Optimal Dietary Status in Couples Trying to Conceive

Is There a Role for Antioxidant Vitamins?

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Overview

- Background on fertility and diet
- Reactive oxygen species, oxidative stress, and antioxidant vitamins
- The prospective *ISIS* study
- Next …..a vitamin supplementation trial?

Diet and fertility

- There is a great deal of interest in understanding the impact of diet on women’s ability to conceive.

Yet we know surprisingly little…….

- Evaluation of the impact of nutrients and dietary patterns on women’s fertility and early pregnancy represents a significant gap in scientific research.

Scientific evidence

- Animal and laboratory data
  - Suggest ‘environmental’ insults on germ cells ameliorated by antioxidant vitamins
  - Useful for hypothesis generating
  - See Ruder, *et al., Human Reproduction Update*, July-August 2008 for a review

Scientific evidence

- Studies in men
  - Semen parameters
  - Oxidative damage to DNA causes impaired spermatogenesis and an increase in sperm anomalies
  - See Tremellen K, *Human Reprod Update*, May-June, 2008 for a review

Scientific evidence

- Epidemiologic studies in women
  - Case-control studies of infertility (endometriosis, tubal factor, ovulatory)
  - Studies of preconceptional multivitamin supplementation and enhanced menstrual cycle regularity/fertility
  - Studies of time to pregnancy and early pregnancy loss

Associations reported in women

- Ovulatory infertility
  - BMI, exercise, alcohol
- Tubal infertility
  - PID, STD's, caffeine, smoking
- Endometriosis
  - Menstrual characteristics, smoking, exercise, alcohol
- Delayed time to pregnancy
  - Smoking, caffeine, obesity, fat distribution, alcohol

Goldman, MB, *et al.,* Infertility, and Baird and Strassman, Women's Fecundability and Factors Affecting It, *Woman and Health,* Academic Press, 2000.

Clinical question:

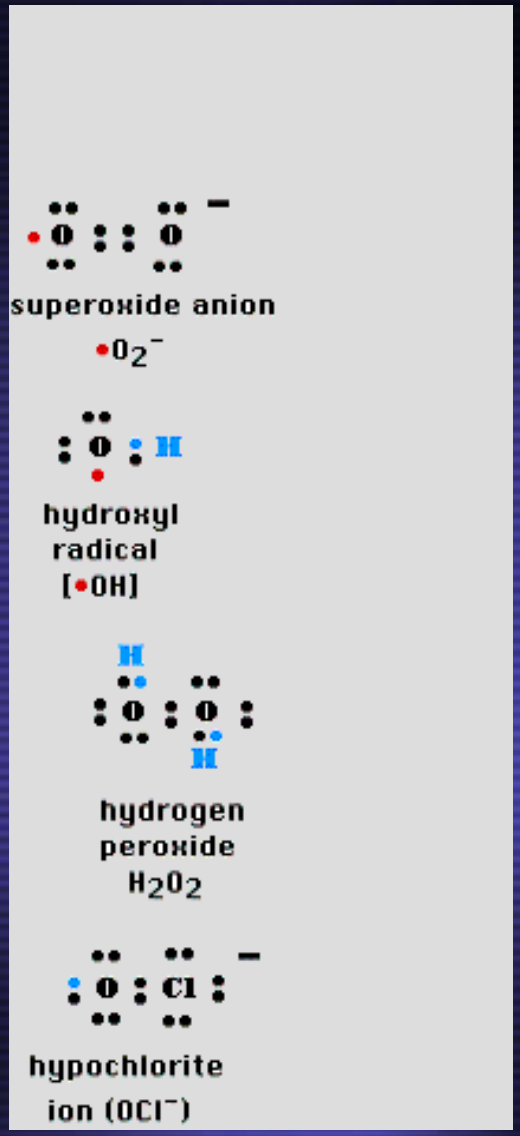
Are low pre-conception intakes of antioxidant vitamins (C and E and the carotinoids) associated with delayed time to pregnancy or early unrecognized pregnancy loss?

Reactive oxygen species (ROS)

- Form as a consequence of both biochemical reactions and external factors such as radiation, infection, alcohol use, smoking.

Reactive oxygen species

- Oxygen containing molecules, oxygen atoms, and free radicals such as:
  - Superoxide anion
  - Hydroxyl radical
  - Hydrogen peroxide
  - Hypochlorite anion

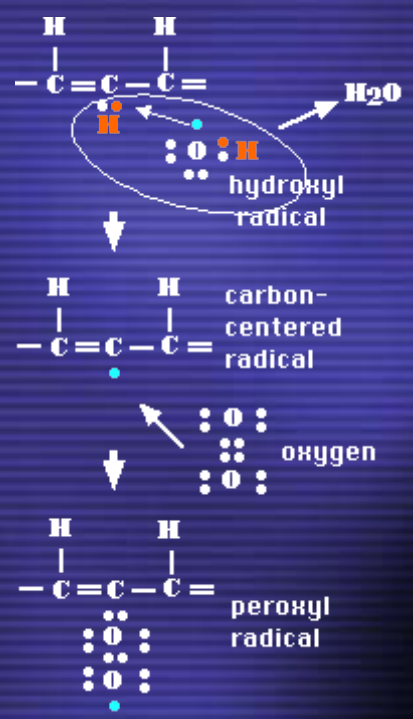


Reactive oxygen species

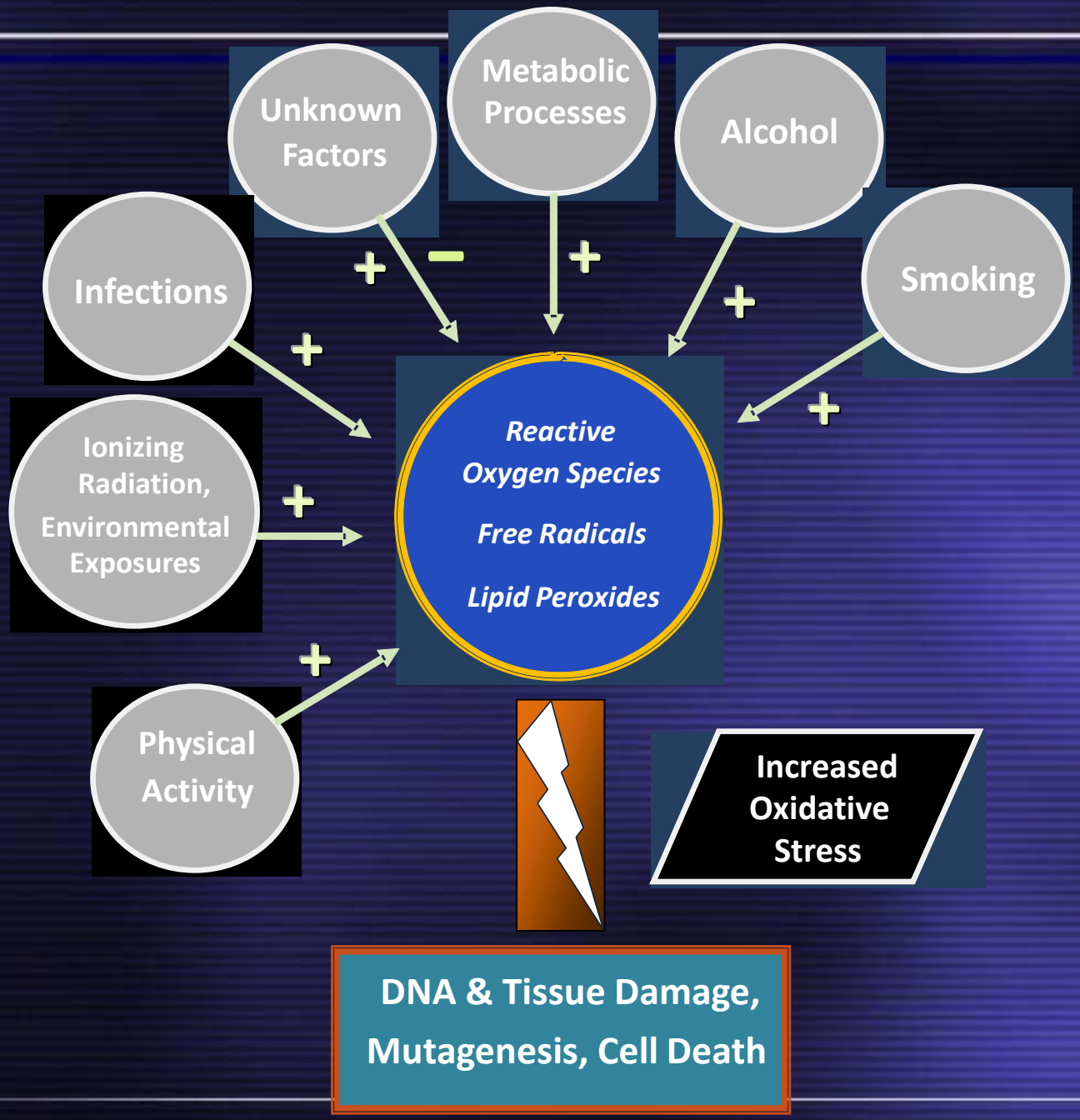
- Associated with adverse reproductive outcomes
  - Increased risk of spontaneous abortion, PPROM, preeclampsia, fetal growth restriction
  - Impaired spermatogenesis and sperm anomalies

Oxidative stress

- Occurs when formation of reactive oxygen species exceeds scavenging by antioxidants
  - Decreased intake
  - Increased antioxidant utilization



Production of Oxidative Stress



Defenses against ROS

- Compounds with a chemical affinity for free radicals:
  - Enzymes
  - Peptides
  - Phenolic compounds - Alpha-tocopherol (Vitamin E)
  - Nitrogen compounds
  - Carotenoids
  - Other agents – Vitamin C

Pilot data

- Nutritional status in couples with unexplained infertility enrolled in the Fast Track and Standard Treatment *(FASTT)* Trial
- Intake of selected antioxidant nutrients and fruits and vegetables

Fast Track and Standard Treatment *(FASTT)* Trial

- A two-arm, unmasked, randomized, controlled clinical trial
- Primary aim was to compare time to pregnancy between
  - an accelerated infertility treatment regimen (clomiphene/IUI $\longrightarrow$ IVF)
  - and stepwise conventional treatment (clomiphene/IUI $\rightarrow$ FSH/IUI $\rightarrow$ IVF)

FASTT study population

- Couples with unexplained infertility, mild male factor, or mild endometriosis who were treated at Boston IVF or HVMA between September, 2001 and April, 2005 (N = 503).

FASTT inclusion/exclusion criteria

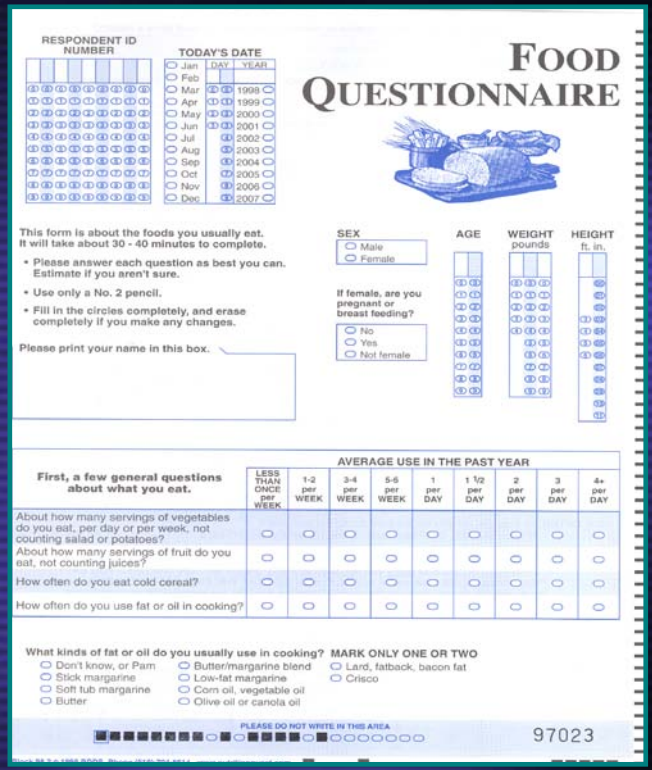
- Eligibility:
  - Female partner
    - 21 to 39 years of age
    - no previous infertility treatment
    - normal ovarian reserve
    - BMI < 39
  - Minimum of one year of infertility
  - Participating insurance

Data collection included

- Male and female personal history (including dietary supplement use)
- Infertility, medical, and obstetric records
- Nutritional assessment at baseline in women and men
- Treatment outcome

Nutrition data collected at baseline

- Nutritional assessment
  - 1998 Block Food Questionnaire
  - Validated nutrition survey included 110 food items
  - Analysis for 36 macro- and micronutrients
    - Vitamins C & E
    - Food group servings
    - Vitamin supplement use



RESPONDENT ID NUMBER

TODAY'S DATE

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

DAY YEAR

1998 1999 2000 2001 2002 2003 2004 2005 2006 2007

FOOD QUESTIONNAIRE

This form is about the foods you usually eat. It will take about 30 - 40 minutes to complete.

- Please answer each question as best you can. Estimate if you aren't sure.
- Use only a No. 2 pencil.
- Fill in the circles completely, and erase completely if you make any changes.

Please print your name in this box.

SEX: Male, Female

If female, are you pregnant or breast feeding? No, Yes, Not female

AGE

WEIGHT pounds

HEIGHT ft. in.

First, a few general questions about what you eat.	AVERAGE USE IN THE PAST YEAR								
	LESS THAN ONCE per WEEK	1-2 per WEEK	3-4 per WEEK	5-6 per WEEK	1 per DAY	1 1/2 per DAY	2 per DAY	3 per DAY	4+ per DAY
About how many servings of vegetables do you eat, per day or per week, not counting salad or potatoes?									
About how many servings of fruit do you eat, not counting juices?									
How often do you eat cold cereal?									
How often do you use fat or oil in cooking?									

What kinds of fat or oil do you usually use in cooking? MARK ONLY ONE OR TWO

Don't know, or Pam; Stick margarine; Soft tub margarine; Butter; Butter/margarine blend; Low-fat margarine; Corn oil, vegetable oil; Olive oil or canola oil; Lard, fatback, bacon fat; Crisco

PLEASE DO NOT WRITE IN THIS AREA

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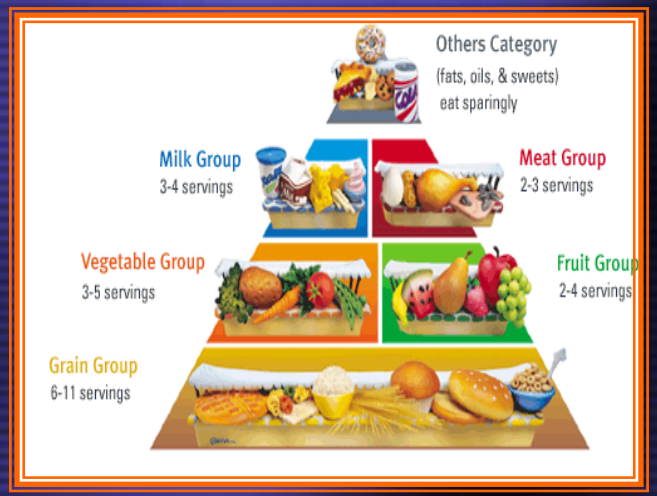
FASTT Trial: first 250 couples

Characteristic	*N (%) or Mean ± SD*	
	Female	Male
Age at randomization (y)	33.0 ± 3.5	34.3 ± 4.6
White race	228 (91.2)	228 (91.2)
Non-Hispanic	234 (93.6)	232 (92.8)
Married	243 (97.2)	
Body mass index (kg/m²)	23.8 ± 4.3	
Gravida 0	146 (58.4)	
Para 0	199 (79.6)	
Smoking	17 (6.8)	29 (11.6)
Alcohol use	194 (77.6)	223 (89.2)
Nutritional data available	241 (96.4)	229 (91.6)
Vitamin supplement use	207 (83.1)	100 (40.5)

FASTT Trial

We compared baseline dietary intake of the antioxidant vitamins C & E, and fruits and vegetables to national recommendations.



National recommendations

- Participants' intakes were compared to the US Estimated Average Requirement (EAR)
- Daily intake value estimated to meet the requirement in half of healthy individuals in each age and sex category
- For pregnant women 19-30 years of age the EAR for Vitamin C is 70 mg/day and for Vitamin E is 12 mg/day

Dietary intake (compared to US recommendations)

Baseline intakes for Vitamins C, E, fruits & vegetables

		Estimated Mean Intakes and % Lower than EAR				
		Females (n = 241)		Males (n = 229)		Couples Both Low
Vitamin C (mg/day)	**Diet**	103.8	34%	112.4	32%	15%
	Diet & Supp	198.3	12%	243.1	21%	4%
Vitamin E (mg/day)	**Diet**	6.9	94%	8.7	83%	78%
	Diet & Supp	30.4	33%	31.9	51%	21%
Low in C or E	**Diet**		94%		85%	79%
	Diet & Supp		34%		53%	22%
Fruits & Vegetables	**Servings**	4.1	68%	4.0	74%	52%

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Summary

- In a population of infertile couples (where participants may have already made dietary changes to improve their chances of conception), many consumed less than the recommended amounts of antioxidant nutrients and fruits and vegetables.

Antioxidant Status, Diet, and Early Pregnancy *(ISIS)* Study

- A prospective cohort study of healthy couples planning pregnancy
  - to directly evaluate the roles of oxidative stress and antioxidant vitamins on fertility in women

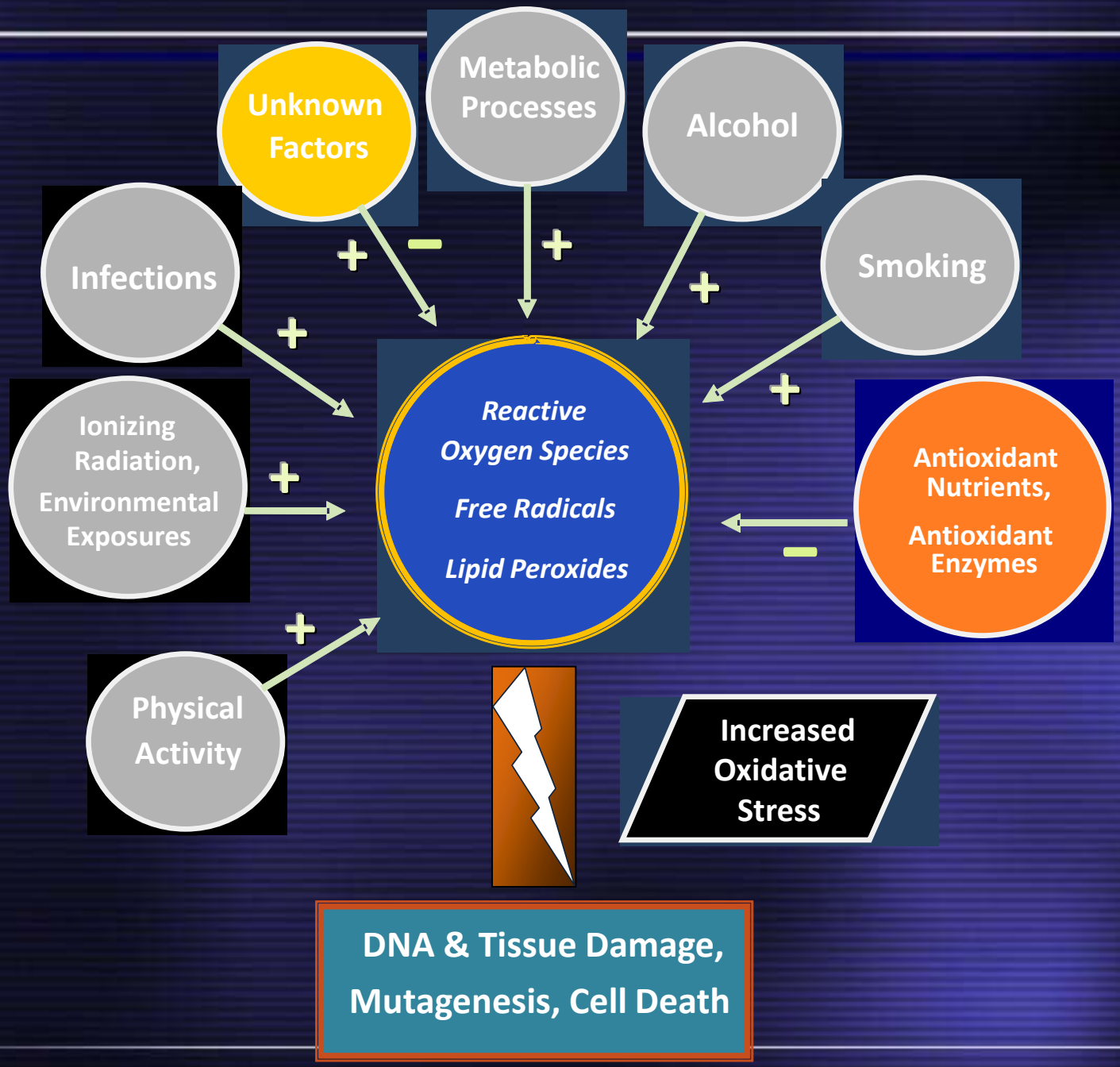
Clinical question:

Are low pre-conception intakes of the antioxidant vitamins C and E and the carotinoids associated with delayed time to pregnancy and/or early unrecognized pregnancy loss?

Antioxidant Status, Diet, and Early Pregnancy *(ISIS)* Study

- *Specific aim 1:*
  - to evaluate whether low pre-conception intakes of vitamins C, E and the carotinoids, as assessed by serum measurement and dietary recalls, are associated with delayed time to pregnancy or early unrecognized pregnancy loss.

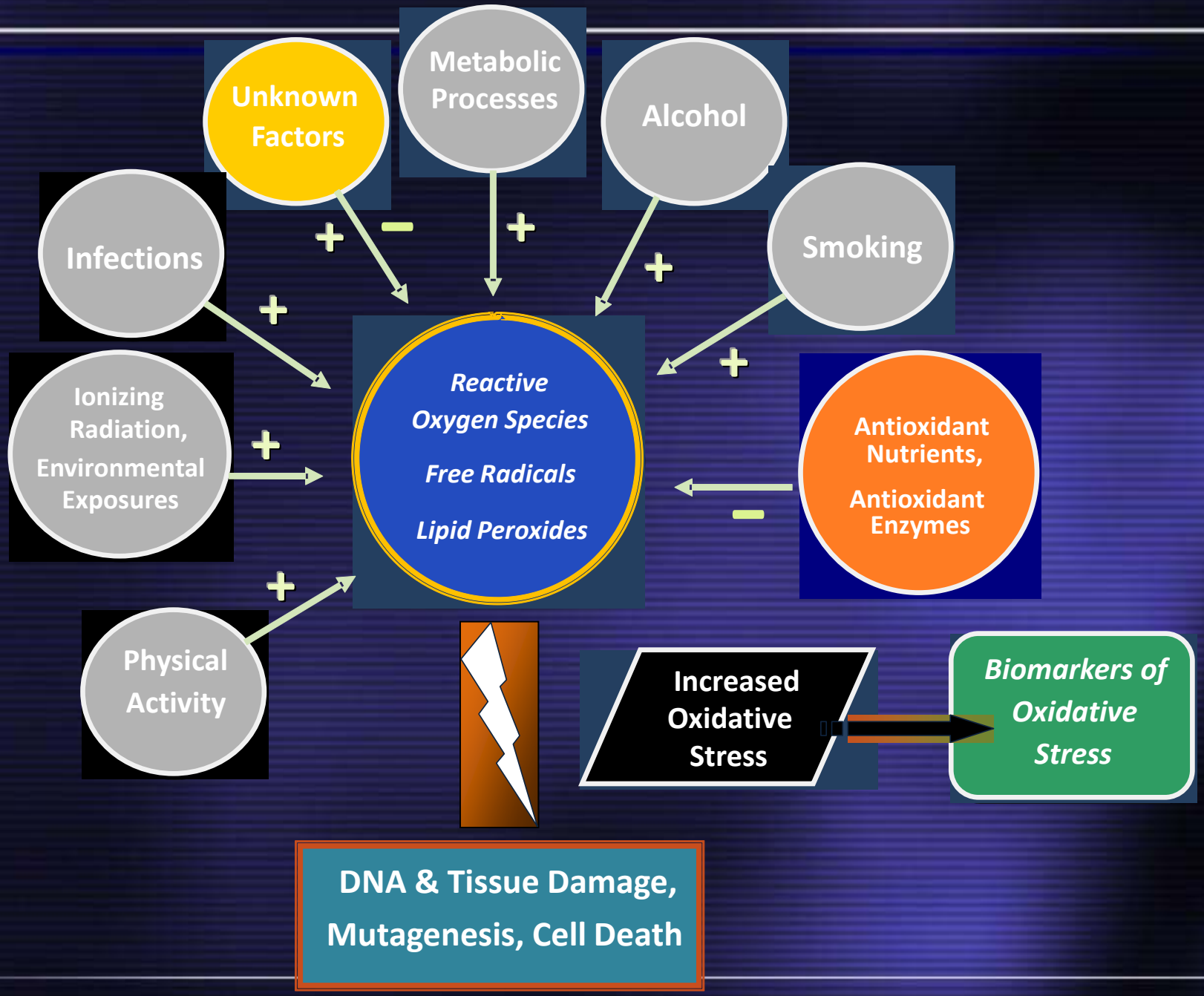
Antioxidant Compounds



Antioxidant Status, Diet, and Early Pregnancy *(ISIS)* Study

- *Specific aim 2:*
  - to evaluate whether an increase in oxidative stress, measured by the urinary biomarkers F2α-isoprostanes and 8-hydroxy-2'-deoxyguanosine (8OH2'dG), is associated with delayed time to pregnancy and/or early unrecognized pregnancy loss.

Biomarkers of Oxidative Stress



Study population

- Married or partnered women between 18 and 39 years of age who plan to stop contraception in order to conceive
- Recruitment in eastern MA, western PA, and NH

Prospective data collection

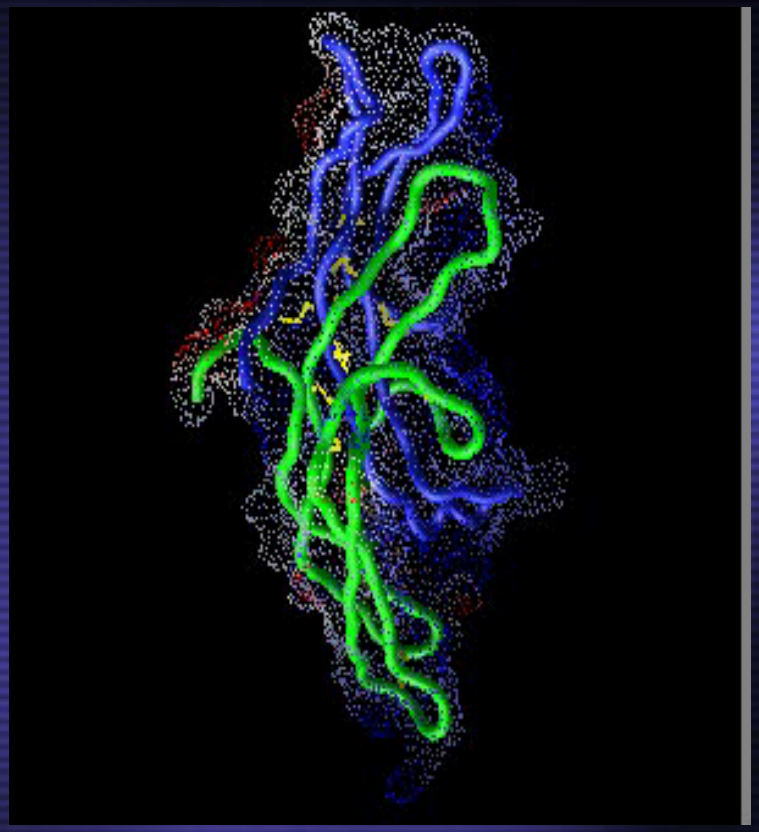
- *Baseline visit*
  - Heath questionnaire (♀,♂)
  - Anthropometric measurements (♀,♂)
  - Fasting serum glucose and lipid profile (♀)
  - Daily urine collection supplies and menstrual diary (♀)

Prospective data collection

- Baseline nutritional data, antioxidant, and oxidative stress evaluation
  - Diet recalls – 3 day, Diet Assessment Center at Penn State
  - Block Food Frequency Questionnaire (on-line)
  - Serum antioxidants at CDC Inorganic Toxicants and Nutrition Branch
  - Urinary oxidative stress assays (♀ only) at Tufts Antioxidants Research Laboratory

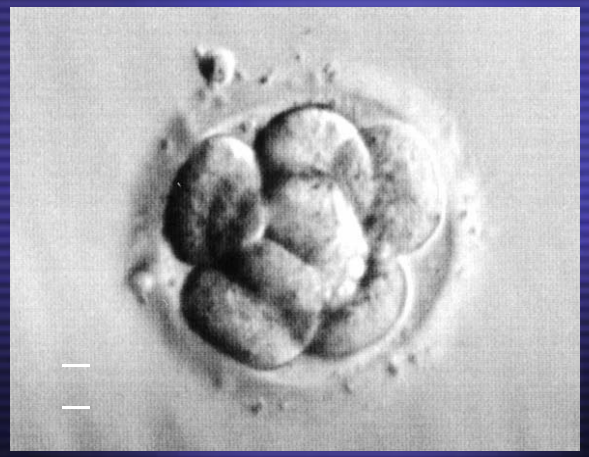
ISIS protocol during the preconception period

- Daily urine collection for hCG analyses until conception occurs or for 6 months or menstrual cycles
- Documentation of ovulation
- Pregnancy testing and menstrual diary



ISIS protocol during the preconception period

- Repeat diet recalls at 3 and 6 months
- Repeat urinary oxidative stress assays at 3 and 6 months (♀)
- Semen analysis offered at 6 months



Sample size requirements

Enrollment of 500 couples would yield 372 women with one implanted pregnancy within 6 months and 80% power to detect a 30% reduction in fecundability if 30% of the population have an adverse level of a dietary component.

Sample size requirements

With a sample size of 500 women, the study will have at least 80% power to detect a 55% increase in early pregnancy loss if at least 30% of the population are exposed.

Consider study designs for developing scientific evidence

- Case report, case series
- Case-control studies
- Animal and laboratory investigations
- Prospective cohort studies
- Clinical trials
  - » Treatment, prevention, vaccine
  - » Equipoise

Designing a trial……..questions to ask:

- What are the aims of the study?
- What intervention(s) will be studied?
- What is the primary outcome? Secondary outcome(s)?
- What design would be optimal?
- What funding sources are available?

Designing the trial……..questions to ask:

- Who will we study? Sample size?
- What are the inclusion/exclusion criteria?
- When will participants be enrolled?
- What baseline data will be collected?

Designing the trial……..questions to ask:

- How long will participants be followed?
- What challenges will arise?
- How will the data be analyzed?
- Where will the results be published and presented?
- What challenges will arise?

ISIS Study Investigators

- Marlene Goldman, ScD
- Terry Hartman, PhD, MPH
- Jeffrey Blumberg, PhD
- Paul Jacques, SD
- Diane Mitchell, MS, RD
- Nancy Williams, ScD
- ISIS Study Staff:
  - Caitlin Simili, MPH
  - Becca Liss
  - Nicholas Lehman-White
  - Erica Wellington
- Boston IVF
  - Michael Alper, MD, Medical Director
  - Kim Thornton, MD
- Harvard Vanguard Medical Associates
  - Martha Richardson, MD
  - Mary Fischer, NP
  - Department of Obstetrics & Gynecology Physicians & Staff

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Questions & Comments