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Identifying women with a narrow window of embryo implantation using the endometrial receptivity assay

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Abstract
The window of implantation (WOI) is believed to be at least several days wide. It should not make a difference in pregnancy rates if an IVF program chooses to perform frozen embryo transfers (FET) after 120 hours of progesterone or after 144 hours of progesterone exposure. The endometrial receptivity assay (ERA) has been used to improve pregnancy rates in women who have failed to achieve pregnancy after multiple embryo transfers. Since the ERA utilizes an endometrial biopsy performed after 120 hours exposure to progesterone, it is not clear if it can be applied to women in an IVF program performing FET after 144 hours of exposure to progesterone.

An ERA was performed on a series of patients who had failed to achieve pregnancy after transfer of multiple good quality embryos. FET in these women had been executed after approximately 144 hours of progesterone exposure to estrogen prepared endometria. The ERA produced a recommendation for a change in the duration of progesterone exposure in 70.8% of the women tested. Changes in progesterone duration based on ERA testing led to an implantation rate of 50% and a live birth rate of 76.5%. This compares to an implantation rate of 3.4% and a live birth rate of 1.1% for embryo transfers before ERA testing.

Keywords: Endometrial receptivity assay, ERA, FET, frozen embryo transfer, repeated implantation failure, RIF, window of implantation, WOI

Introduction

The window of receptivity or implantation (WOI) is defined as the time period in the menstrual cycle during which the endometrium is receptive to normal embryo implantation [1]. Clinical observations have suggested that this WOI may last as long as four to six days [2-4]. Understanding the optimal duration of progesterone administration to an artificially prepared endometrium before transfer of frozen/thawed or vitrified/warmed cleavage embryos or blastocysts (FET cycle) is a related, but different problem and has been the subject of much investigation [5, 6]. Mackens *et al*. undertook a comprehensive review of the optimal time interval of progesterone exposure prior to FET [5]. This review suggested that approximately 120 hours of progesterone exposure was optimal. However, this hypothesis has not been universally accepted and blastocyst transfer after 144 hours of progesterone exposure is routinely used in some IVF programs.

Implantation failure is a complex issue [7]. Recurrent implantation failure (RIF) is defined as the failure of high quality embryos to implant after several ART cycles. The endometrial receptivity assay (ERA: Igenomix, Miami, FL) was an attempt to determine the optimal WOI by defining the transcriptomic signature for endometrial receptivity [8]. Such a test potentially provides a rational basis for evaluating and treating RIF [8-10]. The ERA was originally designed to evaluate the expression of 238 genes based on mRNA in tissue obtained from a timed endometrial biopsy taken 120 hours after the addition of progesterone in a woman with an estrogen primed endometrium. It uses a logical algorithm based on this transcriptomic profile to determine a woman's WOI. The ERA has been applied by some to mean that the normal WOI is a narrowly defined time period occurring after around 120 hours of progesterone exposure [4, 9]. ERA testing finds that about 88% of women having a first IVF cycle, will have receptive endometrium occurring after about 117 to 123 of progesterone exposure [9]. Prima facie, this suggests that the ERA may not applicable in an IVF program which routinely transfers embryos after 144 hours of progesterone exposure.

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This retrospective cohort review involved women with multiple failures to achieve a live birth after transfer of high quality blastocysts. FET had been done to estrogen prepared endometria after 144 to 148 hours of progesterone exposure. That is, blastocyst transfer was routinely performed in a setting in which the ERA could be expected to find the endometrium non-receptive about 88% of the time. What was the utility of the ERA in this setting? This study looked at women with RIF after FET at 144 hours of progesterone exposure to answer that question.

Materials and Methods

The FET protocol utilized oral contraceptives for cycle timing with a one week overlap of a GnRH agonist. Patients were then treated with escalating doses of oral micronized estradiol to a maximum of 6 mg per day. Progesterone in oil (50 mg) was started in the morning approximately 144 hours prior to planned embryo transfer. The clinic's routine involved transferring two embryos if acceptable to the couple and if two embryos were available.

Because of the timing of the program's blastocyst transfer, the ERA was not an evaluation offered to patients. However, in response to few patients desperate to "do something differently" with the transfer of their last remaining cryopreserved blastocysts, ERA testing was offered on a limited basis. All patients given the opportunity of ERA testing had at least two failed IVF cycles (including at least one FET cycle) with the transfer of at least three good prognosis embryos.

Results

Twenty-four patients had ERA testing done during mock FET cycles over a period of three years. Their average age was 36.6 ± 5.7 years. These patients had previously undertaken 94 embryo transfers involving 174 embryos which resulted in five clinical pregnancies and one delivered pregnancy. After several failed transfers, many of these patients had turned to the use of donor oocytes from good prognosis donors. Specifically, fifteen of these patients with failed transfers had utilized 76 donor oocytes in some of their transfers.

Of the initial 24 patients tested, 21 (87.5%) provided interpretable ERA results. Of these 21 cases, seventeen patients (81%) obtained ERA results that could be used to alter the timing of their subsequent transfers. The mean optimal duration of progesterone exposure for these 17 patients was 121 hours with a range of 108 to 149 hours and an intra-quartile range of 6. Twelve of these patients previously had failed FETs using donor oocytes or known euploid embryos. The subsequent FET cycles of these seventeen women resulted in thirteen (76.5%) clinical pregnancies and eleven women (64.7%) with viable deliveries. Of these pregnancies, three resulted in the delivery of twins. The implantation rate for the seventeen ERA-modified transfers was 50%. This compares to an implantation rate of 3.4% for these 24 patients prior to ERA testing. The live birth rate prior to ERA testing was 1.1%.

Discussion

The ERA proved to be surprisingly cost effective in providing useful information in 70.8% of the patients tested and 81% of those with a result. The ERA results needed to be interpreted in the context of the transfer times previously used for these patients. Since the routine FET transfer time was about 144 hours in all prior FETs, three (14.3% with interpretable results) of the women had a peak receptivity appropriate with the timing of their prior transfers (at 144, 144 and 146 hours). These

women were not offered a change in the timing of their FET.

If the WOI was at least two days wide in all women, it would not make any difference if embryos were transferred at either 120 or 144 hours after starting progesterone for an FET cycle. One must reconcile the evidence showing that the WOI is four to six days wide [2-4] with the findings of this review suggesting that the WOI is less than 24 hours. A logical interpretation of this contradiction is to conclude that most women have a "wide" WOI, but a small subset have a "narrow" WOI. This is consistent with Ruiz-Alonso et al., noting that only 88% of good prognosis IVF patients have a receptive endometrium [8]. A subset of the 12%, whose endometrium is not receptive, may have a narrow WOI.

One interpretation of the ERA is that it specifies the optimal time for FET. It may identify the optimal time for those women with a narrow WOI, but since our program has a successful history of FET transfer at 144 hours with a clinical pregnancy rate 59.1% after FET, the WOI unlikely to be narrow in most patients. Yet, the findings of this presented case series, in which after multiple failed ART cycles, women experienced a 64.7% live birth rate and a 50% implantation rate, supports the idea that some women do have a narrow WOI. In our population of women with multiple failed FET cycles, the ERA appeared to be a useful tool in identifying those women.

Ethics Approval

This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of Baptist Hospital (Baptist Research Institute, Jacksonville, FL, USA) approved this study.

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