SHORT COMMUNICATIONS

LOS ANGELES, CALIFORNIA

Assessing the Importance of Endometrial Biopsy Prior to Oocyte Donation

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INTRODUCTION

For more than 10 years oocyte donation has been used to combat female infertility. It has greatly increased in popularity due to its broad applicability and therapeutic merits. Initially oocyte and embryo donations were offered principally to women with either premature ovarian failure or carrying a genetic illness. More recently, patients include younger women who have repeatedly failed attempts at standard in vitro fertilization and older women with age-related infertility (1).

Often a timed endometrial biopsy is obtained prior to an actual attempt at oocyte donation. The patient follows a prescribed regimen of hormone replacement similar to that planned for the actual transfer. Usually biopsies occur on the twenty-first day of medications or 7 days after beginning progesterone. This date coincides with the period known as the "window" of embryo implantation, granting an opportunity to survey the intrauterine environment during this critical interval.

We required an endometrial biopsy of prospective recipients at the University of Southern California. Initially, the purpose of the biopsy was to assess whether or not a receptive endometrial environment had been established. However, as time passed it became apparent that additional benefits were gained from performing the mock cycle. This report details our cumulative experience.

MATERIALS AND METHODS

The protocol for oocyte donation at the University of Southern California in vitro fertilization program (USC-IVF) was approved by the Institutional Review Board of the California Medical Center, Los Angeles. Between July 1987 and July 1995, recipients ($n = 450$) were asked to undergo a "practice transfer cycle" using the same medications prescribed for the planned attempt. Patients were between 24 and 59 years of age. Their primary diagnoses included premature ovarian failure, surgical castration, advanced reproductive age (>45 years), multiple failed attempts at in vitro fertilization, and natural menopause. Medications consisted of oral micronized estradiol administered in a stepwise and incrementally increased dosage followed by and in conjunction with intramuscularly injected progesterone, as described previously (2). On the twenty-first day of the artificial cycle an endometrial biopsy was performed using a pipelle uterine aspirator. Specimens were fixed in formaldehyde and processed using standard hematoxylin and eosin staining. Endometrial samples were dated according to criteria published by Noyes *et al.* (3). All biopsies were reviewed by the same pathologist (D.L.M.) blinded to the patients' clinical data.

Prior to biopsy a transvaginal ultrasound examination was performed using a 5-MHz probe. Endometrial thickness was measured and grading of the echogenic pattern was performed in accordance with criteria published by Grunfeld *et al.* (4).

RESULTS

Biopsies were successfully performed in 443 of 450 attempts (98.4%). In seven instances endometrial sampling was not obtained secondary to severe cervical stenosis. All of these women had previously undergone either cryosurgery ($n = 4$) or cone biopsy ($n = 3$) for treatment of cervical dysplasia.

The majority of specimens (283; 63.9%) were "in phase" for day 21 of an idealized cycle. Table I

Table I. Results of 443 Consecutively Performed Endometrial Biopsies

Histologic dating	
Specimens with histologic dating within 2 days of day 21	283 (63.9%)
Appearance of glands	19.5 $\pm$ 0.2 days
Appearance of stroma	20.3 $\pm$ 0.2 days
Normal gland-to-stromal ratio	83.0%
Specimens with dating out of phase >2 days	74 (16.7%)
Specimens with absent secretory response	9 (2.0%)
Specimens with hyperplastic features	77 (17.4%)

describes the histologic characteristics of the processed specimens. Within this group a lack of synchrony between glands and stroma was noted in 81 instances (28.6% of "normal" biopsies), with the glandular compartment lagging behind the stroma in development by 1–2 days. This represented the most common aberrancy.

In 74 cases (16.7%) the specimens were out of phase by more than 2 days. Of these, 52 (70.3%) exhibited glandular maturational lag while the stromal compartment was appropriate in its development. In 22 other cases (29.7%) both the glands and the stroma were lagging >2 days.

A poor response, interpreted by us as a lack of adequate estrogen stimulation, was present in nine instances (2.0%). Six of these patients were menopausal for >5 years and had never taken hormone therapy. In each of these cases a normal biopsy was obtained on repeat attempts after estrogen priming. In three instances no endometrial response was evident despite repeated adjustments of the hormone replacement therapy (up to 10 mg of micronized estradiol per day), with biopsies appearing weakly proliferative and exhibiting areas of fibrosis. All of these patients had previously been treated for malignancy with pelvic radiation, and their lack of response was likely secondary to the permanent damaging effects of radiotherapy upon the uterus.

Gross hyperplasia was observed in 77 cases (17.4%). These specimens were characterized by glandular crowding, an increase in the normal gland-to-stroma ratio, and papillary excrescences within the glandular lumens.

No correlation was evident between the ultrasonographically measured endometrial thickness or pattern and the corresponding histology. Thickness ranged from 4 to 18 mm, with a mean $\pm$ SD of 9.7 $\pm$ 1.6 mm. Most often a pattern II image was visible (71.0%). However, in cases where hypoplastic responses were detected the endometrial echo was <4 mm.

Occasionally difficulties were encountered in following the prescribed treatment regimen. Despite careful instruction, both verbal and written, 26 women (5.8%) were unable to complete the biopsy cycle correctly, requiring the cycle to be repeated. The most common error was in the administration of oral micronized estradiol. Usually patients missed doses during the "step-up phase" of the medications.

DISCUSSION

The endometrium is directly involved in the process of implantation and is the principal target organ for estrogen and progesterone. Therefore, endometrial morphology may be viewed as an *in vivo* bioassay, reflecting the adequacy of hormone replacement therapy. Most cases of delayed endometrial development are associated with normal levels of serum progesterone (5). This has also been noted in functionally agonal women failing to respond to exogenous hormone replacement despite reaching pharmacologic levels of sex steroid in their serum. Thus, serum measurements cannot be reliably used to assess endometrial normalcy. Furthermore, other abnormalities such as endometrial polyps and hyperplasia will not be discovered without resorting to more direct means.

Artificial cycles provide a unique opportunity to study the precise relationship between administered sex steroids and endometrial morphology. Navot *et al.* investigated the effect of varying the duration of estrogen priming and reported that endometrial development during the artificial cycle was similar in women with a short duration (6 days), a standard duration (14 days), or a long duration (3–5 weeks) of priming (6). Devroey *et al.* found that normal endometrial maturity in the midluteal phase of artificial cycles was demonstrable despite a rather wide range of serum progesterone levels (7). Given the elevated steady-state levels of both estradiol and progesterone in women taking these medications, serum determinations are of little benefit in predicting endometrial response.

Biopsies are commonly heterogeneous in appearance. It is often difficult to date specimens since one area may possess features of a well-developed endometrium, while adjacent areas may appear "lagging" in glandular development or even grossly hyperplastic. Our attempt at dating represents a general assessment related to overall appearance, with a full understanding that few biopsies are truly "normal" by strict criteria. For this reason women were required to undergo repeat

biopsy cycles only if "poor" responses were seen. Repeat biopsies were typically improved in appearance, with the exception of patients previously irradiated in whom fibrosis was prominent and minimal maturational responses were noted. We have not noted any differences in pregnancy outcomes with this approach and do not suggest modification of the treatment regimens when glandular-stromal dyssynchrony is present.

Endometrial biopsy has been criticized as an invasive procedure. Possible complications include immediate and delayed pelvic discomfort, uterine perforation, infection, and interruption of an early conception. However, using the 3-mm pipelle aspirator, we found the procedure to be safe and well tolerated. Few patients required analgesia, and in most cases the instrument passed into the fundus without using a tenaculum. At the time of biopsy other important information was obtained related to the ease of catheterization, angle of passage, and uterine depth. These landmarks are of critical importance in planning the attempt at embryo transfer.

As the method of oocyte donation becomes more standardized and efficient, the apparent need for pre-transfer visits lessens. Yet the biopsy cycle assesses patient compliance following the introduction of a treatment plan to which few patients are familiar. The greatest difficulty for patients appeared to be remembering split dosages. Complaints over the use of intramuscular progesterone, including severe localized allergic reactions, also occasionally occurred. The return visit allowed time to address questions that invariably arose as couples proceeded through the program. Inquiries related to the results of medical or reproductive testing, and the upcoming donor matching and synchronization schedule were handled during this time.

Ultrasound characterization of the endometrium was not entirely predictive of histologic response. Yet the ability to visualize the ovaries in patients undergoing "mock cycles" demonstrated that many "functionally agonadal" women still have residual ovarian activity. Follicles >10 mm in diameter were occasionally noted. When folliculogenesis was found, women were placed on gonadotropin releasing hormone agonists for their actual attempt at oocyte donation. The occurrence of untimely ovulation or unexpected uterine bleeding may disrupt planned cycles in women with ovaries, especially if serum levels of follicle stimulation hormone are fluctuating.

SUMMARY

The importance of performing an endometrial biopsy in women preparing for oocyte donation goes beyond confirming the histologic response to hormone replacement therapy. Additional information related to uterine architecture, ease of embryo transfer, status of the ovaries, and patient compliance is also gained. Finally, the return visit provides an opportunity to discuss plans for the upcoming cycle. Whereas this report does not specifically address the question as to how many pregnancies were contingent upon the satisfactory performance of the mock cycle, we estimate that due to a combination of factors (i.e., lack of endometrial response, patient noncompliance, difficult embryo transfer), the likelihood of pregnancy in many cases would have been substantially reduced had the preliminary cycle not been attempted.

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