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Spermatozoa in seminal plasma were fixed, permeabilized and incubated with deoxynucleotidyl tranferase and nucleotides incorporated with fluoresceine- as well as DNA-binding propidium iodide. Images were captured (Slidebook Application 4.1.0) using fluorescence microscopy with transmission filter for fluoresceine (green) and propidium iodide (red), and contrast enhancing filter to identify the sperm. Images were analyzed using Picsara 8.9 and spermatozoa were classified as positive or negative. Statistical analyses were performed using GraphPad Prism 5.01.
Results: Only 15-53 % of the sperm in DNA-ase treated positive controls were able to respond to the TUNEL method. The proportion of TUNEL positive sperm in the samples increased with longer time of abstinence and with longer time between ejaculation and analysis. A negative correlation between semen zinc concentration and frequency TUNEL positive spermatozoa
Conclusions: The TUNEL assay only measures a subpopulation of the spermatozoa in a given semen sample. Insufficient availability of DNA has not earlier been considered when TUNEL has been used to determine the frequency of sperm DNA damage and calls for a positive control for every analyzed sample. Zn2+ in semen can either protect the sperm DNA or reduce the access to the sperm DNA of the TUNEL assay, since TUNEL positivity decreases with higher seminal Zn2+.

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THE IMPACT OF CIGARETTE SMOKING ON SPERM DNA AND OXIDATIVE STRESS IN NON-LEUKOCYTOSPERMIC INFERTILE MEN

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Introduction: Oxidants in cigarette smoke are thought to damage sperm DNA, and smokers have more oxidative DNA damage in their sperm than do nonsmokers. Because oxidative sperm DNA damage can be induced either by cigarette smoking oxidants or by leukocytes, non-leukocytospermic infertile men smoker may reveal the effects of smoking on sperm DNA integrity, oxidants and antioxidants levels.
Objective: To evaluate the effects of cigarette smoking on sperm DNA integrity, oxidants and antioxidants levels in non-leukocytospermic infertile men.
Methods: Semen samples from non-leukocytospermic infertile men (n = 79; subdivided into infertile smokers (n=43) and infertile non-smokers (n=36) subgroups. A fertile men group (n = 18) were included as a control. All semen samples were subjected to standard semen analysis according to WHO guidelines, sperm DNA analysis by flow cytometry, oxidants (malondialdehyde (MDA) concentration), and antioxidant (superoxide dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH)) analysis.
Results: Sperm DNA-fragmentation index (DFI) was significantly higher in the infertile smokers group than in non-smokers infertile and fertile groups (P= 0.032; P=0.001 respectively). Cigarette smoking was significantly correlated with increased MDA, DFI and decreased SOD levels (r=0.796, P=0.0001; r=0.371, P=0.033; r= -0.545, P=0.013 respectively).
Conclusions: Cigarette smoking in non-leukocytospermic infertile men is associated with increased susceptibility of DNA to denaturation causing increased sperm DNA fragmentation that may be sufficient to cause subfertility in the male. These effects may be attributed to increased oxidative stress and insufficient scavenging antioxidant enzymes in the seminal fluid of non-leukocytospermic infertile patients.

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PROSPECTIVE STUDY OF FERTILAID VITAMIN IN MEN WITH LOW SPERM QUALITY

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Introduction and Objectives: A 90-day, randomized, double-blind, placebo controlled study determined if treatment with the vitamin FertilAid for Men (Fairhaven Health, Bellingham, WA) improved sperm quality in men.
Methods: Adult males were enrolled with abnormal sperm parameters defined as one or more: low counts, low percentage of motility; or low percentage of normal morphology. Eligible subjects (including no vitamin ingestion within 30 days) provided two baseline (initial) semen samples. Routine semen analysis was performed according to current WHO guidelines (World Health Organization, 1999) to determine: sperm count per ml and per total ejaculate; percent motile sperm and speed of progression for the motile sperm; and strict assessment of sperm morphology. From these parameters the total motile sperm count and total normal-motile sperm counts for each ejaculate were determined. Following analysis of the baseline semen samples, subjects were randomly assigned to FertilAid or placebo. Following the 90 days of vitamin or placebo therapy, subjects again provided two semen samples. The same laboratory performed the initial and final analyses for each man. Statistical analysis was performed using the Mann Whitney U test.
Results Obtained: No differences existed for sperm parameters between the groups at baseline. Results. A total of 14 subjects completed the trial (initially 10 per treatment were recruited). Eight were randomized to FertilAid and 6 were randomized to placebo. Total normal-motile sperm numbers in the ejaculate improved for men using FertilAid (p=0.05), versus men on placebo. Additionally, the total motile sperm count also showed a tendency towards improvement (p=0.09). Other parameters did not differ between the two groups. Conclusions: In spite of the small sample size in this study, significant improvements were found for men taking FertilAid, with regards to the total number of normal-motile sperm in the ejaculate. Larger studies should be done to confirm the results seen here. Use of FertilAid by the male partner may improve the ejaculate quality in some men, specifically with regards to the number of normally shaped, motile sperm produced. Funded in part by Fairhaven Health.

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DEATH OF HUMAN SPERMATOZOA MAY BE REGULATED SEPARATELY BY MITOCHONDRIAL AND CYTOSOLIC PATHWAYS

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Introduction and Objective: As a terminal cell, cell death is a normal function of spermatozoa. Calcium ionophore A23187 allows divalent cations to cross the cell membrane which leads to the acrosome reaction. A23187 also inhibits mitochondrial ATPase and uncouples oxidative phosphorylation. It is reasonable that A23187 may also induce apoptosis. Staurosporine has been used to induce apoptosis in both somatic cells and spermatozoa. Staurosporine induces apoptosis by non-selective inhibition of protein kinases. The purpose of this preliminary study was to determine if the effects of A23187 and Staurosporine can be differentiated using the common semen parameters of motility, viability, linearity, velocity and acrosome reaction.
Materials and Methods: Aliquots of sperm from normal donors were incubated either in media alone (control), DMSO, staurosporine (10iM final concentration), or A23187 (10iM final) for five hours. At designated time intervals samples were taken and motility, velocity, and linearity were determined using a computer assisted semen analyzer (CASA). Viability was determined using eosin live/dead stain. The state of the acrosome was determined using peanut agglutinin conjugated with FITC.

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PROSPECTIVE STUDY OF FERTILAID® VITAMIN IN MEN WITH LOW SPERM QUALITY

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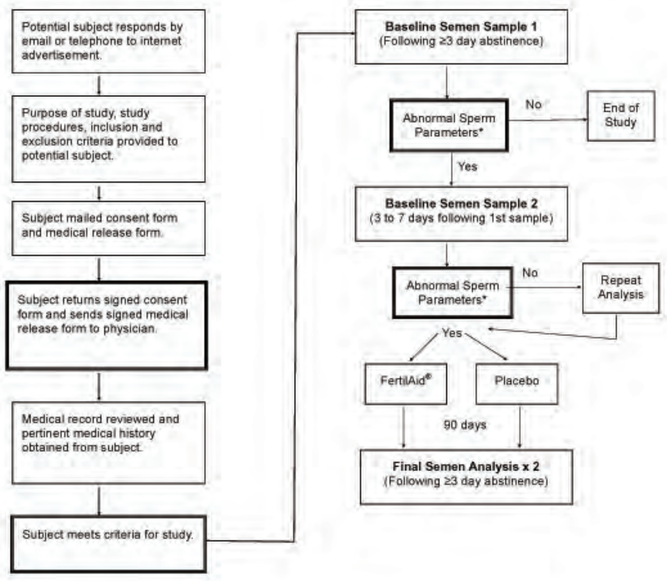
Abstract
Introduction and Objectives: A 90–day, randomized, double–blind, placebo controlled study determined if treatment with the vitamin FertilAid® for Men (Fairhaven Health, Bellingham, WA) improved sperm quality in men. Methods: Adult males were enrolled with abnormal sperm parameters defined as one or more: low counts, low percentage of motility; or low percentage of normal morphology. Eligible subjects (including no vitamin ingestion within 30 days) provided two baseline (initial) semen samples. Routine semen analysis was performed according to current WHO guidelines (World Health Organization, 1999) to determine:sperm count per ml and per total ejaculate; percent motile sperm and speed of progression for the motile sperm; and strict assessment of sperm morphology. From these parameters the total motile sperm count and total normal–motile sperm counts for each ejaculate were determined. Following analysis of the baseline semen samples, subjects were randomly assigned to FertilAid® or placebo. Following the 90 days of vitamin or placebo therapy, subjects again provided two semen samples. The same laboratory performed the initial and final analyses for each man. Statistical analysis was performed using the Mann Whitney U test. Results Obtained: No differences existed for sperm parameters between the groups at baseline. A total of 14 subjects completed the trial (initially 10 per treatment were recruited). Eight were randomized to FertilAid® and 6 were randomized to placebo. Total normal–motile sperm numbers in the ejaculate improved for men using FertilAid® (p=0.05), versus men on placebo. Additionally, the total motile sperm count also showed a tendency towards improvement (p=0.09). Other parameters did not differ between the two groups. Conclusions: In spite of the small sample size in this study, significant improvements were found for men taking FertilAid®, with regards to the total number of normal–motile sperm in the ejaculate. Larger studies should be done to confirm the results seen here. Use of FertilAid® by the male partner may improve the ejaculate quality in some men, specifically with regards to the number of normally shaped, motile sperm produced. Funded in part by Fairhaven Health.

Purpose
The purpose of this trial was to determine whether 90-day treatment with the vitamin FertiliAid® for Men improves sperm quality, in men with low sperm quality, as compared to placebo treatment.

Study Design
90-day, randomized, double-blind, placebo controlled study.

Subject Recruitment, Screening, Treatment Assignment, and Data Collection:



Participants
Adult, trying-to-conceive men, with abnormal sperm parameters (according to 1999 WHO Guidelines) defined as one or more of the following*:
- abnormally low sperm counts
- low percentage of motile sperm or
- low percentage of normal morphology.

Exclusion Criteria
- Ingestion of any fortified antioxidant vitamin formulation within 30 days prior to enrollment
- Azoospermia
- Active urogenital tract infections
- Diabetes
- Varicocele

Sample Collection and Analysis
Ejaculates were collected by masturbation into a sterile specimen cup either at home or at the laboratory. Samples collected at home were received at the laboratory within 30 minutes of collection. Routine sperm analysis was performed according to current WHO guidelines. The same laboratory performing the initial 2 analyses also performed the final analyses. For each subject, an average outcome for the sperm parameters at each time was determined using the average of the two ejaculates initially and at post treatment

Treatment
Subjects were instructed to ingest 3 capsules of the assigned treatment (FertilAid® for Men or matching Placebo) by mouth with 6 ounces of water once each morning. Telephone calls were made at 2-week intervals to assess compliance.

Data Analysis
Because of the variability in reporting methods used by the various laboratories, a non-parametric statistical method was performed to evaluate differences between the treatment and placebo group. The following parameters were analyzed:
- total ejaculate sperm count
- percent motile sperm
- percent forward progression of motile sperm
- total motile sperm count
- percent of sperm with normal morphology and
- total normal-motile sperm count.

Any changes in outcome for the average sperm parameters between the initial and post treatment were scored as: (2) for a parameter increase of 20% or more; (1) for an increase of <20% or >5%; (0) for no change (<5% deviation either way); (-1) for a decrease of <20% or >5%; and (-2) for a decrease of 20% or more. Statistical analysis was performed using the Mann Whitney U test.

Results
Fourteen subjects (age 35.6 ± 9.2 years) completed the 90-day trial. Eight of the 14 subjects were randomized to FertilAid® and 6 were randomized to matching Placebo. No significant difference existed for demographics or sperm parameters between the groups at initial analysis.

- When taken together, the overall ejaculate outcome for total normal-motile sperm significantly improved for men using FertilAid® (p=0.05), versus men receiving placebo.
- The total motile sperm count in the ejaculate also showed a strong tendency towards improvement (p=0.09) in those receiving FertilAid® versus Placebo.
- These differences were due to a higher percentage of men with improvements of 20% or more in their ejaculates (following FertilAid® use), for the total normal-motile sperm count and total motile sperm count.
- Individual parameters of overall counts for sperm in the ejaculate, percent motile sperm and percent of sperm with normal shapes did not significantly differ between the two groups.

Conclusion
In spite of the small sample size, significant improvements were found for men taking FertilAid® for Men, with regards to the total number of normal-motile sperm in the ejaculate.

These data suggest that larger studies into the effect of this vitamin supplement should be done to confirm the trends seen here. Other studies have also shown the value of vitamin-antioxidant supplements for subfertile men. This may be in part due to the lower levels of endogenous antioxidants found in the semen of subfertile men, which appear to make these men's sperm more susceptible to reactive oxygen species damage, including damage to sperm chromatin.

Ingredient	Amount
Vitamin A (as beta-carotene)	5,000 IU
Vitamin C (ascorbic acid)	250 mg
Vitamin D (as cholecalciferol)	400 IU
Vitamin E (as d-alpha tocopheryl succinate)	150 IU
Vitamin K	80 mcg
Thiamin	1.5 mg
Riboflavin	1.7 mg
Niacin	20 mg
Vitamin B6 (pyridoxal 5-phosphate)	2 mg
Folate	500 mcg
Vitamin B12 (as methylcobalamin)	25 mcg
Pantothenic Acid (d-calcium pantothenate)	10 mg
Iodine (kelp)	150 mcg
Magnesium (magnesium oxide)	120 mg
Zinc (zinc gluconate)	50 mg
Selenium (selenomethionine)	100 mcg
Copper (copper gluconate)	2 mg
Manganese	2 mg
Chromium	120 mcg
Proprietary Blend	**875 mg**
L-carnitine (as tartrate)	
Maca root (Lepidium meyenii g.)	
Grape seed extract (standardized to 90-95% proanthocyanidins)	
Panax ginseng (standardized to 2.1% ginsenosides)	

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