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A prospective evaluation of the effect of salpingectomy on endometrial receptivity in cases of women with communicating hydrosalpinges

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BACKGROUND: We aimed to assess whether salpingectomy in women with communicating hydrosalpinges influenced endometrial receptivity. METHODS: The inclusion criteria were: women with communicating hydrosalpinges, absence of other confounding infertility factors and aged <40 years. Patients were scheduled for laparoscopy during the putative window of implantation (cycle days 19–21). In patients in whom salpingectomy was decided upon due to the severity of tubal disease (n = 10), an intra-operative endometrial biopsy was performed. Post-treatment endometrial sampling was done between day 19–21 of the fourth consecutive cycle. Pre-treatment and post-treatment samples were assessed by both conventional histologic criteria and $\alpha v\beta_3$ integrin immunostaining, where histological score (HSCORE) was used for quantification. RESULTS: Despite normal histological maturation assessed by conventional criteria, 8/10 hydrosalpinx cases yielded an epithelial HSCORE of <0.7, which was below the accepted threshold. Following salpingectomy, luminal endometrial epithelium demonstrated a significantly increased $\alpha v\beta_3$ integrin expression (Wilcoxon's signed rank test, P = 0.017). Although the mean HSCORE for glandular epithelia improved, it failed to reach statistical significance. Ultrasound visible hydrosalpinges (n = 5) and non-visible cases (n = 5) were also compared. However, neither the pre-treatment integrin expression, nor the postoperative improvement were significantly different between these groups. CONCLUSIONS: We conclude that the surgical treatment of communicating hydrosalpinges may improve endometrial receptivity as assessed by $\alpha v\beta_3$ integrin expression. Women with hydrosalpinges may undergo endometrial evaluation by the molecular markers of implantation, such as $\alpha v\beta_3$ integrin. This evaluation may be decisive in determining the optimal management of cases, and may also be used to assess the efficacy of the treatment. The expression of the implantation markers should be correlated with implantation and clinical pregnancy rates in IVF–embryo transfer programs.

Keywords: $\alpha v\beta_3$ integrin/endometrial receptivity/hydrosalpinx/salpingectomy

Introduction

Tubal disease is one of the major causes of female infertility (Westrom, 1980). IVF–embryo transfer was initially developed as a method to overcome tubal infertility (Edwards *et al*., 1984). Recent IVF–embryo transfer studies reported low pregnancy and implantation rates in the presence of tubal disease accompanied by hydrosalpinx (Andersen *et al*., 1994; Kassabji *et al*., 1994; Strandell *et al*., 1994; Katz *et al*., 1996). These studies were complemented by reports demonstrating increased implantation rates and decreased miscarriage rates after surgical extirpation, neosalpingostomy or proximal ligation of hydrosalpinges (Vandromme *et al*., 1995; Levy *et al*., 1996). Recently, a prospective, randomized multicentre trial demonstrated the benefit of surgical treatment of hydrosalpinges prior to IVF (Strandell *et al*., 1999).

A direct embryotoxic effect of hydrosalpinx fluid (Mukherjee *et al*., 1996), physical interference due to the intrauterine accumulation of refluxed fluid (Mansour *et al*., 1991), or altered endometrial receptivity (Lessey *et al*., 1994b) have been among the proposed mechanisms by which a hydrosalpinx exerts an adverse influence on development and/or implantation of embryos.

Lessey *et al*. and Tabibzadeh evaluated the expression of integrin molecules in human endometrium throughout the menstrual cycle (Lessey *et al*., 1992, 1994a; Tabibzadeh, 1992). The vitronectin receptor, $\alpha v\beta_3$, was found to appear abruptly on cycle day 19 or 20, coincident with the opening of the putative window of implantation. The expression of $\alpha v\beta_3$ was absent in endometrial biopsies with maturational delay. Hence, $\alpha v\beta_3$ integrin appeared to be a consistent internal

marker of luteal phase maturation and receptive endometrium (Lessey *et al*., 1992, 1994a). Lessey *et al*. also documented the presence of an endometrial dysfunction in patients with hydrosalpinges by means of histological delay and lower integrin expression (Lessey *et al*., 1994b).

This prospective study was undertaken to assess whether surgical treatment of communicating hydrosalpinges resulted in a significant change in endometrial maturation and $\alpha v\beta_3$ integrin expression.

Materials and Methods

During a 2 year period, infertile women with unilateral or bilateral communicating hydrosalpinges, detected by hysterosalpingography, were evaluated for possible inclusion. Among these subjects, women <40 years of age and those having regular menstrual cycles with no ovulatory dysfunction (mid-luteal progesterone >10 ng/ml; conversion factor to SI units, 3.18) were included. Male factor was excluded by the presence of normal semen parameters according to the World Health Organization definition (World Health Organization, 1992) (sperm count $\geqslant 20\times10^6$/ml, progressive motility ⩾40%, normal sperm morphology ⩾40%). The study was approved by the Institutional Review Board of Hacettepe University School of Medicine.

After all the patients were informed and written consent obtained, they were assigned to laparoscopy scheduled during the putative window of implantation (cycle day 19–21). Following laparoscopic confirmation of hydrosalpinges and associated severe tubal disease, salpingectomy and endometrial sampling were performed in 10 patients. These women were subjected to post-treatment endometrial sampling on the corresponding days of the fourth consecutive cycle. The Pipelle device (Laboratoire C.C.D., Paris, France) was used for all biopsies. Transvaginal ultrasounds performed prior to surgery revealed visible hydrosalpinges in 5/10 women.

The endometrial samples were transported on ice and snap frozen in liquid nitrogen to be maintained at –70°C until cryo-sectioning. Serial cryo-sections 5 μm thick were made on poly-L-lysine-coated slides. These slides were then fixed in +4°C acetone for 10 min, and stained using immunofluorescent techniques. One slide for each case was also stained with haemotoxylin and eosin for endometrial histological dating, according to the criteria of Noyes *et al*. (Noyes *et al.*, 1950).

Immunofluorescent staining

An indirect immunofluorescent technique was employed. The primary antibody, $\alpha v\beta_3$ mouse monoclonal IgG_1 (Santa Cruz Biotechnology, sc-7312, California, USA), was placed on cryosections after blocking with 1% bovine serum albumin in phosphate buffered saline (PBS) and incubated at room temperature for 1 h in a dark chamber. A PBS (pH = 7.2–7.4) rinse was followed by incubation with the secondary antibody (anti-mouse IgG-FITC, Santa Cruz Biotechnology, sc-2010) for 45 min at room temperature in a dark chamber. Subsequently, the samples were washed in PBS and mounted (Immu-mount, Shandon Inc, Pittsburgh, PA, USA). The staining was evaluated under a Nikon microscope by a single blinded observer. Photomicrographs were made using Kodak 400 ASA film.

An intestinal biopsy specimen known to express luminal and glandular epithelial $\alpha v\beta_3$ was used as a positive control slide. As a negative control slide, a normal non-immune serum was used instead of the primary antibody.

Immunostaining was evaluated by the histological score (HSCORE). HSCORE was calculated using the following equation: HSCORE = Σ Pi (i + 1); where i is the intensity of staining with a value of 1, 2 or 3 (weak, moderate or strong respectively), Pi is the percentage of stained epithelial cells for each intensity varying from 0 to 100%, and 1 is a correction for optical density. This yielded a range of results from 0 for no staining to 4 for maximal staining. Luminal and glandular epithelial HSCORE were assessed separately for a detailed evaluation of the endometrium. The HSCORE has been shown to yield low inter- and intra-observer variation (Budwit-Navotny *et al*., 1986). Based on a previous receiver operator characteristic curve analysis (Lessey *et al*., 1994c), HSCORE >0.7 was used as the threshold of a positive test for the evaluation of epithelial $\alpha v\beta_3$ immunostaining.

Table I. Demographic characteristics of cases

Before salpingectomy	After salpingectomy
Number of cases	10
Age (years; mean ± SD)	30.1 ± 4.0
Infertility duration (years; mean ± SD)	6.7 ± 3.6
Basal FSH (mIU/ml; mean ± SD)	5.4 ± 2.1
Previous pelvic surgery	6
Episode of pelvic inflammatory disease	2
Previous IVF failure	4
Bilateral hydrosalpinges	6
Unilateral hydrosalpinx	4
Ultrasound visible hydrosalpinges	5

Statistical evaluation

Wilcoxon's signed rank test was used for the comparison of both luminal and glandular endometrial HSCORE of the pre- and post-treatment samples. The pre- and post-treatment HSCORE of ultrasound visible and non-visible hydrosalpinges were separately compared by the Mann–Whitney *U*-test. $P < 0.05$ was considered as statistically significant.

Results

The demographic characteristics of the cases are shown in Table I.

Histopathological evaluation of all salpingectomy specimens confirmed chronic salpingitis with distal tubal occlusion and hydrosalpinx, irrespective of ultrasound visibility.

The conventional histological dating of endometrial biopsies was in concordance with the chronologic dating in all cases. Hence, no case of out-of-phase endometrium in either pre- or post-treatment samples was observed.

The immunofluorescent staining for $\alpha v\beta_3$ integrin was observed on both the luminal and glandular epithelium. Diffuse cytoplasmic and membranous staining patterns were noticed. There was no $\alpha v\beta_3$ expression in the mesenchyme. Occasionally, vascular structures showed linear fluorescence.

The pre-treatment endometrial samples exhibited an average of 25% luminal epithelial $\alpha v\beta_3$ staining with a median staining intensity of 1. The mean HSCORE for the luminal epithelium was 0.8. Eight out of 10 cases revealed an HSCORE <0.7, which was below the accepted cut-off for positive staining. The post-treatment samples revealed an average of 75% luminal epithelial immunostaining with a median staining intensity of 2. The corresponding mean HSCORE was 2.1. Nine out of 10 cases resulted in an improvement of HSCORE

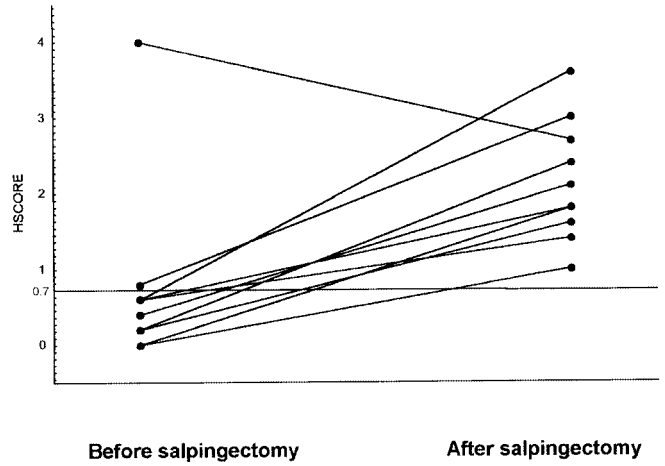

Figure 1. Scattergram of the luminal epithelial HSCORE before and after salpingectomy. HSCORE ⩾7 indicates positive $\alpha v\beta_3$ immunostaining. $P = 0.017$, before versus after salpingectomy.

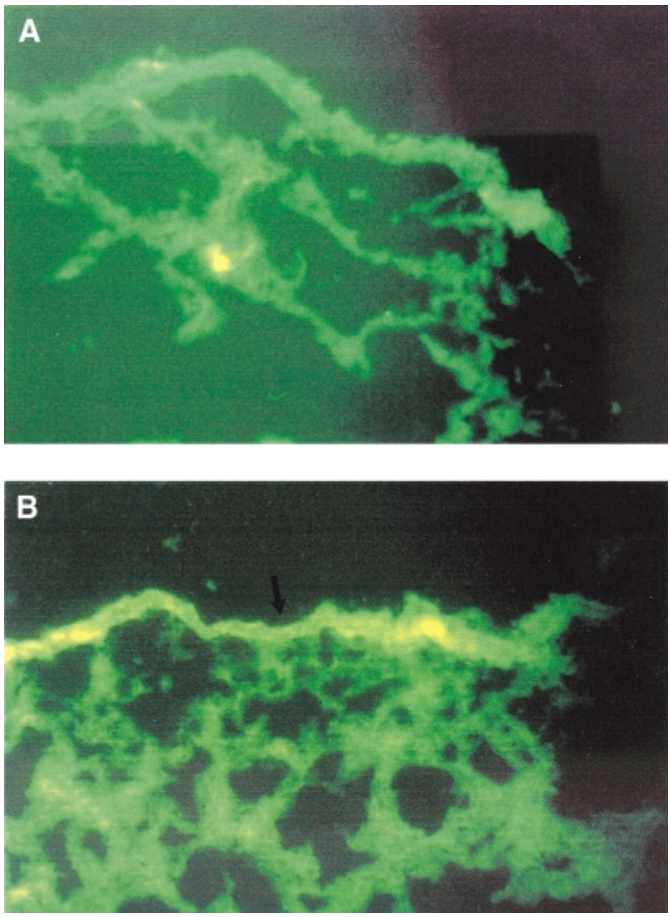

Figure 2. Photomicrographs of the immunofluorescent staining for the integrin $\alpha v\beta_3$ in endometrial samples. The staining in the luminal epithelium was weak prior to salpingectomy (**A**) but quite pronounced (arrow) following salpingectomy (**B**). Original magnification x200.

($P = 0.017$) (Figure 1). Finally, all of the post-treatment biopsies yielded a luminal epithelial HSCORE >0.7. Figure 2A illustrates weak luminal epithelial $\alpha v\beta_3$ immunostaining prior to salpingectomy, and Figure 2B shows increased staining after the treatment.

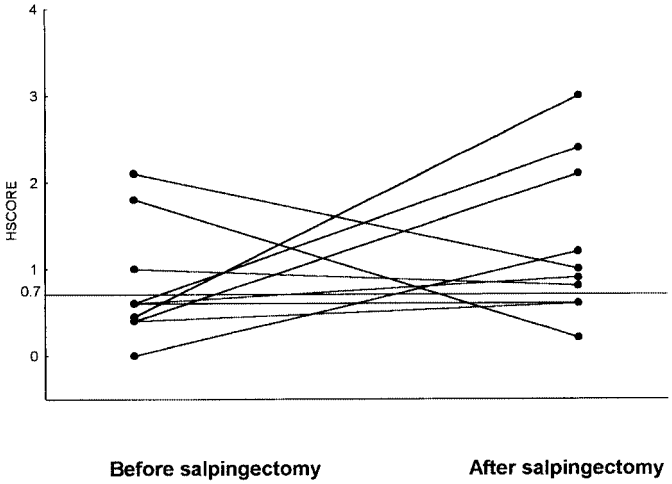

Figure 3. Scattergram of the glandular epithelial HSCORE before and after salpingectomy. HSCORE ⩾7 indicates positive $\alpha v\beta_3$ immunostaining. Before versus after salpingectomy, P is non-significant.

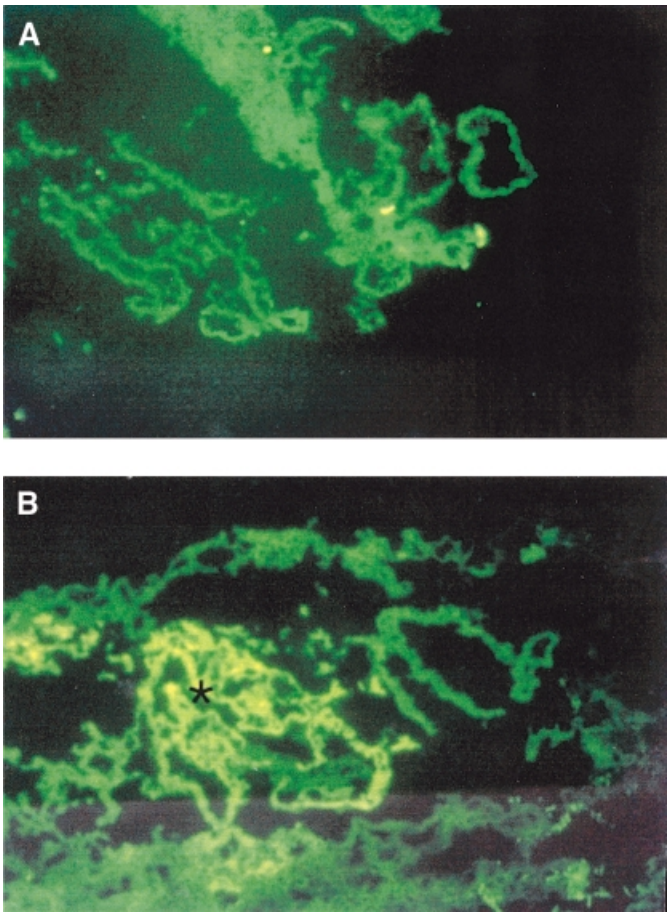

Figure 4. Immunofluorescent staining of $\alpha v\beta_3$ in glandular endometrial epithelium. The staining was largely absent in a patient with hydrosalpinx (**A**) compared with increased immunofluorescence (asterisk) following salpingectomy (**B**). Original magnification x100.

The pre-treatment endometrial samples yielded an average of 30% glandular epithelial staining and a mean HSCORE of 0.8. Corresponding figures for post-treatment samples

were 47% and 1.3 respectively (not significant). Prior to salpingectomy, glandular epithelial HSCORE was <0.7 in 7/10 cases, whereas 7/10 cases resulted in an HSCORE above the cut-off following salpingectomy (Figure 3). Figure 4A demonstrates a pre-treatment glandular epithelium lacking $\alpha v\beta_3$ immunostaining and Figure 4B demonstrates post-treatment enhancement of glandular immunofluorescence.

The pre-treatment HSCORE was comparable among subjects with ultrasound visible and non-visible hydrosalpinges (not significant). Of the five patients with ultrasound visible hydrosalpinges, four resulted in an HSCORE below the cut-off, and of the five patients with hydrosalpinges that were not seen on ultrasound, four also resulted in an HSCORE <0.7.

All post-treatment endometrial biopsies of women with ultrasound visible hydrosalpinges ($n = 5$) resulted in an improved HSCORE. All but one post-treatment endometrial biopsy in ultrasound non-visible subjects ($n = 5$) also revealed a higher HSCORE. The post-treatment HSCORE for each group did not significantly differ from one another.

Discussion

Our data further support the effect of hydrosalpinx and its surgical treatment on the endometrial receptivity marker, $\alpha v\beta_3$ integrin. Interestingly, conventional histological evaluation of the endometrium did not reveal any maturational delay. However, the impaired endometrial epithelial $\alpha v\beta_3$ immunofluorescent staining was followed by its improvement after salpingectomy. This finding may suggest the presence of an unknown endometrial defect in patients with communicating hydrosalpinges. Unlike our series, Meyer *et al*. reported a combination of histological delay and aberrant integrin expression in women with hydrosalpinges (Meyer *et al*., 1997).

Following salpingectomy, endometrial luminal epithelial $\alpha v\beta_3$ expression was significantly increased. Although an improvement was observed in the post-treatment glandular integrin expression, it did not reach statistical significance. The limited number of cases and a slower glandular response, which was not completed after a 3-cycle interval, might be possible explanations.

We decided to perform our post-treatment endometrial biopsies somewhat arbitrarily, in the fourth sequential cycle. This policy was employed according to some conventional knowledge. The normalization of endometrium has been accepted to take place following three cycles of continued treatment in certain disorders, such as dysfunctional uterine bleeding (Speroff, 1999). Likewise, a repeat biopsy has been recommended following 3 months of medical therapy in cases of endometrial hyperplasia (DiSaia, 1997). Ideally, the progressive change would have been shown by sequential monthly biopsies, but this was not possible due to ethical concerns. Hence, the timing of optimal improvement in endometrial receptivity markers following salpingectomy remains undetermined.

One of the challenges in the management of the hydrosalpinx is to determine who would benefit from salpingectomy. Vasquez *et al*. suggested salpingectomy in thick-walled hydrosalpinges with wall fibrosis and hydrosalpinges with mucosal adhesions (Vasquez *et al*., 1995). Puttemans *et al*. supported the use of tubal endoscopy in the decision-making process (Puttemans *et al*., 2000). According to several authors, only the ultrasound visible hydrosalpinges would be responsible for the impaired implantation and pregnancy rates (Andersen *et al*., 1994; de Witt *et al*., 1998). The findings of some studies (Meyer *et al*., 1997), including this one, linked the possibly impaired endometrial receptivity by means of reduced expression of the $\alpha v\beta_3$ integrin to the presence of hydrosalpinx. Therefore, the evaluation of endometrial $\alpha v\beta_3$ integrin expression for the selection of salpingectomy candidates might be a promising alternative.

Since the transvaginal ultrasound was routinely employed in the pre-treatment evaluation of our patients, we were able to compare the influence of ultrasound visible and non-visible hydrosalpinges on endometrial integrin expression. Neither pre-treatment nor post-treatment integrin expression was different among cases with and without ultrasound visible hydrosalpinges. However, due to the limited number of cases in each group, the power is inadequate to reach strong conclusions as to whether ultrasound visibility of hydrosalpinx affects endometrial receptivity or not.

Ultrasound evaluation of hydrosalpinges has several potential limitations. A false negative interpretation is possible due to intermittent intrauterine drainage and on the other hand, an unrecognized hydrosalpinx may be stimulated during ovulation induction (Schiller and Tsuchiyama, 1995). Sonographic evaluation may not provide substantial information regarding endometrial receptivity and a possible toxicity of hydrosalpinx fluid, the latter of which is in fact a dilemma.

Mukherjee *et al*. reported embryotoxic effects of hydrosalpinx fluid on murine embryogenesis and suggested prophylactic salpingectomy (Mukherjee *et al*. 1996). However, several subsequent studies in mouse models failed to show obvious embryotoxicity (Beyler *et al*., 1997; Murray *et al*., 1997; Koong *et al*., 1998; Spandorfer *et al*., 1999). In addition, hydrosalpinx fluid was not found to exertjyany major negative effects on in-vitro development of human embryos (Granot *et al*., 1998; Strandell *et al*., 1998).

We conclude that the surgical treatment of communicating hydrosalpinges improves endometrial receptivity as assessed by immunofluorescent $\alpha v\beta_3$ integrin staining. The initial impaired integrin expression and its improvement following salpingectomy is independent of the conventional histological dating of the endometrium. Therefore, women with communicating hydrosalpinges may undergo endometrial evaluation with the help of molecular markers of implantation, such as $\alpha v\beta_3$ integrin. This evaluation may be both decisive in determining the optimal management of cases, and used to assess the efficacy of the treatment. Finally, the expression of implantation markers should be correlated with implantation and clinical pregnancy rates in IVF–embryo transfer programs.

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