different culture vessels were essentially the same and our results provide support from a second laboratory that microdrops performed at least as well as tissue culture tubes in supporting fertilization and overall embryo development using the techniques described above. Our study had a statistical power of 80% to detect at least a 10% difference in the two groups, assuming a 65% fertilization rate in the tissue culture tube group. Furthermore, the use of microdrops saves a substantial amount of time during oocyte/embryo handling procedures and greatly reduces the risk of losing or damaging the oocytes/embryos.

CONCLUSIONS

Our laboratory has corroborated previously published work demonstrating that tissue culture tubes and microdrops perform equally well for in vitro fertilization and culture of human oocytes and embryos.

ACKNOWLEDGMENTS

We thank Martin M. Crane, Ph.D., for his help with the statistical aspects of the manuscript.

REFERENCES

1. Boone WR, Shapiro SS: Quality control in the in vitro fertilization laboratory. *Theriogenology* 1990;33:23-50
2. Fiorentino A, Magli MC, Fortini D, Feliciani E, Ferraretti AP, Dale B, Gianaroli L: Sperm:oocyte ratios in an in vitro fertilization (IVF) program. *J Assist Reprod Genet* 1994;11:97-103
3. Boone WR, Johnson JE, Price TM, Bowers BK, Morris MD, Crane MM, Bates GW: In vitro fertilization and embryo transfer (IVF-ET) comes to the upstate. *J SC Med Assoc* 1994;90:360-366
4. World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, 3rd ed. New York, Cambridge University Press, 1992

William R. Boone¹

Jane E. Johnson

Greenville Hospital System

Department of Obstetrics and Gynecology

Division of Reproductive Endocrinology and Infertility

890 West Faris Road, Suite 470, Box 32

Greenville, South Carolina 29605

¹ To whom correspondence should be addressed.

NEW YORK, NEW YORK

Differences in Ovarian Stimulation Among Older and Younger Women with a Diminished Gonadal Reserve¹

Submitted: September 3, 1996

Accepted: December 2, 1996

INTRODUCTION

The transition to menopause is commonly referred to as the climacteric. During this time, a number of events occur prior to complete cessation of menses. Subtle changes include a diminution in the number of oocytes, elevations in serum gonadotropins, specifically follicle stimulating hormone (FSH), and lower levels of sex steroids (1).

Age has long been known to be associated with a natural decline in fertility (2). This relationship has been noted in patients undergoing assisted reproduction (ART) (3). However, there is individual variation within the population with respect to response. For instance, occasional older women respond to controlled ovarian hyperstimulation (COH). Conversely younger women may experience unexpectedly poor responses.

Serum FSH has been demonstrated to have prognostic predictive value for women undergoing ART and, when found to be abnormal, is a more sensitive marker of ovarian reserve than age alone (4). However, both age and serum hormone values need to be considered in evaluating the overall prognosis for undergoing therapy.

Since age and serum FSH are independent predictors of cycle quality and outcome, we hypothesized that younger and older women with elevated FSH attempting COH may demonstrate different clinical responses. Patients with elevated baseline hormone levels planning to undergo in vitro fertilization are typically canceled. However, we offer patients with a diminished ovarian reserve (elevated FSH levels) COH and IUI prior to moving to donor gametes since it is less invasive, is less costly, and occasionally results in pregnancy. We were interested in reviewing their

¹ Presented at the 44th Annual Meeting of the Pacific Coast Fertility Society, April 1996, Palm Springs, California.

stimulation characteristics in order to determine if younger patients with abnormal profiles respond differently than women older of with a similar elevation.

MATERIALS AND METHODS

Subjects. We reviewed 136 patients who underwent 201 consecutive cycles of COH and IUI. All patients had cycle day (CD)-3 serum FSH levels, had documented tubal patency, and were without semen abnormalities.

Patients were grouped according to age and CD-3 FSH: Group I ($n = 96$), <40 years of age (mean $\pm$ SE; 32.5 ± 0.5 years) with normal CD-3 FSH (<15 mIU/ml previously determined using receiver-operated curve analysis) (5) (10.2 ± 0.5 mIU/ml); Group II ($n = 16$), <40 years (34.5 ± 0.5 years) with abnormal CD-3 FSH (20.0 ± 0.5 mIU/ml); Group III ($n = 13$), ≥ 40 years (41.3 ± 0.4 years) with normal CD-3 FSH (11.9 ± 0.7 mIU/ml); and Group IV ($n = 11$), ≥ 40 years (41.8 ± 0.5 years) with abnormal CD-3 FSH (22.3 ± 1.0 mIU/ml).

COH Protocol. Human menopausal gonadotropin (Serono, MA) was given daily by intramuscular injection beginning with 3 or 4 ampoules. Dosing was individualized to maximize the patient's response until the lead follicle reached >16 mm in diameter and estradiol ≥ 200 pg/ml for each follicle exceeding 14 mm. Human chorionic gonadotropin (hCG) was administered im and an IUI sperm preparation using a standard swim-up technique performed 36 to 40 hr later. Clinical pregnancies were confirmed by transvaginal ultrasound.

Assays. Serum samples were assayed for FSH on CD-3 and estradiol while on COH using 125 IRIA kits. For FSH (Diagnostic Products, LA, CA), calibrated against the WHO second IRP-hMG, intra- and interassay CVs were 3.4 and 4.2%, respectively. For estradiol (Pantex, Santa Monica, CA) intra- and interassay CVs were 5.0 and 8.4%, respectively.

Statistical Analysis. Statistical analysis was performed using chi-square and ANOVA with Bonferroni t test for post hoc analysis. Significance was defined as $P < 0.05$.

RESULTS

CD-3 FSH was significantly greater in Groups II and IV compared to Groups I and III ($P < 0.05$).

Groups were similar with respect to the number of days of stimulation (DOS). However, Groups I and II required significantly fewer ampoules of hMG compared to Groups III and IV (Group I, 24.6 ± 1.3 amps; Group II, 32.4 ± 1.7 amps; Group III, 47.9 ± 3.1 amps; and Group IV, 59.0 ± 3.6 amps; $P < 0.05$). Figure 1 depicts the number of lead follicles (<14 mm), peak serum estradiol, pregnancy rates per cycle (PR), and miscarriage rates. Group I recruited significantly greater numbers of dominant follicles (4.1 ± 0.4 ; $P < 0.05$) compared to Group II (2.7 ± 0.3), Group III (1.9 ± 0.3), and Group IV (1.5 ± 0.3). Peak serum estradiol was significantly higher in Groups I and II (844 ± 74 and 621 ± 55 pg/ml; $P < 0.05$) compared to Group IV (276 ± 53 pg/ml). No difference was seen between Group II and Group III (481 ± 77 pg/ml). As expected, Group I had significantly greater ongoing-delivered pregnancy rates per cycle (18.3%; 26/142; $P < 0.05$) than either Group II (0.0%; 0/22), Group III (8.3%; 3/36), or Group IV (3.1%; 1/32). It is worth noting that only 1 of 54 cycles resulted in a pregnancy in women with an elevated FSH. While the miscarriage rate was significantly less in Group I (21.2%; 7/33; $P < 0.05$) than Group II (100%; 3/3), differences in Group III (0.0%; 0/3) and Group IV (0.0%; 0/1) were not seen, which probably reflects a Type II error because of the small sample size.

DISCUSSION

Prior to menopause, a transition period occurs where compensated ovarian function is seen. Changes are initially subtle, and often subclinical, detectable only using serum markers. Later alterations in menstrual function occur. These changes are accompanied by decreasing fertility rates. Typically, this affects women in their late 30s and seems pronounced for women in their forties. The observed rise in FSH appears to precede changes in LH by almost a decade, with different patterns of gonadotropin secretion noted from normal to postmenopausal levels. Several authors have also described normal to lower levels of serum estradiol even in the presence of elevated serum gonadotropins. Serum progesterone in ovulatory cycles appears to be normal and corpus luteal function is one of the last changes to occur prior to menopause (6–8).

A diminished ovarian reserve primarily represents a decreased quantity and quality of oocytes. We observed a profound decrease in viable pregnancies in women with elevated CD-3 FSH levels regardless of age. There does appear to be an age-related decline in

Fig. 1. Peak serum estradiol, number of dominant follicles, and pregnancy rate.

ovarian response as evidenced by peak serum estradiol levels. This suggests that a diminishing ovarian reserve initially represents reduced oocyte and ultimately embryo quality in younger women. However, with advancing age there appears to be a reduction in both oocyte quality and granulosa cell steroid hormone production.

In summary, it is clear that our data show that elevated CD-3 FSH is a poor prognostic indicator for outcome success and demonstrates a lack of overall efficacy of COH in these patients. However, an age-related difference in stimulation response to COH was seen. This further supports that waning ovarian function is a gradual process, with a series of events leading to menopause, including one's response to COH.

REFERENCES

- Sherman BM, West JH, Korenman SG: The menopausal transition: Analysis of LH, FSH, oestradiol and progesterone concentrations during menstrual cycles of older women. *J Clin Endocrinol Metab* 1976;42:629-634
- Menken J, Trussell J, Larsen U: Age and infertility. *Science* 1986;23:1389-1391
- Piette C, deMouzon J, Bachelot A, Spira A: In-vitro fertilization: influence of woman's age on pregnancy rates. *Hum Reprod* 1990;5:56-59
- Toner JP, Philput C, Jones GS, Mausher SJ: Basal follicle stimulating hormone level is a better predictor of in vitro fertilization performance than age. *Fertil Steril* 1991;55(4):784-791
- Witt BR, Barad DH, Barg P, Cohen BC, Lindheim SR, Testaiuti L, Amin HK: Basal serum follicle stimulating hormone (FSH) and estradiol levels as predictors of pregnancy in unstimulated donor insemination cycles. *J Assist Reprod Genet* 1995;12:157-160
- Van Look PFA, Lothian H, Hunter WM, Michie EA, Baird D: Hypothalamic pituitary ovarian function in perimenopausal women. *Clin Endocrinol (Oxf)* 1977;7:13-31
- Papanicolaou AD, Loraine JA, Dove GA, Loudon NB: Hormone excretion patterns in menopausal women. *Br J Obstet Gynecol* 1969;76:308-316
- Adampoulis DA, Loraine JA, Dove GA: Endocrinologic studies in women approaching the menopause. *Br J Obstet Gynaecol* 1971;76:62-79

Steven R. Lindheim²

Edward C. Dittkoff

Mark V. Sauer

Department of Obstetrics and Gynecology

Division of Reproductive Endocrinology

Columbia-Presbyterian Medical Center

New York, New York 10032

Michael Zinger

The North Shore University Hospital

Manhasset, New York

² To whom correspondence should be addressed at Columbia Presbyterian Medical Center, Department of Obstetrics and Gynecology, 622 West 168th Street, New York, New York 10032.