Case report

Ongoing pregnancies from early retrieval of prematurely developing antral follicles after DHEA supplementation



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Abstract

Two patients with severely diminished ovarian reserve who were refractory to aggressive ovarian stimulation conceived with oocytes from prematurely developing antral follicles after dehydroepiandrosterone supplementation. The first patient had 11 and 14.5 mm, and the second patient had 13 mm antral follicles on cycle days 2 and 3 respectively. In the first case, no ovarian stimulation was performed, while the second case received one dose of gonadotrophins with a gonadotrophin-releasing hormone antagonist. Following very early human chorionic gonadotrophin (HCG) triggering on cycle day 5, when antral follicles reached 15 and 18.5 mm in the first case, and 19 mm in the second case, IVF intracytoplasmic sperm injection treatment resulted in pregnancies in both cases, which are currently ongoing at 35 and 14 weeks of gestation. The results in these patients show that pregnancy can be achieved in poor responder patients with prematurely developing antral follicles following early HCG triggering based on follicle size rather than cycle day, with no or minimal stimulation. Whether DHEA supplementation had any impact on the success of these cycles remains to be determined.

Keywords: *antral follicle, DHEA, poor ovarian reserve*

Introduction

Thanks to the development of gonadotrophins and gonadotrophin-releasing hormone (GnRH) analogues, there have been significant improvements in assisted reproductive techniques. Stimulation protocols have been constantly optimized since their first introduction into clinical use. For optimal ovarian stimulation, it is generally preferred that baseline serum oestradiol is <50 pg/ml, endometrial lining is <5 mm, and that no dominant follicle >10 mm exists during the early follicular phase (Barash *et al.*, 1998). Aspiration of dominant follicle/s, sustaining GnRH analogues at the same doses until high serum oestradiol declines below desired values, or cycle cancellation, are among the usual practices when desired hormonal or ultrasonographic values are not met.

However, clinical management of patients with poor ovarian reserve (POR) might differ from those with a normal ovarian reserve because of accelerated follicle growth due to high FSH concentrations. Despite a high oestradiol concentration at baseline, this may reflect advanced follicle maturation and not necessarily alter oocyte quality even though these two go hand in hand in older patients.

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Dehydroepiandrosterone (DHEA) supplementation has recently been introduced as a method to improve success rates in women with premature ovarian ageing (Sönmezer *et al.*, 2009). This study reports on two patients with severely diminished ovarian reserve who were refractory to aggressive ovarian stimulation and became pregnant after the retrieval of oocytes from prematurely developing dominant follicles following DHEA supplementation.

Case 1

A 31-year-old patient was evaluated in the IVF centre for infertility of 12 years duration; she had previously undergone several intrauterine insemination cycles. Initial examination revealed normal semen parameters and a normal hysterosalpingography; however, day 3 FSH and oestradiol concentrations were 21.5 IU/l and 29.2 pg/ml, respectively. On transvaginal ultrasound, a total of three antral follicles and a simple ovarian cyst measuring 5 × 4 cm were observed. One month later, her day 2 serum FSH and oestradiol were 10 IU/l and 79 pg/ml, respectively. Ovarian stimulation was commenced with a combination of 225 IU human menopausal gonadotrophin (HMG, Menogon; Ferring, Turkey) and 225 IU recombinant FSH (rFSH; Puregon, Schering-Plough, Turkey), but the patient did not respond. Three months later, when day 2 serum FSH was 12.7 IU/l and oestradiol was 16.9 pg/ml, she was started on ovarian stimulation with the same protocol, but there was no response again. The patient was recommended to use DHEA 25 mg t.i.d. Following 6 months of DHEA supplementation, the serum FSH was 8.9 IU/l and oestradiol was 210 pg/ml on day 2 of the cycle. Transvaginal ultrasound revealed two follicles measuring 14.5 and 11 mm. It was decided to monitor follicle growth without ovarian stimulation. On cycle day 5, her serum oestradiol was 712 pg/ml, the dominant follicles reached 18.5 and 15 mm in diameter, and endometrial thickness was 9 mm (**Figure 1**). Oocyte maturation was triggered by 10,000 IU of HCG (Pregnyl; Schering Plough), and oocyte retrieval was performed at 35.5 h, yielding two metaphase II oocytes. Two grade A embryos were transferred on day 4 by a soft embryo transfer catheter under ultrasonographic guidance (Swemed; Vitrolife, Kungsbacka, Sweden). The luteal phase was supported with natural micronized progesterone vaginal capsules 200 mg t.i.d (Progestan; Koçak Pharmacy, Turkey). Serum beta-HCG concentration was 118 IU/ml on day 12 after the embryo transfer, and a singleton intrauterine pregnancy with a fetal heart beat was observed by transvaginal ultrasonography in gestational week 6. Currently the pregnancy is ongoing in week 35 without any complications.

Case 2

A 30-year-old patient with unexplained infertility of 8 years duration was referred to the IVF centre. She had previously undergone three IUI cycles in another clinic. Basal evaluation revealed cycle day 3 serum FSH and oestradiol concentrations of 16.6 IU/l and 370 pg/ml respectively, and three antral follicles were observed on ultrasound. Ovarian stimulation was commenced with a combination of 225 IU rFSH (Puregon; Schering-Plough) and 150 IU HMG (Menogon; Ferring, Turkey). Only one follicle developed and a mature egg was retrieved which was fertilized by intracytoplasmic sperm injection (ICSI); however, transfer of the developing embryo did not result in pregnancy. Another cycle was again cancelled due to no response with the same protocol. After supplementation of DHEA 25 mg t.i.d for 4 months, her cycle day 3 serum FSH and oestradiol concentrations were 5.1 IU/l and 139 pg/ml. Transvaginal ultrasound on the same day revealed two antral follicles in the right ovary and one antral follicle and a 13 mm dominant follicle in the left ovary with an endometrial thickness of 6.8 mm. On cycle day 4, a minimal ovarian stimulation was commenced with 150 IU HMG along with a GnRH antagonist (Cetrotide; Merck Serono, Geneva, Switzerland) 0.25 mg/day when serum oestradiol was 176 pg/ml. On cycle day 5, her serum oestradiol was 294 pg/ml and there were two follicles measuring 19 and 13 mm with an endometrial thickness of 9 mm (**Figure 2**; dominant follicle only). Only GnRH antagonist was administered and oocyte maturation was triggered by 250 µg recombinant HCG (Ovitrelle; Serono). An MII oocyte was retrieved 36 h later and was fertilized by ICSI. Blastocyst transfer was performed on day 5 using a soft embryo transfer catheter under ultrasonographic guidance. Serum

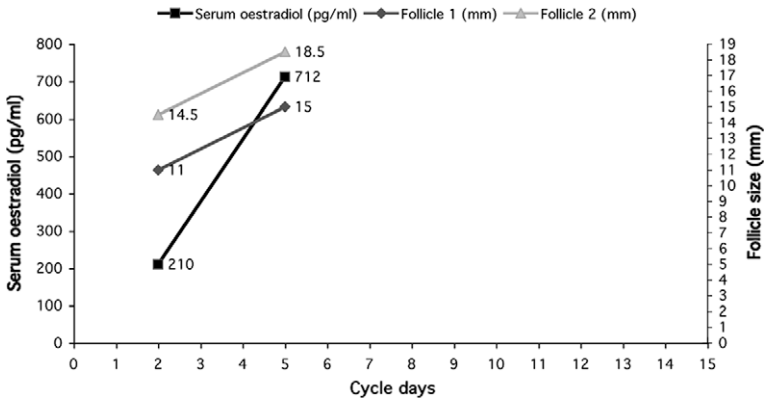


Figure 1. Schematic representation of oestradiol concentrations and follicle sizes in relation to menstrual day in case 1. HCG = human chorionic gonadotrophin.

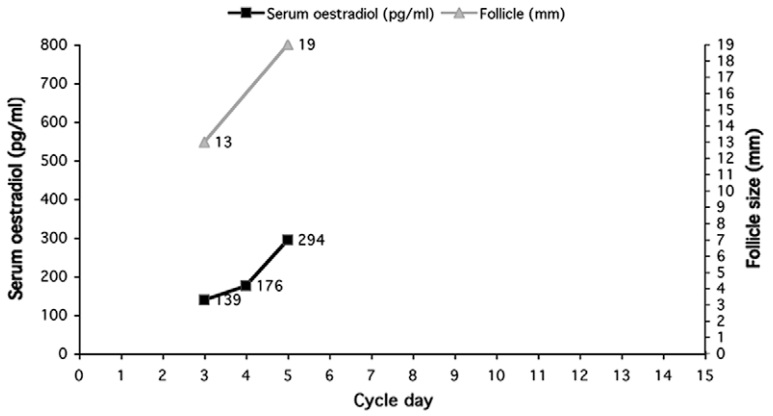


Figure 2. Schematic representation of oestradiol concentrations and follicle sizes in relation to menstrual day in case 2.

beta-HCG was 129 IU on day 12 after the transfer. Fetal heart rate was confirmed on gestational week 6 and the pregnancy is still ongoing in its 14th week.

Discussion

Ovarian reserve is made up of primordial follicles, the number of which inexorably declines with advancing age; this becomes more prominent after the age of 38 (Speroff and Fritz, 2005). As ovarian reserve diminishes, there is a progressive decline in inhibin B secretion by granulosa cells, with a consequential increase in FSH concentrations, notably in the early follicular phase. The rising FSH concentrations during the period of luteofollicular transition stimulate earlier follicle development, which is associated with advanced follicular maturation and early oestradiol rise (Klein *et al.*, 2002; van Zonneveld *et al.*, 2003). Elevated basal serum FSH is associated with diminished numbers of growing follicles, but advanced age is more strongly associated with decreased oocyte quality.

It is advocated that the likelihood of pregnancy, either with IVF or spontaneous, is different in young women with elevated FSH compared with women of advanced age (van Montfrans *et al.*, 2000; van Rooij *et al.*, 2004). Levi *et al.* observed that more pregnancies resulted in live birth in younger women with elevated serum FSH compared with that in women older than 40 years of age with elevated serum FSH (Levi *et al.*, 2001). Among the most important points to underline is that the implantation window may begin at an earlier time in such patients due to early increasing serum FSH and accelerated follicle growth, as occurred in both of the present cases. In a stimulated cycle, advanced proliferative aspects and early secretory changes were observed even before any rise in serum progesterone in the pre-ovulatory phase (Marchini *et al.*, 1991), and the day of oocyte retrieval is generally accepted as equivalent to day 14 in a natural cycle (Develioglu *et al.*, 1999; Creus *et al.*, 2003). The window of implantation can be different due to altered hormonal environment, accelerated follicle growth and early rise in serum oestradiol in patients with diminished ovarian reserve. In both of the presented cases the HCG trigger was strikingly early compared with a stimulated cycle, on cycle day 5. Moreover, ICSI of these oocytes resulted in very good quality embryos that were transferred on cycle days 11 and 12, which was again earlier in comparison with a stimulated cycle.

Both of the presented patients received DHEA for at least 3 months with the aim of improving ovarian response. It was demonstrated that DHEA increases follicular FSH receptor expression in granulosa cells, promotes initiation of primordial follicular growth, and increases the number of pre-antral and small antral follicles (Vendola *et al.*, 1998, 1999). Oral administration of physiological DHEA resulted in marked augmentation of serum IGF-I concentrations (Casson *et al.*, 1998). It has been previously demonstrated that patients with POR can benefit from long term DHEA supplementation, as reflected by enhanced ovarian response, reduced cycle cancellation, and increased embryo quality (Barad and Gleicher, 2006; Sönmezer *et al.*, 2009). In the present study, significant decreases were found in day 3 serum oestradiol concentrations after at least 3 months of DHEA supplementation. Moreover, mean number of follicles >17 mm and MII oocytes increased after DHEA supplementation. Another recent interesting report presented five successful pregnancies in five patients with premature ovarian failure after at least 2 months of DHEA supplementation (Mamas and Mamas, 2009). Of the pregnancies, four conceived naturally and one by intrauterine tuboperitoneal insemination.

In conclusion, altered follicular dynamics and its subsequent impact on oocytes and endometrium should be taken into account when managing a patient with diminished ovarian reserve. Young patients with elevated basal FSH may have a good chance to achieve a pregnancy, and when appropriate, patients should not be discouraged from undergoing assisted reproductive technologies. Whether DHEA has a direct effect on the growth or recruitment of these follicles or there is a synergistic action with early rising serum FSH remains to be clarified. With the presented cases here, it is suggested that when prematurely developing dominant follicles are observed in young patients undergoing IVF with seemingly diminished ovarian reserve along with elevated serum oestradiol, a natural cycle or minimal ovarian stimulation can be performed to achieve a pregnancy instead of cycle cancellation. However, the

proposition that triggering the ovulation with HCG at cycle day 5 is effective for these patients should be modulated by the fact that the menstrual phase of the cycle is variable from one woman to another and that endometrial receptivity may not always be adequate according to whether patients were still bleeding or not. A larger series of cases is necessary to challenge this concept.

References

Barad D, Gleicher N 2006 Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF. *Human Reproduction* **21**, 2845–2849.

Barash A, Weissman A, Manor M *et al.* 1998 Prospective evaluation of endometrial thickness as a predictor of pituitary down-regulation after gonadotropin-releasing hormone analogue administration in an in vitro fertilization program. *Fertility and Sterility* **69**, 496–499.

Casson PR, Santoro N, Elkind-Hirsch K *et al.* 1998 Postmenopausal dehydroepiandrosterone administration increases free insulin-like growth factor-I and decreases high-density lipoprotein: a six month trial. *Fertility and Sterility* **70**, 107–110.

Creus M, Ordi J, Fabregues F *et al.* 2003 The effect of different hormone therapies on integrin expression and pinopode formation in the human endometrium: a controlled study. *Human Reproduction* **18**, 683–693.

Develioglu OH, Hsiu JG, Nikas G *et al.* 1999 Endometrial estrogen and progesterone receptor and pinopode expression in stimulated cycles of oocyte donors. *Fertility and Sterility* **71**, 1040–1047.

Klein NA, Harper AJ, Houmard BS *et al.* 2002 Is the short follicular phase in older women secondary to advanced or accelerated dominant follicle development? *Journal of Clinical Endocrinology and Metabolism* **87**, 5746–5750.

Levi AJ, Raynault MF, Bergh PA *et al.* 2001 Reproductive outcome in patients with diminished ovarian reserve. *Fertility and Sterility* **76**, 666–669.

Mamas L, Mamas E 2009 Premature ovarian failure and dehydroepiandrosterone. *Fertility and Sterility* **91**, 644–646.

Marchini M, Fedele L, Bianchi S *et al.* 1991 Secretory changes in preovulatory endometrium during controlled ovarian hyperstimulation with buserelin acetate and human gonadotropins. *Fertility and Sterility* **55**, 717–721.

Sönmezer M, Özmen B, Çil AP *et al.* 2009 Dehydroepiandrosterone supplementation improves ovarian response and cycle outcome in poor responder patients. *Reproductive BioMedicine Online* **19**, 508–513.

Speroff L, Fritz MA 2005 *Clinical Gynecologic Endocrinology and Infertility*. Philadelphia: Lippincott Williams and Wilkins; 2005, pp. 1013–1067.

van Montfrans M, Hoek A, van Hooff MH *et al.* 2000 Predictive value of basal follicle-stimulating hormone concentrations in a general subfertility population. *Fertility and Sterility* **74**, 97–103.

van Rooij IA, De Jong E, Broekmans FJ *et al.* 2004 High follicle-stimulating hormone levels should not necessarily lead to the exclusion of subfertile patients from treatment. *Fertility and Sterility* **81**, 1478–1485.

van Zonneveld P, Scheffer GJ, Broekmans FJ *et al.* 2003 Do cycle disturbances explain the age-related decline of female fertility? Cycle characteristics of women aged over 40 years compared with a reference population of young women. *Human Reproduction* **18**, 495–501.

Vendola K, Zhou J, Adesanya OO *et al.* 1998 Androgens stimulate early stages of follicular growth in the primate ovary. *Journal of Clinical Investigation* **101**, 2622–2629.

Vendola K, Zhou J, Wang J *et al.* 1999 Androgens promote oocyte insulin-like growth factor I expression and initiation of follicle development in the primate ovary. *Biology of Reproduction* **61**, 353–357.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 24 June 2009; refereed 20 July 2009; accepted 4 September 2009.