Meta-Analysis

Seven out of the eight studies included in the systematic review, assessing the difference in DHEAS levels at initiation of ovarian stimulation between pregnant and non-pregnant women after IVF provided data to answer the research question and were included in the meta-analysis. *Harbord-Egger's* test was not performed due to the low number of studies in the groups evaluated [16].

Main Analysis

No significant difference in DHEAS levels at initiation of ovarian stimulation was observed between pregnant and non-pregnant women after IVF (SMD: +0.02, 95% CI: -0.27 to +0.31, p=0.88, seven studies, 2630 patients) (Figure 2) [9-11,25-27].

Subgroup Analysis According to The Type of Population

Expected Poor Responders

Supplementary Table 4: Association between dehydroepiandrosterone sulphate (DHEAS) levels at initiation of ovarian stimulation and the achievement of pregnancy (clinical pregnancy) in patients undergoing IVF/ICSI stratified by type of population.

Expected Poor Responders	
Pregnant vs non-pregnant patients, SMD (95%CI)	+0.87 (+0.32 to +1.41)
Women without PCOS	
Pregnant vs non-pregnant patients, SMD (95%CI)	-0.15 (-0.47 to +0.16)
General Population	
Pregnant vs non-pregnant patients, SMD (95%CI)	+0.37 (-0.25 to +0.99)

Note*: Results are presented as SMD (95% confidence interval) for the eligible studies assessing the difference in DHEAS levels at initiation of ovarian stimulation between patients who achieved or not pregnancy (clinical pregnancy) after IVF. Cells in bold indicate statistical significance while cells are left blank where no relevant information s present.

Significantly higher DHEAS levels at initiation of ovarian stimulation were observed in women who achieved clinical pregnancy compared with those who did not after IVF (SMD: +0.87, 95% CI: +0.32 to +1.41, p=0.002, single study, 129 patients) [11] (Supplementary Table 4).

Although no significant difference was observed in body mass index (BMI) in women who achieved clinical pregnancy compared with those who did not, this was not the case regarding age which showed that women who achieved clinical pregnancy after IVF/ICSI were significantly younger compared with those who did not (Supplementary Table 5) [11].

Supplementary Table 5: Difference in female age or BMI between patients who achieved or not pregnancy after IVF.

AGE (years) WMD (95%CI)		
Expected poor responders	-2.30 (-4.24 to -0.36)	1 study, n=129
Women without PCOS	-1.78 (-4.00 to +0.45)	3 studies, n=712
BMI (kg/m^2) WMD (95%CI)		
Expected poor responders	-0.50 (-1.74 to +0.74)	1 study, n=129
Women without PCOS	-0.37 (-1.03 to +0.30)	2 studies, n=666

Note*: Results are presented as WHD (95% confidence interval). Cells in bold indicate statistical significance while cells are left blank where no relevant information is present.

Women Without PCOS

No significant difference in DHEAS levels at initiation of ovarian stimulation was observed between pregnant and non-pregnant women after IVF (SMD: -0.15, 95% CI: -0.47 to +0.16, p=0.35, five studies, 2448 patients) Supplementary Table 4 & Figure 3 [9,10,12,26,27]. In these studies, no significant difference was observed in age or BMI between women between pregnant and non-pregnant women after IVF Supplementary Table 4.

General Population

No significant difference in DHEAS levels at initiation of ovarian stimulation was observed between pregnant and non-pregnant women after IVF (SMD: +0.37, 95% CI: -0.25 to +0.99, p=0.25, single study, 53 patients) Supplementary Table 4 & Figure 3 [28].

Discussion

This systematic review and meta-analysis, including eight eligible studies and 2747 women, showed that similar DHEAS levels are present at initiation of ovarian stimulation between women who achieve or not clinical pregnancy after IVF. This is true both for women without PCOS as well as for women in the general population. However, there is weak evidence to suggest that higher DHEAS levels at initiation of ovarian stimulation are present in poor responders who achieve clinical pregnancy compared with those who did not. The underlying mechanism regarding the increased DHEAS levels at initiation of ovarian stimulation observed in expected poor responders who achieved clinical pregnancy compared to those who did not remains unclear. It should be, however, taken into consideration that women who achieved clinical pregnancy were significantly younger compared with those who did not. It is well known that DHEAS levels decrease profoundly with age [29,30], which is negatively associated with the achievement of pregnancy [31,32]. Thus, it could be argued that the positive association between DHEAS levels at initiation of ovarian stimulation and the achievement of pregnancy might be explained by female age, although this requires further investigation.

It has been shown that DHEAS levels play an important role in folliculogenesis and follicular development, by stimulating the initiation of primordial follicles and enhancing the development of pre-antral and early antral follicles [33]. Furthermore, in studies performed in rats with diminished ovarian reserve, levels of atresia were reduced after DHEAS treatment [34]. In addition, a positive correlation between DHEAS levels in the follicular fluid and clinical pregnancy as well as live birth rates has been reported in non-PCOS women [35]. On the other hand, DHEAS supplementation prior to IVF in poor responders has been associated with improved aneuploidy rates [36], whereas no significant difference was observed in the number of oocytes retrieved, clinical pregnancy and live birth rates compared to no treatment [37,38]. Even though most of the studies included in the current systematic review and meta-analysis where of good quality, as assessed by the relevant quality tools, most of these studies were of retrospective nature with considerable heterogeneity regarding patient population and type of ovarian stimulation protocol used. Furthermore, although a lack of difference in the comparisons performed may represent a true lack of difference, it may also reflect a type b error due to the insufficient number of patients included in the eligible studies.

Conclusion

In conclusion, the present systematic review and meta-analysis showed similar DHEAS levels at initiation of ovarian stimulation between women who achieved pregnancy after IVF/ICSI compared with those who did not. This is true both for women without PCOS as well as for women in the general population. However, there is weak evidence to suggest that higher DHEAS levels at initiation of ovarian stimulation are present in poor responders who achieve clinical pregnancy compared with those who did not. If this finding is confirmed in future studies, it highlights the importance of taking into consideration DHEAS levels at initiation of ovarian stimulation in expected poor responders in case DHEA administration is proposed, since a differential effect of DHEA, depending on DHEAS levels might be present.

Declaration of Interest

None.

References

1. Mertens HJ, MJ Heineman, J Koudstaal, P Theunissen, JL Evers, et al. (1996) Androgen receptor content in human endometrium. Eur J Obs Gyn Rep Biol 70(1): 11-13.
2. Simitsidellis I, PTK Saunders, DA Gibson (2018) Androgens and endometrium: new insights and new targets. Mol Cell Endocrinol 465: 48-60.
3. Bonser J, J Walker, A Purohit, MJ Reed, BV Potter, et al. (2000) Human granulosa cells are a site of sulphatase activity and are able to utilize dehydroepiandrosterone sulphate as a precursor for oestradiol production. J Endocrinol 167(3): 465-471.
4. Vendola K, J Zhou, J Wang, OA Famuyiwa, M Bievre et al. (1999) Androgens promote oocyte insulin-like growth factor I expression and initiation of follicle development in the primate ovary. Biol Reprod 61(2): 353-357.
5. Weil S, K Vendola, J Zhou, CA Bondy (1999) Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. J Clin Endocrinol Metab 84(8): 2951-2956.
6. Astapova O, BMN Minor, SR Hammes (2019) Physiological and Pathological Androgen Actions in the Ovary. Endocrinology 160(5): 1166-1174.
7. Haning RV, RJ Hackett, RI Boothroid, JA Canick (1990) Steroid sulphatase activity in the human ovarian corpus luteum, stroma, and follicle: comparison to activity in other tissues and the placenta. J Steroid Bio 36(1-2): 175-179.
8. Labrie F, V Luu The, C Labrie, A Belanger, J Simard, et al. (2003) Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. Endocr Rev 24(2): 152-182.
9. Check JH, A Nazari, C Dietterich (1995) Comparison of androgen levels in conception vs. non-conception cycles following controlled ovarian stimulation using the luteal phase gonadotropin-releasing hormone agonist protocol. Gynecol Endocrinol 9(3): 209-214.
10. Zollner U, K Lanig, T Steck, J Dietl (2001) Assessment of endocrine status in patients undergoing in-vitro fertilization treatment. Is it necessary? Arch Gynecol Obstet 265(1): 16-20.
11. Alebić M, N Stojanović, M Zuvić Butorac (2013) The IVF Outcome Counseling Based on the Model Combining DHEAS and Age in Patients with Low AMH Prior to the First Cycle of GnRH Antagonist Protocol of Ovarian Stimulation. Int J Endocrinol 2013: 637919.
12. Guo J, Q Zhang, Y Li, J Huang, W Wang, et al. (2014) Predictive value of androgens and multivariate model for poor ovarian response. Rep Biomed Onl 28(6): 723-732.
13. Page MJ, JE McKenzie, PM Bossuyt, I Boutron, TC Hoffmann, et al. (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 372: n71.
14. Wells G, B Shea, DO Connell, J Peterson, V Welch, et al. (2013) The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa Hospital Research Institute.
15. DerSimonian R, N Laird (1986) Meta-analysis in clinical trials. Control Clin Trials 7(3): 177-188.
16. Higgins J, J Thomas, J Chandler, M Cumpston, T Li, et al. (2020) Cochrane Handbook for Systematic Reviews of Interventions. Recommendations on testing for funnel plot asymmetry.
17. Harbord RM, M Egger, JA Sterne (2006) A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. Stat Med 25(20): 3443-3457.
18. Frattarelli JL, EH Peterson (2004) Effect of androgen levels on in vitro fertilization cycles. Fertil Steril 81(6): 1713-1714.
19. Orvieto R, V Yulzari Roll, A La Marca, J Ashkenazi, B Fisch, et al. (2005) Serum androgen levels in patients undergoing controlled ovarian hyperstimulation for in vitro fertilization cycles. Gynecol Endocrinol 21(4): 218-222.
20. Alebić M, N Stojanović (2014) Dehydroepiandrostendione sulphate and prediction of live birth after IVF in young women with low anti-Müllerian hormone concentration. Reprod Bio Onl 28(2): 191-197.
21. Abide Yayla C, E Ozkaya, S Kayatas Eser, I Sanverdi, B Devranoglu, et al. (2018) Association of basal serum androgen levels with ovarian response and ICSI cycle outcome. Ir J Med Sci 187(2): 409-415.
22. Findeklee S, P Sklavounos, L Stotz, RM Sima, I Iordache et al. (2019) Influence of androgen levels on conception probability in patients undergoing fertility treatment: a retrospective cohort study. Arch Gyn Obs 299(5): 1481-1485.
23. Chimote BN, NM Chimote (2021) Dehydroepiandrosterone sulphate (DHEAS) concentrations stringently regulate fertilisation, embryo development and IVF outcomes: are we looking at a potentially compelling 'oocyte-related factor' in oocyte activation? J Assist Rep Genet 38(1): 193-202.
24. Frattarelli JL, MD Gerber (2006) Basal and cycle androgen levels correlate with in vitro fertilization stimulation parameters but do not predict pregnancy outcome. Fertil Steril 86(1): 51-57.
25. Hossein Rashidi B, Hormoz B, E Shahrokh Tehraninejad, Shariat M, Mahdavi A, et al. (2009) Testosterone and dehydroepiandrosterone sulphate levels and IVF/ICSI results. Gynecol Endocrinol 25(3): 194-198.
26. Kunicki M, K Lukaszuk, G Jakiel, J Liss (2015) Serum dehydroepiandrosterone sulphate concentration is not a predictive factor in IVF outcomes before the first cycle of GnRH agonist administration in women with normal ovarian reserve. PLoS One 10(3): e0118570.
27. Guo J, Q Zhang, Y Li, W Wang, D Yang, et al. (2016) Low level of basal testosterone: a significant risk factor for poor oocyte yield after ovulation induction. Reprod Fertil Dev 28(3): 286-292.
28. Rashidi BH, B Hormoz, E Shahrokh Tehraninejad, M Shariat, A Mahdavi, et al. (2009) Testosterone and dehydroepiandrosterone sulphate levels and IVF/ICSI results. Gynecol Endocrinol 25(3): 194-198.
29. De Pergola G, VA Giagulli, G Garruti, MR Cospite, F Giorgino, et al. (1991) Low dehydroepiandrosterone circulating levels in premenopausal obese women with very high body mass index. Metabolism 40(2): 187-190.
30. Sulcova J, M Hill, R Hampl, L Starka (1997) Age and sex related differences in serum levels of unconjugated dehydroepiandrosterone and its sulphate in normal subjects. J Endocrinol 154(1): 57-62.
31. George K, MS Kamath (2010) Fertility and age. J Hum Reprod Sci 3(3): 121-123.
32. Provost MP, KS Acharya, CR Acharya, JS Yeh, RG Steward, et al. (2016) Pregnancy outcomes decline with increasing body mass index: analysis of 239,127 fresh autologous in vitro fertilization cycles from the 2008-2010 Society for Assisted Reproductive Technology registry. Fertil Steril 105(3): 663-669.
33. Narkwichean A, K Jayaprakasan, WE Maalouf, JH Hernandez Medrano, et al. (2014) Effects of dehydroepiandrosterone on in vivo ovine follicular development. Hum Reprod 29(1): 146-154.
34. Hassa H, Y Aydin, O Ozatik, K Erol, Y Ozatik, et al. (2015) Effects of dehydroepiandrosterone (DHEA) on follicular dynamics in a diminished ovarian reserve in vivo model. Syst Biol Rep Med 61(3): 117-121.
35. Chimote NM, NM Nath, NN Chimote, BN Chimote (2015) Follicular fluid dehydroepiandrosterone sulfate is a credible marker of oocyte maturity and pregnancy outcome in conventional in vitro fertilization cycles. J Hum Reprod Sci 8(4): 209-213.

36. Gleicher N, DH Barad (2011) Dehydroepiandrosterone (DHEA) supplementation in diminished ovarian reserve (DOR). Rep Biol Endocrinol 9: 67.

37. Neves AR, P Montoya Botero, NP Polyzos (2022) Androgens and diminished ovarian reserve: the long road from basic science to clinical implementation. A comprehensive and systematic review with meta-analysis. Am J Obs Gyn 227(3): 401-413 e418.

38. Wang Z, A Yang, H Bao, A Wang, X Deng, et al. (2022) Effect of dehydroepiandrosterone administration before in vitro fertilization on the live birth rate in poor ovarian responders according to the Bologna criteria: A randomised controlled trial. BJOG 129(7): 1030-1038.