Journal of Assisted Reproduction and Genetics, Vol. 19, No. 4, April 2002 (© 2002)

Clinical Assisted Reproduction

Ascorbic Acid Supplement During Luteal Phase in IVF

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Submitted August 13, 2001; accepted December 10, 2001

***Purpose*:** To evaluate the impact of ascorbic acid of different doses as additional support during luteal phase in infertility treatment by means of a prospective, randomized, placebo-controlled, group comparative, double-blind study.
***Methods*:** Voluntary daily oral intake of either ascorbic acid (1, 5, or 10 g/day) or Placebo for 14 days after follicle aspiration for IVF-ET procedure. Data was obtained on 620 cases of women, age <40 years, undergoing first IVF-embryo transfer cycles in two private outpatient infertility clinics. All women were stimulated by the same protocol. The mean age was 31.73 (±4.4 SD) years.
***Results*:** No differences in clinical pregnancy rate and implantation rate were noted in statistical logistic regression analysis between the four intake groups.
***Conclusions*:** There was no clinical evidence of any beneficial effect, as defined by main outcome measures, of ascorbic acid on IVF-ET. Our data suggest there is no obvious value of high dosed intake of vitamin C during luteal phase in infertility treatment.

KEY WORDS: Ascorbic acid; fertility; IVF; luteal phase.

INTRODUCTION

Ascorbic Acid (AA) has long been associated with fertility (1–5). Endocrine tissues like the adrenal and pituitary gland as well as the theca interna, granulosa, follicular fluid, and the luteal compartment of the ovary accumulate high concentrations of AA (6–8). Ovarian vitamin C is an essential cofactor for the biosynthesis of collagen, that is required for the high rates of tissue remodeling that attend follicle growth, ovulation, and corpus luteum development (9–11). Consequently, scorbutic guinea pigs are anovulatory and show ovarian atrophy, marked degeneration of follicles, a failure of implantation, and an increase in spontaneous abortion (12). Synthesis and secretion of steroids, catecholamins, and neuroendocrine peptides appear to be ascorbate-dependent (13–15). However, the ascorbic acid of the ovary is under endocrine control and rapidly depleted by high levels of LH, ACTH (16,17), or Prostaglandin, a luteolytic agent. Luteal regression is associated with ascorbate depletion and the generation of reactive oxygen species, which inhibit the action of LH and block steroidogenesis (18,19). AA is the preeminent water-soluble antioxidant (20). Women with unexplained infertility have a lower total antioxidant status in their peritoneal fluid (21). In animal studies AA was shown to inhibit follicular apoptosis (22) and to protect ovarian cells from oxidative damage by detoxifying H_2O_2 (23). With the advancement of pregnancy, increasing amounts of AA correlate with a gradual suppression of oxygen toxicity in human placental tissues (24).

Large quantities of ascorbate are utilized during conception. Thus, a daily supply of at least 500 μg of vitamin C, starting as early in pregnancy as possible,

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has been suggested (25). Although a significant influence of AA on reproduction has been proposed by several authors (26), until now, little has been done to examine these proposals in practice. Oral administration of daily 400 mg of ascorbic acid enhanced the ovulation-inducing effects of clomiphene in otherwise anovulatory women (27). Moreover, an early study reported conception in infertile cows after AA administration at estrus (28). Antioxidant compounds are commonly added to culture media in IVF and protection of the embryo by nonenzymatic antioxidants in the tubal fluid is considered (29).

Humans are dependent on the dietary supply of vitamin C (RDA 60 mg/day), and requirements of ascorbic acid have been estimated from measurement of ascorbate pool size (22 mg/kg) and from doses of ascorbate required to prevent scurvy. Yet, ascorbate is synthesized by most mammals at a rate of ∼40 to 275 mg/kg/day (30). These synthetic rates imply that optimal requirements for ascorbic acid in humans may exceed those merely to prevent scurvy, especially in different metabolically high active states. Therefore, the authors hypothesized, that higher than RDA intake would better fit maintenance of ascorbate homeostasis in the female reproductive system during luteal phase and thereby support embryo implantation and early embryonic development.

MATERIALS AND METHODS

Subjects and Treatment

This prospective randomized double-blind study analyzes a total of 620 consecutive patients with the mean age of 31.7 (±4.4 SD) years, who underwent IVF-ET. Data were collected in two private outpatient infertility clinics: Institut für Sterilitätsbetreung, Vienna and Kaali Intezet, Budapest. In Vienna 383 patients (61.77%) took part in the study, in Budapest 237 (38.23%).

All women were stimulated by the same protocol (31), using Clomiphen citrate in combination with HMG. Selection criteria were age younger than 40 years and undergoing first IVF cycle. A study on vitamin supplement is not subject to IRB approval in Austria and Hungary, but all subjects were counseled concerning the nature and purpose of the study and signed a detailed consent form. Cases with tubal (80%), idiopathic (10%), and male (10%) infertility were included in the study. Excluding criteria, namely, were patients with repeated IVF cycles and patients suffering from renal or gastrointestinal disease.

Patients were randomly allocated to either of the four groups: 1 g, 5 g, and 10 g daily oral intake of AA or placebo (consisting of lactose and citric acid) intake. Vitamin or placebo intake started on the day of follicle aspiration and was given together with a luteal support of 30 mg (3 × 10 mg) Dydrogesteron for 14 days.

Samples for both clinics were prepared and coded by number by a pharmacy in Vienna, thus avoiding patients and administering doctors knowing the respective contents.

Outcome measures were clinical pregnancy rate and implantation rate, defined as fetal heart beat seen on 8 weeks ultrasound in relation to embryos transferred. There was no further follow-up of pregnancies.

After completing the study the relevant IVF data was given to the Institute of Medical Statistics at the University of Vienna, which received a list of the sample codes and their subdivision directly from the pharmacy.

RESULTS

Clinical pregnancy rates for the four treatment groups are summarized in Table I. As can be seen, no positive influence of the amount of administered AA is found. Table II summarizes mean and standard error of implantation rate by the four treatment groups and accordingly there is obviously no positive trend for the treatment group. Since the study involves 619 patients (620 with one missing value) this can be interpreted as evidence against a positive effect of AA administration during luteal phase on the outcome of IVF-ET.

For the probability to become pregnant differences caused by the treatment groups were also explored by a logistic regression analysis that accounted for the covariables age and the number of transferred

Table I. Rates of Clinical Pregnancy in the Four Treatment Groups

Group	Total	Clinical pregnancy rate
Placebo	158	0.28
Ascorbic acid (g)		
1	172	0.22
5	153	0.24
10	136	0.21

Table II. Mean and Standard Error of the Implantation Rates by the Treatment Groups

Group	Implantation rate (mean ± SE)
Placebo	14.77 ± 2.18
Ascorbic acid (g)	
1	10.03 ± 1.59
5	12.36 ± 1.97
10	10.29 ± 1.90

embryos. As expected, a significant decrease with age ($p = 0.0009$) and a significant increase with the number of transferred embryos ($p = 0.0026$) could be observed, whereas in agreement with our observations given in Table I and Table II, no significant influence of the amount of administered AA was found ($p = 0.186$).

DISCUSSION

The imbalance of oxidative stress and the antioxidant defense has been implicated in the pathogenesis of several diseases, including recurrent abortion, unexplained infertility, and defective embryo development (29,32,33). Although the effect of dietary antioxidant supply on many aspects of human health has been investigated extensively, studies have produced conflicting results. It should also be noted that reactive oxygen species play a physiological role and may influence fertilization and regulate embryo development (34,35). In our study the implantation rate suggests an inhibitory effect of high dosed AA; however, this did not reach statistical significance.

Estimates of human ascorbate requirements are to afford stringent protection against scurvy, yet these data reflect only minimum requirements. Furthermore, optimal requirements may be dependent on homeostasis (6). In his best selling books and numerous scientific papers (36,37), Linus Pauling, two times Nobel laureate, proposed high intake of the vitamin. He stated that only megadoses of vitamin C "can improve your general health . . . can help in controlling various diseases and in slowing down the process of aging" (38). He himself reportedly ingested at least 12.000 mg/day and made vitamin C the most popular dietary supplement, with his megavitamin claims being accepted by large numbers of people.

AA-concentration in plasma responds rapidly to changes in vitamin C intake and with increasing doses the absorption and half life decreases, the plasma ascorbate increases, and the total body pool tends to increase (39). In this study we administered mega vitamin doses to ensure the respective high plasma levels and fast saturation of tissue stores. The safety of doses up to 10.000 mg/day for up to 3 years has been confirmed in several clinical trials (40). Since we did not measure AA serum levels, we had to rely on the compliance of subjects for taking the assigned amount of the vitamin. In a recent depletion–repletion study bioavailability in humans was complete for 200 mg of vitamin C as a single dose. At single doses of 500 mg and higher, bioavailability declined and the absorbed amount was rapidly excreted (41). Accordingly, only transient high plasma levels can be achieved by high dosed AA intake, that can exert anti-inflammatory and immunostimulant effects in studies in humans and animals (42,43). We considered that these effects of AA would benefit embryo implantation, which can be negatively affected by urogenital infections at the time of transfer (44).

The relatively high bioavailability of vitamin C inside the Graafian follicle (8) and the clinical trial by Igarashi (27) suggest an important role of the vitamin in follicular genesis and ovulation (45). Many preliminary studies have also emphasized the importance of AA in luteal formation and regression, but no examination of dietary supplementation during luteal phase has been reported. The efficacy of supplemental use of vitamin C above a level that can be supplied by means of diet alone has been the source of considerable controversy in the medical and scientific community. This study shows that a short-term supplementation with high doses of AA, as proposed by Linus Pauling, is of no obvious benefit to IVF-ET. To further improve the outcome of infertility treatment, an evaluation of the clinical potential of dietary supplements like vitamin C and other antioxidants would be of great practical interest.

CONCLUSION

The present study shows no positive impact of short-term supplementation of high doses of AA on clinical pregnancy rate and implantation rate in infertility treatment. However, a positive effect of AA on human reproduction cannot be ruled out until further studies on supplementation during follicular phase, ovarian stimulation, and early pregnancy are realized and the role of dietary AA in ovulation, conception, and pregnancy is further evaluated.

ACKNOWLEDGMENTS

The authors thank the staff of the Institute of Medical Statistics at the University of Vienna, particularly Dr Werner Brannath, Mag. Dr Wehrstein of Flora Pharmacy in Vienna, and Monika Deutner for her help with the text.

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