

Hyperbaric oxygen and ovarian follicular stimulation for in vitro fertilization: a pilot study

Our objective was to assess the safety and tolerability of hyperbaric oxygen therapy (HBO) as an adjunct to IVF therapy in women with a poor prognosis for pregnancy in a prospective observational pilot study. We conclude that HBO is well tolerated by women undergoing IVF treatment and that further study is required to determine whether this is an efficacious adjuvant therapy for women being treated by IVF. (*Fertil Steril*® 2005;83:226–8. ©2005 by American Society for Reproductive Medicine.)

Angiogenesis and blood flow have been shown to be important in human ovarian follicular development. The evidence for this has largely come from ultrasound and doppler techniques that have consistently demonstrated increased systolic blood flow in the preovulatory ovary as compared with in the early follicular ovary (1). Several studies have demonstrated increasing perifollicular blood flow during follicular development for IVF cycles (2, 3). Interestingly, oocytes retrieved from follicles that have ultrasound evidence of good blood flow tend to have improved embryonic development in vitro (2). Recent studies have also demonstrated the importance of oxygen in oocyte meiosis. In humans, reduced oxygen content in ovarian follicular fluid has been associated with an increased occurrence of abnormalities in the organization of chromosomes on the metaphase spindle. This could lead to segregation disorders and mosaicisms in the early embryo (4, 5). Thus, sufficient oxygen supply appears to be necessary to allow for normal egg maturation and alignment of chromosomes during meiosis.

We hypothesize that part of the reduced oocyte recruitment, reduced pregnancy rates with IVF, and increased chromosomal abnormalities found in oocytes of women as they age may be due to impaired follicular angiogenesis and oxygenation. Furthermore we hypothesize that this can be reversed by hyperbaric oxygen therapy (HBO) during follicular stimulation for IVF. To begin to test this hypothesis, we conducted this pilot study to determine the safety, tolerability, and effects of HBO when used during ovarian stimulation for IVF.

Study approval was obtained from the University of Iowa Human Subjects Committee. We chose to study patients with a relatively poor prognosis for pregnancy

through IVF. We included infertile women aged 40 years or older and women aged 35–39 years who had at least one previous IVF cycle canceled because of a poor stimulation. Women were excluded for medical contraindications to HBO therapy.

All patients entering the study had the same microdose-flare protocol for ovulation induction after 1 month of birth control pills to help time the cycle. On the 3rd day after stopping birth control pills, leuprolide acetate injections (40 µg twice per day) were started and continued until hCG was administered. After 2 days of leuprolide alone, injections of recombinant FSH were begun at a dose of 5 ampules (total of 375 IU/d) for 3 days. After 3 days of FSH alone, a split protocol of gonadotropin injections consisting of FSH and hMG was begun. Follicular development was monitored by ultrasound measurements and by serum estradiol levels. Cycles were canceled for a serum E₂ of <75 pg/mL on stimulation day 6, or for two or fewer maturing follicles sized >12 mm in mean diameter on stimulation day 8. Human chorionic gonadotropin was administered when there were at least two follicles sized ≥18 mm in mean diameter and the serum E₂ was >500 pg/mL.

Up to four cleavage-stage embryos or two blastocyst embryos were transferred after 3 or 5 days in culture, respectively. Pregnancy tests were obtained 2 weeks after oocyte retrieval, and pregnancies were confirmed by ultrasound.

For purposes of comparison, cycle outcomes from women who were eligible for this study but declined HBO therapy were recorded (concurrent controls). We also recorded outcomes from historical controls, who were women meeting study entrance criteria in our program in the 6 years before the start of this study. Protocols and pregnancy rates for poor responders have been stable over the past 6 years in our program. These women had either a microdose-flare stimulation protocol or a standard luteal-phase leuprolide down-regulated cycle with gonadotropin

Received January 12, 2004; revised and accepted May 10, 2004.

Reprint requests: Bradley J. Van Voorhis, M.D., Department of Obstetrics and Gynecology, 200 Hawkins Drive, University of Iowa Hospitals & Clinics, Iowa City, Iowa 52242 (FAX: 319-353-6659; E-mail: brad-van-voorhis@uiowa.edu).

TABLE 1**Outcomes of IVF cycles.**

Variable	HBO	Concurrent controls	Historical controls
Cycles (n)	10	13	144
Age (y)	40.4 ± 2.3	39.1 ± 2.3	40.2 ± 1.9
Parity	.4 ± .7	.5 ± .9	.8 ± 1.0
Canceled cycles, n (%)	5 (50)	6 (46)	52 (36)
Peak E ₂ ^a (pg/mL)	2,092 ± 797	1,751 ± 915	1,224.85 ± 688 ^c
Follicles ^a (n)	14.4 ± 5.2	14.4 ± 7.8	13.4 ± 9.2
Oocytes retrieved (n)	11.0 ± 2.6	8.1 ± 5.5	8.8 ± 6.4
Mature oocytes (n)	9.4 ± 2.1	6.9 ± 3.9	7.6 ± 5.7
Embryos (n)	6.8 ± 1.1 ^b	3.3 ± 2.8	5.1 ± 4.0
Implantation rate, n (%)	4/18 (22.2)	4/15 (26.6)	26/265 (9.8)
Clinical pregnancy rate per transfer, n (%)	2/5 (40)	3/7 (43)	19/86 (22.1)
Delivery rate, n (%)	2/5 (40)	3/7 (43)	14/86 (16.3)

Note: Data are ± SD.

^aIn noncancelled cycles.

^b*P* ≤ .05 compared with concurrent controls.

^c*P* ≤ .05 compared with HBO cycles.

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injections starting on cycle day 3, depending on the physician's preference.

Hyperbaric oxygen therapy was given for each day for 2 hours, Monday through Friday. The treatments (referred to as *dives*) were performed in a pressurized compartment resembling a submarine at 2.4 atmospheres of pressure, and patients breathed 100% oxygen during 90 minutes of this time. This is the standard HBO treatment time and dose for most indications. The dives continued until the day before oocyte retrieval unless this was on a weekend. All stimulation cycles were timed to begin on Monday (1st day of leuprolide injection), so HBO commenced with the onset of ovarian stimulation.

Pure follicular fluid (no visible blood contamination) was aspirated from the first follicle punctured on each ovary from women in the study. A separate study consent form allowing us to collect follicular fluid was signed by some of the women who were eligible for this study but declined HBO therapy. Follicular fluid was immediately frozen in liquid nitrogen and then stored at -70°C until analysis. Fluid was then thawed, processed by centrifuge, and assayed in duplicate for VEGF concentration with a commercially available ELISA measuring free VEGF165 (Quantikine human VEGF; R and D systems, Minneapolis, MN).

The median number of HBO dives was 9.5 (range, 4–11). HBO was well tolerated during ovarian stimulation for IVF. One woman quit after four HBO dives because of new sinus headaches that resolved shortly after stopping. Otherwise, only minor side effects were noted. Cycle can-

cellation rates for poor ovarian responsiveness remained high after HBO therapy (Table 1). In noncanceled cycles, comparisons with historical but not concurrent controls (who tended to be younger than HBO subjects) suggest improved E₂ response, implantation rates, and pregnancy rates after HBO therapy. Comparisons with concurrent controls suggest that HBO may result in a higher number of embryos from the IVF cycle. Follicular fluid VEGF levels were higher in women who had HBO therapy as compared with in follicular fluid obtained from concurrent controls (4,249 ± 2,358 pg/mL vs. 1,906 ± 1,048 pg/mL, *P* = .04).

Two women conceived after HBO therapy and IVF. The first woman was 36 years old, had low ovarian volume, and had a previous IVF cycle canceled for poor ovarian response. She had 10 HBO treatments, and the IVF cycle resulted in eight embryos with two blastocysts transferred. She conceived twins who were induced at 38 weeks of gestational age. The second woman was 41 and had two previous unsuccessful IVF cycles. She had 11 HBO treatments, and IVF resulted in six embryos. Four embryos were transferred on day 3, and she also conceived twins. Her delivery was by cesarean section at 38 weeks of gestational age. There were no complications of pregnancy for either woman, and no birth defects were noted in the children.

Hyperbaric oxygen—100% oxygen at two to three times the atmospheric pressure at sea level—can result in tissue oxygen tensions of 15 times that seen under normal physiologic conditions (6). High tissue levels of oxygen induced by HBO treatments stimulate angiogenesis in poorly vascularized tissues, and HBO is commonly used for treatment

of poorly healing wounds, particularly in previously irradiated tissues or in diabetic patients (7, 8). The mechanism of increased angiogenesis appears to be both by supplying needed oxygen and by the stimulation of production and release of cytokines, including VEGF (8). Studies have shown that HBO-induced hyperoxia significantly increases levels of VEGF in wounds (9). It appears that VEGF is stimulated by both hypoxia as well as by hyperoxia induced by HBO therapy.

Angiogenesis is critical to follicular development, oocyte quality, and early embryo development. Reduced oxygen delivery may also be a mechanism behind aneuploidy in the oocyte. In 1992, Gaulden (10) hypothesized that the cause of the increased incidence of Down's syndrome as women age was a compromised microcirculation to ovarian follicles as they develop in relatively older women.

For the reasons above, we hypothesize that HBO therapy might improve outcomes for IVF in women who are at high risk for a failed cycle. In this pilot study, we have demonstrated that HBO is well tolerated by women undergoing IVF. This study population was too small to prove or disprove our hypothesis. There was a disappointingly high cycle cancellation rate despite HBO. The five women who completed therapy had reasonably high ovarian stimulation parameters, embryo implantation rates, and pregnancy rates. Both sets of twins resulting from this therapy went to full term with no apparent obstetric or neonatal complications. However, the conclusion of this pilot study must be that no benefits of HBO treatments on the outcomes of IVF cycles were seen. Much larger studies will be required to adequately test our hypothesis. Unfortunately, HBO therapy is quite expensive (~\$500–\$700 per treatment), so this will be a costly trial.

We suggest that perhaps the timing and dose of HBO therapy given may not have been optimal. Earlier initiation of treatments may lead to a larger cohort of ovarian follicles for stimulation. Studies in wounds have demonstrated that angiogenesis increases in a linear fashion through 20 treatments before a plateau is reached (11). Our patients received, on average, 9.5 treatments, with the two pregnancies occurring in women who received 10 and 11 treatments. We saw a significantly higher VEGF level in the follicular fluid of women treated with HBO compared with the controls. In general, previous studies have associated higher follicular fluid VEGF levels with older age and a worsened outcome from IVF (12, 13). It is possible that the higher VEGF levels were therefore simply reflective of the older age and poor prognosis of our study patients. On the other hand, because VEGF is important in angiogenesis and HBO increases VEGF at least transiently in wounds, HBO may have begun to stimulate angiogenesis by increasing VEGF levels. Perhaps with more HBO treatments started before ovarian stimulation, this effect may have been completed with better outcomes observed. Methods to

accurately and objectively measure microvasculature of the ovarian follicle in vitro are needed to answer this question.

Bradley J. Van Voorhis, M.D.^a
J. Eric Greensmith, M.D., Ph.D.^b
Anuja Dokras, M.D., Ph.D.^a
Amy E. T. Sparks, Ph.D.^a
Shawn T. Simmons, M.D.^c
Craig H. Syrop, M.D.^a

^a *Department of Obstetrics and Gynecology, University of Iowa Hospitals and Clinics, Iowa City, Iowa;*

^b *Department of Anesthesia, St. Elizabeth Hospital, Appleton, Wisconsin; and* ^c *Department of Anesthesia, University of Iowa Hospitals and Clinics, Iowa City, Iowa*

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