Dehydroepiandrosterone (DHEA) supplementation: an underappreciated cause of premature progesterone elevation detected during frozen embryo transfer

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Introduction

The negative impacts of late follicular elevation of serum progesterone (P) on the clinical outcome of fresh embryo transfers (ET) have been well established [1, 2]. The main detriment of premature P elevation is impaired endometrial receptivity, specifically, the asynchrony between the endometrium and the developing embryos [3, 4]. Therefore, frozen embryo transfer (FET) was almost universally offered to patients in the event of premature P elevation with encouraging outcomes, indicating that follicular P exposure confers no significant impact to the quality of oocyte and the derived embryos [5, 6].

Previously, the origin of P elevation in the follicular phase was attributed to the premature luteinizing hormone (LH) surge during non-down-regulated ovarian stimulations [7]. Nevertheless, with the introduction of various GnRH analogues to stimulation protocols effectively minimizing the event of early LH surge, the incidence of premature P elevation remained prevalent [8, 9]. It is now postulated that primary cause of premature P elevated is from the supernumerary follicle development resulted from prolonged ovarian stimulation with follicular stimulating hormone (FSH), with each of resultant follicle contributing partially to the total serum P concentration [8, 10, 11].

Therefore, according to the above theory, the probability of premature P elevation in FET cycle should be minimal, especially in cases with artificial endometrium preparation since no event of follicular maturation and ovulation would occur. Recently, Franasia et al. have reported the interference of serum P immunoassay in patients treated with oral DHEA supplement during fresh IVF cycles [12]. The authors demonstrated unequivocally that DHEA-S, which is the main metabolite of ingested DHEA, could cross-react with three of the mainstream commercial P immunoassays, resulting in falsely elevated serum P level being reported. The phenomenon can have great clinical implications, as DHEA has become one of the most frequently prescribed supplements to IVF poor responders. In the responses to a worldwide survey from 196 IVF units in 45 countries, which represented a total of 124,700 IVF cycles, DHEA was included in more than a quarter of treatment protocols for patients with diminished ovarian reserve (DOR) [13].

With the recognized negative impact of premature P elevation, the embryo transfer cycle would inevitably be cancelled in such event. In the light of the above discovery, we report two cases of falsely elevated serum P reported during FET cycle by artificial endometrium preparation, with the P levels returned to normal range upon the cessation of DHEA supplement. To the best of our knowledge, these are the first series of cases documenting such phenomenon in a true clinical scenario of FET. Our discovery further strengthened the point that DHEA supplement could interfere the P values detected by commercial assays, and potentially affecting the clinical decision of physicians.

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Case report

Two women of Southeast Asian descent presented to our clinic with diagnosis of primary infertility. Both patients had

underwent three unsuccessful cycles of IVF with fresh ET at other fertility clinics. Our initial assessments included antral follicle count, serum anti-Müllerian hormone, thyroid function, and prolactin assays. Based on above results and their previous response to gonadotropin stimulation, they were both classified as patients with DOR. Hysterosalpingogram and hysteroscopy were also performed to exclude possible uterine pathology. Before their initial visit, they both had been ingesting DHEA supplement continually at a dose of 75 mg/day for the past 3 months under the advice of their previous physicians. Flexible gonadotropin antagonist protocol was used in their subsequent IVF cycle. Premature elevation of P (> 1.0 ng/mL) was noted on the day of the trigger shot for both patients; therefore, fresh embryo transfer was cancelled and all the embryos were vitrified. The FET cycle was prepared with daily oral estradiol valerate at 8 mg/day, commencing on day 3 of the subsequent menstrual cycle. After 10 days of estrogen priming (FET d10), transvaginal ultrasound revealed thick trilaminar endometrium at 9 and 10 mm, respectively. Blood test on the same day revealed elevated serum P. After thorough counselling, the patients were offered two options. Option one was to cancel the present FET cycle. Option two was to check serum DHEA-S level on the same day, then withhold DHEA supplement for 72 h and return to the clinic to recheck P and DHEA-S levels again, as well as endometrial ultrasound appearance. Both patients opted for option two. The blood tests after 72 h (FET d14) showed marked decrement in both P and DHEA-S levels, and the appearance of the endometrium remained the same without signs of luteinization. Furthermore, the calculated slope between the difference of P and DHEA-S levels on FET d10 and FET d14 showed remarkably identical results in both cases (Figs. 1 and 2). Both patients continued with their FET, with one achieving termed live birth and another with ongoing pregnancy presently at 20 weeks of gestations. The P immunoassay platform

used was eCobas 411 (Roche Diagnostics USA, Indianapolis, IN) in patient 1, and Advia Centaur (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY) in patient 2, which concurred with two of three platforms reported by Franasiak et al. [12] that showed cross-reactivity with DHEA-S. Summary of the relevant patient characteristics and endocrine profiles is presented in Table 1.

Discussion

This the first-ever report investigating the effects of DHEA supplement on the perceived serum P level in the clinical setting of FET prepared with artificial endometrium. We have demonstrated again that DHEA-S, the metabolite of DHEA, does have cross-reactivity with some of the popular P immunoassays, thus potentially affecting physician's clinical decisions.

The precise definition of premature P raise is still under great debate. Different follicular phase P concentrations, ranging from 0.8 to 2.0 ng/mL, have been proposed as the threshold which is detrimental to endometrial receptivity [14, 15]. Using $P > 1.2$ ng/mL as the cut-off value, Bosch et al. has demonstrated the incidence of premature P elevation can be as high as 38% in stimulated IVF cycles, resulting in significant reduction in pregnancy and implantation rate in the affected group [8]. Our clinic has adopted a more stringent criterion set at 1.0 ng/mL.

Under physiological conditions, adrenal DHEA serves as the precursor of steroid hormones, and the positive association between DHEA supplement and serum P level has been explored in literatures. In a study consisted of postmenopausal population using low-dose DHEA (10 mg/day) for hormone replacement therapy, significant increase in serum P level was found just after 1 month of supplement [16]. In another case

Fig. 1 The changes of serum progesterone levels during DHEA supplement (FET d10) and after discontinuing DHEA supplement for 72 h (FET d14)

Fig. 2 The changes of serum DHEA-S levels during DHEA supplement (FET d10) and after discontinuing DHEA supplement for 72 h (FET d14)

report by Strauss et al., increased circulating P was observed after 75 mg DHEA was supplemented daily in an oncology patient who underwent ovarian tissue transplantation [17]. In the case control study by Weissman et al., significantly increased follicular phase P level was found in IVF patients with DOR in cycles supplemented with 75 mg of DHEA daily [18]. Interestingly, the clinical pregnancy rate for fresh ET was higher in the DHEA-supplemented cycles (33 vs. 6.6%), despite significantly higher level of P on hCG trigger day (0.58 vs. 1.54 ng/mL). These findings contradicted the proven concept of high follicular phase P interfering with endometrial receptivity. A possible explanation of such contradiction is that in the DHEA-supplemented cycle, the serum P was in fact not elevated but from DHEA-S cross-reacting with P immunoassay. Furthermore, the immunoanalyzer used in the above study (Elecsys; Roche Diagnostics) was a similar model to one of the three platforms reported by Franasiak et al. [12] that showed DHEA-S cross-reactivity.

Table 1 Patient characteristics and endocrine profiles during ovarian stimulation and frozen embryo transfer cycle

	Patient 1	Patient 2
Age	37	38
AMH (ng/mL)	0.14	0.91
hCG day P (ng/mL)	1.97	2.09
hCG day E ₂ (pg/mL)	1157	890
FET d10 P (ng/mL)	1.12	1.36
FET d14 P (ng/mL)	0.187	0.4
Slope ($\Delta Y_{(\Delta P)}/\Delta X_{(\Delta \text{Days})}$)	-2.35	-2.4
FET d10 DHEA-S (µg/dL)	507.6	634.9
FET d14 DHEA-S (µg/dL)	146	246.2
Slope ($\Delta Y_{(\Delta \text{DHEA-S})}/\Delta X_{(\Delta \text{Days})}$)	-90.25	-97.17

FET with artificial endometrial preparation, in the absence of spontaneous ovulation or exogenous gonadotropin stimulation, provides a unique opportunity to examine the effects of DHEA on serum P level in a real clinical scenario. In the study by Franasiak et al., the authors conclusively demonstrated that DHEA-S, whether from a pure commercial preparation or from endogenous production from the sulfation of DHEA, does cross-react with various marketed P assays in a dose-dependent manner; therefore, serum P levels in IVF patients on DHEA supplementation may not truly represent the bioactive progesterone concentration [12]. According to the statement from the testing platforms' manufacturers, such interactions have not been observed under physiological level of DHEA-S, and cross-reaction would probably only occur under supra physiological DHEA-S concentration derived from supplements [12]. If that is indeed the case, given the prevalence of DHEA supplement among IVF patients with DOR, this finding still has significant implications. In order for any test to have clinical significance, the fidelity to which the reported value truly reflects the clinically relevant values must first be assessed carefully.

It has been our clinic's longstanding policy to measure the serum P level before luteal conversion of estrogen-primed endometrium in FET cycle. Only recently have we begun to notice the association between of the elevated P level to DHEA supplement, which prompted us to investigate such phenomenon. Though only two cases are reported here, the possibility of many "misguided" ET cancellation due to perceived premature P elevation cannot be overlooked. Cancellation of ET not only leads to protracted time to live birth but also burdens infertile couples with further stress. Since the occurrence of the premature P elevation during ovarian stimulation is not an uncommon event, prudence must be exercised when supplement patients with DHEA, as many of the commercial P assay may not be able to readily distinguish

DHEA-S from P. In such event, the reported P level would be interpreted incorrectly thus affecting practitioners' clinical decisions. The most appropriate timing of DHEA supplementation during fertility treatments warrants further research. Lastly, efforts should be made to develop P immunoassays that have minimal cross-reactivity with DHEA-S at any concentration.

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