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·*Original Article*·

Combined conventional/antioxidant "Astaxanthin" treatment for male infertility: a double blind, randomized trial

F. H. Comhaire[1,3], Y. El Garem[2], A. Mahmoud[1], F. Eertmans[1], F. Schoonjans[1]

[1]*Department of Medical and Urological Andrology, Ghent University Hospital, Gent B 9000, Belgium*
[2]*Department of Andrology, Alexandria University Hospital, Alexandria, EG 21544, Egypt*

Abstract

Aim: To evaluate the treatment of male infertility with a strong natural antioxidant, in addition to conventional treatment. **Methods:** Using a double blind, randomized trial design, 30 men with infertility of ≥12 months and female partners with no demonstrable cause of infertility received conventional treatment according to the guidelines of the World Health Organization (WHO), and either a strong antioxidant Astaxanthin 16 mg/day (AstaCarox®, AstaReal AB, Gustavsberg, Sweden) or placebo for 3 months. The effects of treatment on semen parameters, reactive oxygen species (ROS), zona-free hamster oocyte test, serum hormones including testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and Inhibin B, and spontaneous or intrauterine insemination (IUI)-induced pregnancies were evaluated. **Results:** ROS and Inhibin B decreased significantly and sperm linear velocity increased in the Astaxanthin group (n = 11), but not in the placebo group (n = 19). The results of the zona-free hamster oocyte test tended to improve in the Astaxanthin group in contrast with the placebo group, though not reaching statistical significance. The total and per cycle pregnancy rates among the placebo cases (10.5 % and 3.6 %) were lower compared with 54.5 % and 23.1 % respectively in the Astaxanthin group (P = 0.028; P = 0.036). **Conclusion:** Although the present study suggests a positive effect of Astaxanthin on sperm parameters and fertility, the results need to be confirmed in a larger trial before recommending Astaxanthin for the complementary treatment of infertile men. *(Asian J Androl 2005 Sep; 7: 257–262)*

Keywords: male infertility; antioxidant; astaxanthin; ROS; treatment; pregnancy

1 Introduction

Evidence has accumulated supporting the pivotal role of reactive oxygen species (ROS) in the pathogenesis of sperm dysfunction among men with infertility. Increased generation of ROS has been documented in a high percentage of subfertile men with diverse diseases of the uro-genital tract, and under the influence of environmental as well as life style factors [1].

Due to their paucity in cytoplasm, spermatozoa possess little defense against oxygen damage and are highly sensitive to ROS inducing changes of the fatty acid composition of the sperm membrane and damage of sperm DNA (for review see [2]). These alterations are usually not reflected in changes in conventional sperm characteristics, but impair acrosome reactivity and decrease the fusogenic

Correspondence to: Professor F. H. Comhaire, Ghent University Hospital, Department of Medical and Urological Andrology, 9k12 IE, De Pintelaan, 185, B 9000, Gent, Belgium.
Tel: +32-9-240-2133, Fax: +32-9-240-3897
E-mail: frank.comhaire@ugent.be
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capacity with the oolemma. Oxidative stress to sperm DNA increases DNA fragmentation [3] and causes transition mutagenesis due to an increased content of 8-hydroxy-2-deoxyguanosin (8-OH-2-dG).

In an open label trial including 27 infertile men, we have demonstrated that the combination of conventional treatment, oral antioxidants and fish oil reduced ROS and the 8-OH-2-dG content of spermatozoa. Also, membrane fluidity was increased and the induced, but not the spontaneous, acrosome reaction was enhanced [2]. A 7 % per month pregnancy rate was attained among couples in whom the male partner was a present or an ex-smoker.

Astaxanthin is a carotenoid extracted from the algae *Hematococcus pluvialis*. The potency of this antioxidant is much higher as a singlet molecular oxygen quencher compared with vitamin E [4].

The objective of the present trial was to assess, for the first time, the effects of complementary treatment with a strong lipophilic antioxidant (Astaxanthin) on sperm function and fertility of subfertile men, using a prospective, double blind, randomized trial design.

2 Materials and methods

Thirty men were enrolled into the trial. The patients were not selected on the basis of sperm parameters or ROS levels. They were the male partners of couples who had been infertile for a period of at least 12 months, in whom the female partner was classified as presenting no demonstrable cause of infertility after investigation in agreement with the World Health Organization (WHO) recommendations [5]. All men were investigated as recommended in the guidelines of WHO [6]. Because of the ethical obligation to always treat patients according to the rules of good medical practice, the patients received conventional treatment as indicated (Table 1). Intra-uterine insemination was applied as indicated by the WHO guidelines [6] and provided semen characteristics fulfilled the minimum criteria suggested by Milingos *et al.* [7], namely, at least 0.3 million progressively motile spermatozoa per mL after preparation of the semen sample over a density gradient column, and 4 % or more spermatozoa with normal morphology in the native semen sample. In addition, 11 patients received capsules containing natural Astaxanthin (Astacarox®, AstaReal AB, Gustavsberg, Sweden), 16 mg per day. Nine patients received identically packaged placebo, and data from an additional ten placebo-treated patients participating in another parallel placebo-controlled study were also included.

Two semen analyses were performed before intervention using the WHO-recommended methods [8] and the best result was used for statistical analysis. The quality of semen analysis was under rigorous internal and external control. In addition, sperm motility was objectively assessed by means of a computer-assisted method (Autosperm, Fertipro, Beernem, Belgium). ROS generation was measured by chemoluminescence in high quality spermatozoa prepared by discontinuous density-gradient centrifugation (Sil-Select, FertiPro NV, Beernem, Belgium) after stimulation with phorbol ester (PMA) as described previously, and spermatozoa were cryopreserved for zona-free hamster oocyte testing [8]. All semen tests were repeated after 3 months of intervention. Gamma glutamyl transpeptidase was measured in seminal plasma as a marker of prostatic function using a spectrophotometric assay [9].

Blood was taken at baseline and at the end of the 3 months intervention period for hormone measurements of testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), Inhibin B. A commercial kit (Medgenics Diagnostics, Fleurs, Belgium) for radioimmuoassay was used to determine the serum concentrations of testosterone, and commercial kits (Medgenics Diagnostics, Fleurus, Belgium) for immunoradiom-etric assays were used for determinations of serum LH and FSH. Intra- and interassay coefficients of variation for all of these assays were less than 10 % and 15 %, respectively. Serum inhibin B was determined in duplicate using a specific double-antibody, enzyme-linked immunoassay (Inhibin-B, dimer assay kit ultra-sensitive, Serotec, Oxford, UK). Intra- and interassay coefficients of variation were 6 % and 9 %, respectively.

The data were collected and analyzed by means of the MedCalc statistical package (Medcalc Inc., Mariakerke, Belgium). The unpaired or paired *t*-test or Wilcoxon test were used to compare variables as appropriate. Because of their skewed distribution, calculations of sperm concentration and ROS used log-transformed data and data on the zona-free hamster oocyte test are described as median and 95 % confidence intervals (95 % CI). The null hypothesis was that the pregnancy rate and the changes in sperm quality would not differ significantly ($P > 0.05$) between Astaxanthin treated and placebo groups.

Complementary treatment by means of intrauterine

insemination (IUI) in FSH stimulated cycles (without hyperstimulation of ovulation; Table 1), but not *in vitro* fertilization (IVF), was permitted and the occurrence of pregnancy during the 3 months' trial period was recorded. Relative risk and 95 % CI for pregnancy incidence were calculated by using an appropriate statistical model [10], and statistical significance was assessed by Fisher's exact test. All patients gave informed consent and the ethical committee of the Ghent University Hospital approved the trial (project number 2000/17).

3 Results

The epidemiological characteristics of the cases are summarized in Table 1. One patient dropped out within one month of recruitment, and was replaced by the subsequent eligible patient. When the code was broken, after completion of the trial, the former case was found to receive placebo whereas the replacement case received Astaxanthin. Hence, the number of cases included in the statistical analysis was 19 in the placebo group and 11 in the Astaxanthin group. None of the epidemiological characteristics of the two groups are significantly different.

Semen characteristics and hormone results at baseline and after 3 months of intervention are shown in Table 2. Baseline characteristics, including duration of abstinence before semen donation (data not shown) were not significantly different in the two groups (unpaired *t*-test), though motile sperm concentration was somewhat higher in the Astaxanthin treated cases than that in the controls. Sperm motility and morphology presented a trend to improvement during treatment but changes did not reach the usual level of significance (paired *t*-test). In addition, there was no difference in the trends seen in both groups. The number of white blood cells in semen did not show significant changes neither in the Astaxanthin nor in the placebo groups (Table 2). ROS significantly decreased and linear velocity increased in the Astaxanthin group (paired *t*-test), but not in the controls. A positive correlation was found between basal ROS levels and the increase of sperm concentration during treatment (calculated as the ratio of sperm concentration after/before treatment) in the Astaxantin treated group ($r = 0.83$, $P = 0.02$) but not in the placebo group ($r = –0.52$, $P = 0.15$). The ratios of other sperm variables and Inhibin B did not show significant correlations.

The number of spermatozoa firmly attached to the zona-free hamster oocytes (median 6.1 per oocyte before, 18.0 during intervention) but not the number of decondensed sperm heads (0.7 per oocyte before, 0.6 during intervention) presented a non significant trend to increase in the Astaxanthin cases, while these tended toward decrease in the placebo cases (2.8 per oocyte attached before, 1.6 during intervention) and almost no change was observed in the number of decondensed sperm heads (0.2 decondensed sperm heads per oocyte before, and 0.3 during placebo).

The mean testosterone concentration increased by 37 % in the placebo group, which may be related to the effect of tamoxifen treatment given to five out of the 19 cases. FSH levels remained unchanged. Inhibin B was significantly decreased in the Astaxanthin group (paired *t*-test) but not in the placebo group.

Two spontaneous pregnancies occurred among the placebo cases for a total pregnancy rate of 10.5 %, and a per months probability of pregnancy of 3.6 %. Among

Table 1. Baseline characteristics of the study population. [a]Mean ± SD, [b]median (range), [c]one patient was treated with both antibiotics and varicocele embolization. IUI: intrauterine insemination.

	Placebo	Astaxanthin
Number of cases	19	11
Male age (years)[a]	33.2 ± 5.6	31.4 ± 4.5
Female age (years)[a]	30.4 ± 3.1	29.1 ± 3.3
Duration of infertility (months)[b]	22.8 (12–132)	20.8 (12–48)
Treatments given		
Varicocele embolization	6 (32 %)	4 (36 %)
Antibiotics[c]	3 (16 %)	1 (9 %)
Tamoxifen	5 (26 %)	3 (27 %)
IUI number of couples	3	1
IUI number of cycles	5	1

Table 2. Semen characteristics and hormone results (Mean±SD) at baseline and after 3 months of treatment. [*]Geometric mean (95 % CI), [#]Median (95 % CI). [b]$P<0.05$, compared with preintervention in Astaxanthin group. [e]$P<0.05$, compared with postintervention in Placebo group. FSH: follicle stimulating hormone; γGT: gamma glutamyl transpeptidase.

	Placebo (n = 19)		Astaxanthin (n =11)	
	Preintervention	Postintervention	Preintervention	Postintervention
FSH (IU/L)	4.38 ± 1.52	4.34 ± 2.16	5.32 ± 2.36	5.08 ± 1.42
Testosterone (nmol/L)	15.6 ± 7.1	21.3 ± 6.1	16.7 ± 4.1	16.0 ± 2.1
Inhibin B (IU/L)	182 ± 89	152 ± 71	186 ± 123[b]	133 ± 52[b]
Sperm concentration (million/mL)	28.3 (16.3–49.2)	28.2 (17.1–46.3)	36.2 (25.1–56.1)	48.6 (29.0–81.4)
Linear velocity (μm/s)	25.3 ± 9.5	22.9 ± 9.8	22.1 ± 5.8[b]	29.6 ± 8.1[b]
Grade (a) motility (%)	15.0 ± 10.5	22.3 ± 14.7	28.5 ± 18.1	31.9 ± 21.4
Sperm morphology (% normal)	8.6 ± 5.2	10.0 ± 5.7	9.6 ± 7.1	11.4 ± 6.8
Ejaculate volume (mL)	3.4 ± 1.4	3.9 ± 2.2	2.4 ± 1.4	2.4 ± 1.6
Seminal γGT (IU/L)	7389 ± 3405	8887 ± 4440	8131 ± 1921	8135 ± 4478
Seminal α-Glucosidase (IU/L)	22.1 ± 4.2	29.7 ± 11.8	24.5 ± 12.9	20.9 ± 5.4
ROS[*] (counts/s)	376 (96–1477)	490 (98–2450)	394 (74–2096)[b]	99 (35.2–279.2)[b]
Seminal WBCs (million/mL)	0.24 ± 0.25	0.49 ± 0.90	0.37 ± 0.55	0.47 ± 1.68
Zona-free hamster oocytes test (n)	15	15	9	9
Spermatozoa firmly attached/oocytes[#]	2.8 (0.5–9.2)	1.6 (0.1–6.9)	6.1 (0.1–29.4)	18.0 (0.0–27.2)
Decondensed sperm heads/oocytes[#]	0.2 (0.0–1.6)	0.3 (0.1–0.5)	0.7 (0.1–0.8)	0.6 (0.0–0.7)
Total pregnancy rate (%)		10.5		54.5[e]
Pregnancy rate/month (%)		3.6		23.1[e]

the 11 cases of the Astaxanthin group, six attained conception for a total pregnancy rate of 54.5 %, of which five were naturally conceived and one resulted from IUI (P = 0.028 for comparison between the two groups). The relative risk (RR) for attaining pregnancy during Astaxanthin intake compared to placebo was 5.2 (95 % CI: 1.25–21.39). The probability of conception per month in the Astaxanthin group was 23.1 % (P = 0.036, compared with the placebo group).

4 Discussion

In the present study we evaluated the effects of a natural potent antioxidant Astaxanthin as complementary treatment to improve the outcome of WHO male infertility treatment guidelines. This particular antioxidant was chosen because of its much higher potency as a singlet molecular oxygen quencher compared with vitamin E [4].

In the guidelines for the standardized diagnosis and management of the infertile male, WHO recommends consensus-based treatment modalities, including treatments aiming at the improvement of the quality and fertilizing potential of his semen, as well as assisted reproductive techniques [6]. Recently, concerns have been raised about possible adverse effects in children born after IVF [11]. Therefore, for the sake of economy of cost-effectiveness [12], treatment of the infertile men must be applied before the implementation of assisted reproductive techniques. This approach may reduce the need of IVF and increase the probability of conception, either naturally or by means of IUI.

In the patient population consulting our center, the conventional treatment of subfertile men results in a natural pregnancy rate of between 3 % to 4 % per month [12], being higher than the treatment independent pregnancy rate of 1.0 % to 1.5 % per month. The observed pregnancy rate of 3.6 % in the placebo group falls within the expected range. Both the total pregnancy rate and the per-month probability of pregnancy are higher in the Astaxanthin group. The differences in pregnancy rates between these two groups compare favorably to the results of placebo-controlled studies in which other antioxidants were used (for a review see [13]).

The observation that these pregnancies occurred within the time period of 3 months after initiation of Astaxanthin intake, among couples with an average duration of infertility of more than 20 months, is suggestive for a causal link with the antioxidant supplementation.

Such rapid effect would be compatible with the improved functional capacity of spermatozoa rather than with changes in the conventional sperm characteristics. Functional improvement may be related to the reduction of ROS resulting in enhancement of linear velocity and reduction of DNA damage. Changes of the phospholipid composition of the sperm membrane produced by antioxidant treatment have been documented to improve the fluidity of the membrane that may explain the higher reactivity in the zona-free hamster oocyte test. The small number of cases included in this study precludes subgroup analysis for a possible beneficial effect of correcting any particular pathology on ROS production. However, the strongest relative increase of sperm concentration was observed in cases with the highest pre-treatment ROS concentration.

In spite of the fact that serum FSH remained unchanged in both groups, and the testosterone concentration tended to increase in the treated cases, the concentration of Inhibin B was significantly decreased in the Astaxanthin group. This suggests that reducing the load of ROS may decrease the secretion of Inhibin B by the Sertoli cells, independently from sperm concentration. Therefore, we are tempted to speculate that excessive ROS may have caused an inappropriate secretion of Inhibin B of subfertile men. Similarly, healthy men who had an increased ROS load resulting from smoking, presented a higher serum Inhibin B level than non smokers [14]. On the other hand, it has been documented that estrogens increase the secretion of Inhibin B by Sertoli cells *in vitro* [15]. The high level of Inhibin B may cause both a direct suppression of spermatogenesis and an indirect effect via the feedback reduction of FSH secretion by the pituitary [16] (Figure 1).

Taken together, these observations sustain the hypothesis that exposure of the Sertoli cells to excessive amounts of estrogens, both endogenous and exogenous including xeno-estrogens [17], and to an overload of ROS may be involved in the impairment of spermatogenesis. In addition, ROS exerts oxidative damage to the sperm membrane, the DNA, and the mitochondrial activity (Figure 1). Hence, it may be considered to associate treatment with an anti-oestrogen and with a food supplement containing a judicious mixture of antioxidants [18, 19] for the complementary management of male infertility.

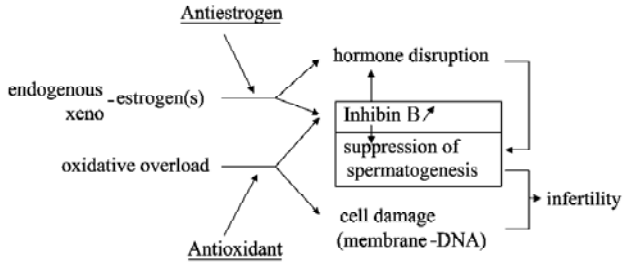

Figure 1. Mechanisms of sperm deterioration.

The results of the present controlled trial confirm those of a previous open label trial [2], but they need confirmation in larger trials. However, the recruitment of couples has become increasingly difficult since assisted reproductive techniques are readily available and commonly demanded by the infertile couples because of their alleged higher efficacy [20].

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References

1 Zalata A, Hafez T, Comhaire F. Evaluation of the role of reactive oxygen species in male infertility. Hum Reprod 1995; 10: 1444–51.
2 Comhaire FH, Christophe AB, Zalata AA, Dhooge WS, Mahmoud AM, Depuydt CE. The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. Prostaglandins Leukot Essent Fatty Acids 2000; 63: 159–65.
3 Fraga CG, Motchnik PA, Wyrobek AJ, Rempel DM, Ames BN. Smoking and low antioxidant levels increase oxidative damage to sperm DNA. Mutat Res 1996; 351: 199–203.
4 Di Mascio P, Devasagayam TP, Kaiser S, Sies H. Carotenoids, tocopherols and thiols as biological singlet molecular oxygen quenchers. Biochem Soc Trans 1990; 18: 1054–6.
5 Rowe PJ, Comhaire FH, Hargreave TB, Mellows HJ. WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple. 1st ed. Cambridge: Cambridge University Press; 1993.
6 Rowe PJ, Comhaire FH, Hargreave TB, Mahmoud AMA. WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male. 1st ed. Cambridge: Cambridge University Press; 2000.
7 Milingos S, Comhaire FH, Liapi A, Aravantinos D. The value of semen characteristics and tests of sperm function in selecting couples for intra-uterine insemination. Eur J Obstet Gynecol Reprod Biol 1996; 64: 115–8.
8 World Health Organization (WHO). WHO Laboratory Manual of the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th ed. Cambridge: Cambridge University Press;

1999.
9 Comhaire FH, Vermeulen L, Pieters O. Study of the accuracy of physical and biochemical markers in semen to detect infectious dysfunction of the accessory sex glands. J Androl 1989; 10: 50–3.
10 Morris JA, Gardner MJ. Calculating confidence intervals for relative risks (odds ratios) and standardised ratios and rates. Br Med J (Clin Res Ed) 1988; 296: 1313–6.
11 Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and *in vitro* fertilization. N Engl J Med 2002; 346: 725–30.
12 Comhaire F. Clinical andrology: from evidence-base to ethics. The 'E' quintet in clinical andrology. Hum Reprod 2000; 15: 2067–71.
13 Agarwal A, Nallella KP, Allamaneni SS, Said TM. Role of antioxidants in treatment of male infertility: an overview of the literature. Reprod Biomed Online 2004; 8: 616–27.
14 Richthoff J, Rylander L, Hagmar L, Giwercman A. Effect of cigarette smoking on reproductive characteristics in an unselected population of young males. Reprod Biomed Online 2003; 7 Suppl 1: 8–9.
15 Depuydt CE, Mahmoud AM, Dhooge WS, Schoonjans FA, Comhaire FH. Hormonal regulation of inhibin B secretion by immature rat sertoli cells *in vitro*: possible use as a bioassay for estrogen detection. J Androl 1999; 20: 54–62.
16 Bame JH, Dalton JC, Degelos SD, Good TE, Ireland JL, Jimenez-Krassel F, *et al*. Effect of long-term immunization against inhibin on sperm output in bulls. Biol Reprod 1999; 60: 1360–6.
17 Turner KJ, Sharpe RM. Environmental oestrogens – present understanding. Rev Reprod 1997; 2: 69–73.
18 Comhaire FH, Mahmoud A. The role of food supplements in the treatment of the infertile man. Reprod Biomed Online 2003; 7: 385–91.
19 Lamond S, Watkinson M, Rutherford T, Laing K, Whiting A, Smallwood A, *et al*. Gene-specific chromatin damage in human spermatozoa can be blocked by antioxidants that target mitochondria. Reprod Biomed Online 2003; 7: 407–18.
20 Krause W, Muller HH, Schafer H, Weidner W. Does treatment of varicocele improve male fertility? Results of the 'Deutsche Varikozelenstudie', a multicentre study of 14 collaborating centres. Andrologia 2002; 34: 164–71.