The incidence of aberrant endometrial $\alpha v\beta_3$ vitronectin expression in a high risk infertility population: could prolonged GnRH agonist therapy play a role?

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Abstract

Purpose Evaluate the incidence of aberrant endometrial integrin ($\alpha v\beta_3$ vitronectin) expression in patients at high risk for implantation defects.

Materials and methods Retrospective case-control trial of 74 consecutive infertile patients with prior failed IVF cycles despite good embryo quality and/or endometriosis who underwent endometrial biopsy 9–11 days after an LH surge to assess the presence or absence of $\alpha v\beta_3$ vitronectin. Patients were separated into two groups for analysis based on the presence (Gr. A) or absence (Gr. B) of integrin expression. A subset of Gr. B patients (86.1%) was treated with a 2 month course of a GnRH agonist prior to IVF (Gr. B1). No Gr. A patients were so treated.

Results Absent $\alpha v\beta_3$ vitronectin expression was noted in 48.6% of patients evaluated. A trend towards more severe endometriosis was noted in Gr. B (57.1 vs 31.5%). Responses to controlled ovarian hyperstimulation and IVF cycle outcomes including ongoing pregnancy rates were similar between Gr. B1 patients untreated Gr. A controls (55.6 vs 63.9%).

Conclusions A high incidence of absent endometrial $\alpha v\beta_3$ vitronectin expression is noted in patients at increased risk for implantation defects. Prolonged GnRH agonist therapy

prior to an IVF cycle resulted in outcomes similar to untreated controls with positive expression.

Keywords Integrin · Implantation · Endometriosis · In vitro fertilization · GnRH agonist

Previous investigators have suggested that endometrial expression of the integrin subtype $\alpha v\beta_3$ vitronectin and its receptor can be detected 6–10 days after ovulation, the presumed window of implantation [1, 2]. This protein has also been demonstrated on the surface of embryos [3]. It has been proposed that $\alpha v\beta_3$ vitronectin and its receptor may act not only as sites of interaction between the embryo and the endometrium, but may also play a role in initiating invasion of the trophoblast [4, 5].

Lessey et al. [6] have shown that aberrant endometrial $\alpha v\beta_3$ vitronectin expression with “in-phase” endometrial biopsies occurred in 39% of women with unexplained infertility. The majority of the patients were noted to have Stage I or II endometriosis. These same investigators demonstrated absent integrin expression in 44% of endometriosis patients [7]. This finding, however, has not been consistently demonstrated [8, 9].

Thomas et al. [10] evaluated a heterogeneous group of patients and reported a significantly increased incidence of aberrant expression of this integrin subtype in patients who had failed to conceive from IVF. Wolz-Lopez et al. [11] reported absent expression in 26.3% of infertile patients who were IVF candidates but did not correlate these results with cycle outcomes.

Return of $\alpha v\beta_3$ vitronectin expression with administration of a GnRH agonist (GnRHa) or danazol has been described in prospective but non-randomized trials [12, 13]. This may represent an explanation for the improved

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Capsule Absent endometrial integrin expression was commonly noted in selected infertility patients who, after prolonged GnRH agonist therapy experienced similar IVF outcomes as integrin positive untreated controls.

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Table 1 Results expressed as mean±SEM unless otherwise indicated (1)

Baseline clinical data										
Group	$\alpha v\beta_3^a$	Patients	Age	Day 3 FSH (mIU/ml)	Antral follicles	Prior failed cycles	Prior laparoscopy (%)	+Endometriosis (%)	Endometriosis stage (%)	
									I/II ^b	III/IV ^b
A	+	38 (51.4%)	35.5±0.6	7.4±0.3	13.6±1.7	1.4±0.2	57.9	39.5	68.5	31.5
B	–	36 (48.6%) ^c	34.7±0.9 ^c	7.1±0.9 ^c	13.8±1.2 ^c	1.5±0.2 ^c	63.9 ^c	52.8 ^c	42.9 ^c	57.1 ^c

^a $\alpha v\beta_3$: $\alpha v\beta_3$ vitronectin^b Percentage of patients with endometriosis who had undergone laparoscopy^c No significant differences vs Group A

pregnancy rates which have been reported in endometriosis patients administered a prolonged course of GnRHa prior to in vitro fertilization [14, 15].

The incidence of $\alpha v\beta_3$ vitronectin expression in IVF candidates specifically at high risk for implantation defects, such as those women with prior failed cycles despite adequate embryo quality and those with endometriosis, has not been evaluated. Similarly, the effect on IVF cycle outcomes of pre-cycle treatment of individuals with absent expression of this integrin subtype with a prolonged course of GnRHa has also not been specifically assessed.

The objective of the current investigation was to evaluate the incidence of aberrant endometrial $\alpha v\beta_3$ vitronectin expression in a targeted high risk population of patients with prior IVF failure and/or endometriosis. The IVF cycle outcomes of patients with absent integrin expression treated with a prolonged course of GnRHa was compared to those of untreated controls with normal expression.

Materials and methods

This investigation is a retrospective case-control trial of 74 consecutive IVF candidates considered to be at high risk for potential implantation defects. The study population consisted of those patients with prior failed IVF cycles despite

good embryo quality and/or a prior surgically documented diagnosis of endometriosis. All patients had evidence of normal early follicular phase serum ovarian reserve testing (day 3 FSH<10 mIU/ml; E₂<60 pg/ml) and bilateral antral follicle count >6. A normal clomiphene challenge test was documented in all women ≥38 years.

Endometrial biopsies were performed in all patients 9–11 days after an LH surge was documented by urine ovulation predictor kits. Tissue was evaluated for the presence or absence of $\alpha v\beta_3$ vitronectin by commercial assay (Adeza, Sunnyvale, CA) using previously described techniques [1]. All biopsy samples were confirmed by the same pathologist to be in phase ±2 days employing standard histologic criteria. All patients subsequently underwent controlled ovarian hyperstimulation employing either a standard GnRHa down-regulation or microdose flare protocol based on ovarian reserve testing and prior response. Indications for day 3 vs 5 embryo transfer were based on previously published protocols [16].

Patients were divided into two groups based on the presence (Gr. A) or absence (Gr.B) of $\alpha v\beta_3$ vitronectin expression. A subset of 31 (86.1%) Group B patients (Gr. B1) was treated with a 2 month course of a depot preparation of the GnRHa leuprolide acetate (TAP Pharmaceuticals, Waukegan, IL) 3.75 mg IM every 28 days immediately prior to initiation of controlled ovarian hyperstimulation (COH)

Table 2 Results expressed as mean±SEM unless otherwise indicated (2)

IVF cycle outcomes								
Group	Cycles	COH dose (units)	COH Duration (days)	MII oocytes	2PN (%)	No. of ET	OPR (%)	IR (%)
A	38	3,367.5±202.5	10.3±0.2	13.4±1.0	72.6±3.2	3±0.2	63.9	29.2
B1	31	3,495±247.5 ^a	10.0±0.3 ^a	10.1±5.3 ^a	69.9±2.9 ^a	2.8±0.2 ^a	55.6 ^d	28.0 ^d

COH Controlled ovarian hyperstimulation, OPR ongoing pregnancy rate, IR implantation rate

^a No significant differences vs Group A

for IVF. No Group A patients were so treated. The outcomes of the IVF cycle performed immediately following in these two groups was evaluated.

Data analyses were performed by Student's group *t* tests and chi-square analyses as appropriate. *P* values <0.05 were considered to be statistically significant. Ongoing pregnancy was defined as the visualization of an intrauterine gestational sac with fetal cardiac activity by ultrasound evaluation. Implantation rate was defined as the number of intrauterine gestational sacs with fetal cardiac activity visualized at ultrasound examination per number of embryos transferred.

Results

Baseline clinical data for Groups A and B are displayed in Table 1. Aberrant endometrial $\alpha v \beta_3$ vitronectin expression was noted in 48.6% of this population of women with endometriosis and/or prior failed IVF cycles. There were no significant differences between the groups with regards to age, serum or ultrasound markers of ovarian reserve and number of prior failed cycles. It is important to reiterate that patients with poor embryo quality in prior cycles were specifically excluded from this trial.

A trend towards a greater incidence of endometriosis in Group B patients did not achieve statistical significance. A higher incidence of more severe disease in those patients diagnosed with endometriosis who were integrin negative was also appreciated. However, the fact that a significant number of patients had not undergone laparoscopy may have lead to an underestimate of the incidence of this disorder in both groups.

Controlled ovarian hyperstimulation and IVF cycle outcomes are displayed in Table 2. There were no differences in any parameters between those patients with absent $\alpha v \beta_3$ vitronectin expression who were pretreated with a prolonged course of a GnRHa prior to IVF and those controls with normal expression who proceeded directly to IVF.

Conclusions

This investigation suggests that aberrant endometrial expression of the integrin subtype $\alpha v \beta_3$ vitronectin occurs with high frequency in patients with prior IVF failure despite good embryo quality and/or endometriosis suggesting an increased likelihood of implantation defects as one explanation for infertility in these specific groups. The incidence reported in the high risk groups evaluated in this trial is slightly higher than previously reported in infertile endometriosis patients and significantly higher than described in a general group of infertile women [7, 11].

The clinical value of the findings reported in this trial is directly associated with the options for correcting the abnormality and, by extension, of enhancing cycle outcomes. It is interesting to note that Thomas et al. [10] have reported that the incidence of positive luminal epithelial staining of endometrial $\alpha v \beta_3$ vitronectin was significantly more common in women who conceived from IVF ($P=0.027$). Evidence suggesting that the use of GnRH agonists may play a role in restoration of expression of this integrin subtype and that pre-IVF cycle treatment of endometriosis patients with this agent enhances cycle outcome may be linked [12, 14, 15]. The fact that Group B patients who were $\alpha v \beta_3$ integrin negative and underwent prolonged GnRHa therapy had similar IVF cycle outcomes as those Group A patients who were $\alpha v \beta_3$ integrin positive and did not receive the agonist is encouraging but in no way definitive. The data do not include the clearly needed control groups of biopsy negative patients not administered prolonged pre-cycle GnRHa and of biopsy positive patients who were administered prolonged GnRHa. To our knowledge, no prospective randomized trial has, to date, directly assessed the predictive value of endometrial $\alpha v \beta_3$ integrin expression as a marker for those who may benefit from this approach. Until the results of such a trial, which is currently ongoing in our center, are analyzed, the role of routine screening of these high risk individuals for endometrial $\alpha v \beta_3$ integrin expression will remain unclear.

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