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Improvement In Markers of Ovarian Reserve After Dehydroepiandrosterone Supplementation

KEYWORDS: Serum antimullerian hormone,Antral Follicular Count, Dehydroepiandrosterone

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ABSTRACT *Objective To determine improvement in S.AMH concentration after oral DHEA administration. Method The study was conducted on 72 infertile women of 20-45 years age who had S.AMH < 2.2 ng/ml.The cases were divided into two groups Group A (36) received oral DHEA 25mg TDS and those in Group B (36) received folic acid 5mg OD for 12 weeks. Day 2 S.FSH ,S.LH ,S.E2 , S.AMH , S.Inhibin B, ovarian volume and AFC were measured at first visit and after 12 weeks of medication.Result After 12 weeks of oral DHEA supplementation,S.AMH concentration was found to be significantly improved (p=0.01). Conclusion DHEA improves the S.AMH concentration suggesting that this molecule can raise the fertility quotient in women with diminished ovarian function.*

Introduction

Ovarian reserve describes a woman's reproductive potential and correlates with ovarian follicle number and oocyte quality.Ovarian factors are responsible for 20-40 per cent cases of infertility. Ovarian reserve gradually decreases with increasing age of the women ,**Te Velde et al [1].** Assessment of antral follicle count(AFC) on Day 2 of menstruation by transvaginal ultrasonography and S.AMH irrespective of day of menstruation , best predict the quantitative and qualitative aspect of ovarian reserve. Antral follicle count is the count of all the follicles measuring 2-10 mm in both the ovaries on day 2 of the menstrual cycle.Normally it is more than 12. AMH is a glycoprotein hormone. It's secretion starts in smaller amount from the granulosa cells of the primordial stage follicle, but major amount is secreted from the granulosa cells of preantral and antral stages during folliculogenesis.Normal S.AMH level ranges between 2-6.8 ng/ml in reproductive age women, irrespective of phase of the cycle.The declining quality of the oocytes with increasing age is basically because of disturbed oocyte environment probably due to decreasing local androgen levels.So, supplementing woman with androgen in the form of DHEA may improve the ovarian environment dramatically with good quality oocyte maturation and ovulation.The present study was conducted with aim to determine improvement in the markers of ovarian reserve after DHEA administration.

Material and Methods

Infertile women visiting the outdoor of Department of Obstetrics and Gynaecology of Motilal Nehru Medical College , Allahabad were subjected to detail history, thorough general , systemic and gynaecological examination.Apart from other investigations of infertility,estimation of S.FSH, LH, Estradiol(E2), AMH and Inhibin B was done on day 2 of menstrual cycle.The study group comprised of 72 infertile women with S.AMH <2.2ng/ml.Informed consent was taken and in selected patients AFC and ovarian volume was also measured on day 2 of menstrual cycle by transvaginal ultrasonography. The study was carried out over a period of twelve months.The cases were divided into Group A (36) and Group B (36).The cases with diabetes mellitus , thyroid disorders , polycystic ovarian syndrome and other medical condition were excluded.Cases in group A received oral DHEA supplementation 25 mg thrice a day and group B received placebo (folic acid, 5 mg once a day) for a period of 12 weeks. After 12 weeks of DHEA and placebo supplementation, the effect was observed by re- measuring Day 2 S.FSH, LH, E2, AMH and inhibin B levels.The AFC and ovarian volume were also observed for any improvement after 12 weeks of oral DHEA administration.S.FSH, S.LH and S.E2 levels were measured by solid phase, two site, chemluminescent immunometric assay.Inhibin B was tested by ELISA method. AMH was estimated by sandwich enzyme immunoassay method. AFC and ovarian volume assessment was done by 7.5 MHz transvaginal ultrasonography probe.

Statistical analysis

The chi-square test was carried out for categorical variables and descriptive statistics were given as mean± SD. For all statistical analysis p value < 0.05 was considered as significant.

Results

The cases in both the groups were divided into three groups, according to age. Majority cases of group A (53.84%) and groupB (50.00%) belonged to 21-30 years of age **(Table 1)**.The difference between study and control groups regarding habitat, socioeconomic status, educational status, occupation , age at marriage and duration of infertility was found to be statistically insignificant, thus avoiding the bias in the ultimate outcome of the treatments in both the groups..

With increasing age, S.AMH levels decreased , in group A from 0.95 ± 0.9 ng/ml (21-30 years) to 0.35 ± 0.11ng/ml (>40 years) and in group B from 1.12 ± 0.87 ng/ml (21-30 years) to 0.34 ± 0.13 ng/ml (>40 years).The statistical difference between group A and B was again insignificant (p=0.79) , hence ,the effect of DHEA and placebo on AMH levels was not biased by their initial S.AMH levels **(Table 2).** 17 cases in group A(65.38%) and 18 in group B (69.23%) had low S.AMH levels; 0.3 – 2.2 ng/ml and rest

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had very low levels; less than 0.30 ng/ml **(Table 3).** During study it was noticed that the maximum cases with very low S. AMH levels belonged to the later quarter of the study age group range.After oral DHEA intake for 12 weeks, the cases in group A showed statistically significant increase in S.AMH levels from 0.65± 0.30 ng/ml to 1.43 ± 0.57 ng/ml (p=0.01); S..FSH levels were also decreased significantly from 12.13 ±2.67 mIU/ml to 3.56 ± 1.2 mIU/ml (p=0.04) and . S.E2 levels again showed statistically significant increase from 29.66 ± 4.9 pg/ml to 57.67 ± 9.6 pg/ml (p=0.03). The levels of S.LH and Inhibin B showed only minimal changes with only minimal decrease in S. LH from 9.37 ± 3.02 mIU/ml to 8.83 ± 2.75 mIU/ml (p value =0.86) and minimal increase in S. Inhibin B from 34.06± 3.61 pg/ml to 37.67± 4.51 pg/ml (P Value-=1) **(Table 4)**.The increase in S.AMH levels after DHEA intake was more pronounced in younger age group than in the older ones. The increase was from 0.95±0.9 ng/ml to 1.98±1.1ng/ml in 21-30years while in >40 years ladies only minimal increase from 0.35±0.11 ng/ml to 0.85±0.17ng/ml was noticed **(Table 5).**Increase in antral follicle count and ovarian volume although not statistically significant, was positively observed in DHEA treated group, with AFC increasing from 6.95± 1.61 to 13.05 ±1.73 (p value=0.56) and ovarian volume from 6.30±0.95 ml to 7.81±0.98 ml. **(Table 6).**

Discussion

Ovarian ageing is responsible for the well-established observation of age related decline in fertility.The concept of ovarian reserve has been based on a presumed remaining follicular pool within ovaries, **Faddy et al [2].**With ovarian ageing, the first change is a decrease in AMH levels, followed by decline in inhibin-B and finally increase in FSH levels with appreciable decrease in antral follicle count.AMH is exclusively produced by granulosa cells of preantral and small antral follicles,La Marca et al [3]. Thus S.AMH better reflects the quantity and quality of pre-antral and antral follicles, whereas later stages of follicular pools are better represented by FSH concentrations.As, AMH better co relates with total quantity and possibly quality of the remaining follicular pool, it is taken as a better marker of declining ovarian reserve, **Tremellen et al [4]**.This study showed that with increasing age, S.AMH level decreased in group A from 0.95 ± 0.9 ng/ml (21-30 years) to 0.35 ± 0.11ng/ml (>40 years) and in group B from 1.12 ± 0.87 ng/ml (21-30 years) to 0.34 ± 0.13 ng/ml (>40 years).In a study carried out by **Seifer et al [5]**, the average yearly decline in the median serum AMH value was found to be 0.2 ng/ml/year through age 35 and that further to 0.1 ng/ml/year after age 35.In the present study also, women with advanced age had very low S.AMH level less than 0.3 ng/ml.Various mechanisms of action of DHEA are suggested.Androgens play a major role in early stages of follicular maturation.There is high concentration of androgen receptors in preantral and growing follicle pool and stimulation of these receptors leads to their transition from dormant primordial follicle pool into growing follicle pool **Gleicher et al [6]**.DHEA improves steroidgenesis, since it is a precursor of estrogen and testosterone. It may also influence ovarian follicular growth by increase in IGF- 1, that in turn stimulating mitosis, proliferation of the granulosa cells and production of AMH as well. This optimizes the hormonal feedback to the pituitary leading to adequate response of the pituitary FSH and thus inducing normal oocyte maturation and good ova quality.The paracrine activity of AMH also regulates FSH stimulated excessive follicle growth, and allows the dominant follicle to emerge.This means that over time DHEA increases the pool of growing follicles, causing a steady improvement in AMH.In our study, DHEA administration showed more pronounced increase in S.AMH levels in younger age group women than in the older women, that is in accordance with the results of the study by **Gleicher et al [7],** that concluded that DHEA supplementation significantly improved ovarian reserve in parallel with longer DHEA use with more appreciable effect in younger women.There are very few studies done to demonstrate the effect of DHEA on ovarian reserve.Though all the studies concluded that DHEA administration in women with decreased ovarian reserve helps in improving the ovarian response **Gleicher et al [7], Hyman et al [8],** the proposed mechanism of action of DHEA differs.**Gleicher et al [6]** performed a retrospective cross sectional and longitudinal analysis of 120 women with DOR and demonstrated improved AMH concentration by approximartely 60% (p value <0.0002) after DHEA administration, that is in coherence with this study, where also a significant rise in AMH was noticed after DHEA administration for 12 weeks (<0.01). Whereas **Hyman et al [8]** performed a prospective, self controlled study on 32 poor responders to IVF treatment, comparing pretreatment day3 serum AMH, Inhibin B and FSH and AFCwith post treatment levels after DHEA supplementation for 3 months duration.The proportion of cancelled cycles due to poor response decreased significantly (<0.02). In contrast to this study they demonstrated stastically significant increase in AFC (p value<0.0003) with no significant change in S AMH, Inhibin B or FSH. Thus they concluded an improved ovarian response but by a mechanism that do not demonstrate the recruitment of preantral follicles, evidenced by rise in AMH, instead by rescue from atresia of small antral follicles, evidenced by increase in AFC.Incresed AMH have a negative feedback on pituitary FSH thus decresing it's level as well as by paracrine action helps in selection of dominant follicle and increase in E2 levels.. Along with statistically significant increase in S AMH ,this study also showed a significant decrese in Day 2 S.FSH levels (p value-<0.04)and increase in E2 level(<0.03) after 12 weeks of oral DHEA intake. In accordance with our study **Sonmezer et al [9]** also found favourable decrease in day 2 serum FSH levels after DHEA supplementation (75.14 +/- 28.93 versus 43.07 +/- 11.77; P < 0.01) and another study done by of **Mamas and Mamas [10],** they concluded mean S.E2 level increases from 26.4±6.65 mIU /ml to 56.6±5.27 mIU /ml (p=0.01) after DHEA supplementation.

Conclusion

By assessing change in S.AMH and S,FSHconcentration,this study presents objective evidence that DHEA supplementation positively affects diminished ovarian reserve by improving AMH concentration.In concordance with prior clinical observations **,**DHEA has emerged as a breakthrough medication for improving ovarian response in women of advanced age and in women responding poorly to ovarian stimulation.This information will improve patient counselling and treatment in women with DOR as DHEA supplementation can have a beneficial effect on patient's ovarian reserve.

Table 1 : Age Distributon

Age Group (in years)	Study Group {N=36}		Control Group {N=36}	
	NO.	%	NO.	%
21 – 30(Group 1)	19	53.84	18	50.00
31 – 40(Group 2)	13	34.61	15	42.30
>40 (Group 3)	4	11.53	3	7.69
Total	36	100	36	100
MEAN ± S.D.	30.80 ± 5.23		30.65 ± 5.08	

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Table 2 : S.AMH Levels in different age groups

Age Group (years)	Group A (N=36)		Group B (N=36)	
	Range (ng/ml)	Mean ± S.D.	Range (ng/ml)	Mean ± S.D.
21-30	0.14-1.56	0.95 ± 0.9	0.29-1.66	1.12 ± 0.87
31-40	0.16-0.98	0.65 ± 0.27	0.28-0.96	0.77 ± 0.38
>40	0.21-0.48	0.35 ± 0.11	0.26-0.53	0.34 ± 0.13

Table 3 : Distribution of patients according to the S.AMH level

S.No.	S.AMH(ng/ml)	Group A (n=36)		Group B (n=36)	
		NO.	%	NO.	%
1	Low level (0.30 - 2.2)	17	65.38	18	69.23
2	Very Low level / Undetectable (0.00-0.30)	9	34.61	8	30.76

Table 4 : Effect of DHEA on serum Hormone Levels

	Group A		Group B		p value
	Baseline	At 12 weeks	Baseline	At 12 weeks	
S.AMH(ng/ml)	0.65±0.30	1.43±0.57	0.76±0.37	0.83±0.42	0.01
S.FSH(mIU/ml)	12.13±2.67	3.56±1.2	11.37±2.45	10.56±2.25	0.04
S.LH(mIU/ml)	9.37±3.02	8.83±2.75	10.1±4.5	10.61±2.99	0.86
S.E2(pg/ml)	29.66±4.9	57.67±9.6	28.66±4.5	30.01±4.3	0.03
S.InhibinB(pg/ml)	34.06±3.61	37.67±4.51	36.11±5.57	37.87±5.10	1

Table 5 : Effect of DHEA on S.AMH Levels (ng/ml) in different age groups

Age Group (in years)	Group A {N=36}		Group B {N=36}	
	Baseline	At 12 weeks	Baseline	At 12 weeks
21 – 30	0.95±0.9	1.98±1.1	1.12±0.87	1.24±0.74
31 – 40	0.65±0.27	1.45±0.8	0.77±0.38	0.86±0.29
>40	0.35±0.11	0.85±0.17	0.34±0.13	0.36±0.15
MEAN ± S.D.	0.65±0.30	1.43±0.57	0.76±0.37	0.83±0.42

Table 6 : Effect of DHEA on AFC and Ovarian volume

	Group A		Group B		p value
	Baseline	At 12 weeks	Baseline	At 12 weeks	
AFC	6.95±1.61	13.05±1.73	7.59±1.02	7.84±0.90	0.56
Ovarian Volume(ml)	6.30±0.95	7.81±0.98	6.63±0.45	6.70±0.75	0.9

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