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# Correlation Between Integrin Alpha-v Beta-3 Expression, Endometrial Thickness and Subendometrial Blood Flow Resistance Index in Unexplained Infertility: A Case Control Study

**Dhai Abdulalazize Rashid[1*], Fadia Jassim Alizzi[2]**

1. *Department of Obstetrics and Gynecology, College of Medicine, Al-Anbar University, Ramadi, Iraq*
2. *Department of Obstetrics and Gynecology, College of Medicine, Al-Mustansiriyah University, Baghdad, Iraq*

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**Corresponding Information:**
**Dhai Abdulalazize Rashid,**
Department of Obstetrics and Gynecology, College of Medicine, Al-Anbar University, Ramadi, Iraq

**Email:** jchr.editor@gmail.com

**ABSTRACT**

**Background & Objective:** Infertility that cannot be explained by anovulation, poor sperm quality, tubal disease, or any other recognized cause of infertility is referred to as unexplained infertility. The aim of this study is to assess the correlation between integrin alpha-v beta-3 expression, endometrial thickness and subendometrial blood flow resistance index in unexplained infertility.

**Materials & Methods:** The study carried out on Kamal – Al-Samurai fertility center in the period from October 2020 - September 2021. Six days after detecting urinary Luteinizing Hormone (LH) surge, all the women were asked to come to do two-dimensional (2D) transvaginal ultrasound to assess endometrial thickness and subendometrial blood flow color Doppler resistance index and endometrial samples was taken and examined immunohistochemically to detect alpha-v beta-3 integrin. The cut-off value of integrin and subendometrial blood flow resistance index, sensitivity and specificity were calculated by applying Receiver Operative Characteristics (ROC) curve.

**Results:** The endometrial thickness was lower and resistance index was higher in case group ($P < 0.001$). The expression of integrin alpha-v beta-3 in infertile group was significantly low ($P < 0.001$) with significant positive correlation between integrin score and endometrial thickness (r= -0.708 & $P < 0.001$) and significant negative correlation between integrin score and subendometrial resistance index (r= -0.786 & $P < 0.001$).

**Conclusion:** Alpha-v beta-3 integrin expressions and endometrial thickness are reduced significantly in mid-luteal phase while the subendometrial blood flow color Doppler resistant index significantly increased, and using them together can possibly be used as a diagnostic predictor of unexplained infertility.

**Keywords:** Integrin αvβ3, Endometrial thickness, Subendometrial blood flow, Resistance index, Unexplained infertility



## Introduction

Infertility that cannot be explained by anovulation, poor sperm quality, tubal disease, or any other recognized cause of infertility is referred to as unexplained infertility. This condition is characterized as the absence of conception despite 12 months of unprotected intercourse **(1)**. Both intrauterine insemination and in vitro fertilization are considered to be the most successful therapies for infertility that cannot be explained medically. Due to the fact that these therapies do not focus on a particular mechanism, but rather raise the likelihood of conception in comparison to the chances of natural conception, it is imperative that their efficacy be evaluated in light of the likelihood of natural conception **(2)**. The utilization of prognostic models might make it easier to determine who would benefit from therapy and who could safely postpone treatment for a period of six months in order to pursue natural conception. This is significant because some treatments can cause adverse effects in both the mother and her child, and the high expense of some treatments can put patients' access to care at risk **(3-5)**. Generally, thirty percent of infertile couples are diagnosed with unexplained infertility where no identifiable causes are seen for the couple to become pregnant for twelve months in spite of regular sexual intercourse and normal standard evaluations **(6, 7)**. The endometrial receptivity is controlled by biomarkers, hormones, growth factors, and cytokines interaction **(8)**, disorders in these biomarkers will affect endometrial receptivity and this may be correlated with unexplained infertility **(9-11)**. Integrins are adhesion molecules that are regulated by cytokines, and they

moderate the embryonic attachment and implantation. Integrin alpha-v beta-3 is expressed in receptive phase of the endometrium **(12, 13)** and this expression may possibly be used as indicator of this receptive phase **(14)**. Many researchers had assessed integrin expression predictive value in unexplained infertility, but ended up with inconclusive results. Some studies revealed under-expression of Integrin alpha-v beta-3 (αvβ3 integrin) **(15-17)**, while others showed no differences **(18-20)**. On the other hand, many researchers assessed the receptivity of the endometrium using 2D & 3D Trans-Vaginal Ultrasound (TVU) with Doppler, and ended up in a conclusion that endometrial factors including endometrial thickness (ET), pattern, uterine artery, and subendometrial blood flow affect the endometrial receptivity in unexplained infertility **(21-24)**. The objective of this study was to assess the correlation between Integrin alphaVbeta3 expressions, ET and subendometrial blood flow resistance index in unexplained infertility.

## Methods

This case control study was conducted in Kamal – Al-Samurai fertility center/ Baghdad in the period between October 2020 to September 2021 after taking the ethical approval from Ethics Research Committee from the Faculty of Medicine.Written consent had been taken from all participants after explaining the purposes of this study and method of work. This case control study included ninety women enrolled in two groups, group one was forty women who previously diagnosed with unexplained infertility and considered as a case group and the second group was parous women who attended the clinic of family planning asking for contraception. Forty women agreed to participate in the study after clear explanation of the purposes of the study and considered as a control group. Other 29 women of both groups were missed from the study because they withdrew their consent or disappeared from the observation. Unexplained infertility was diagnosed when the partner's seminal fluid analysis was normal, and the woman had ovulatory cycle with serum progesterone level ≥ 3mg/ml at mid-luteum, and when the uterine cavity and tubal patency was confirmed by sonography, hysterosalpingogram, hysteroscopy and or laparoscopy **(25, 26)**. All participants were supplied by urinary LH Kit and asked to use urinary LH kit daily from day 11 of their menstrual cycle till LH surge was detected indicating ovulation, and then the women were asked to come to the center 6 days after. Transvaginal ultrasound with color and pulsed Doppler (Mindray, model: DC 30, Mindray Medical International company, China) done for all women to assess ET and to measure subendometrial blood flow resistance index (RI). Endometrial samples were taken simultaneously using the Pipelle biopsy, fixed in 10% formalin-acetic acid and kept in a patient labeled container in deep freeze at -4 °C. At the time of examination, the pathologist embeds the endometrial sample in paraffin and cut it into 4 µm sections, two of them stained with hematoxylin- eosin (H&E) and sent for histopathology to correlate the date of biopsy with secretory changes of endometrium and roll out any endometrial pathology, and another two sections had been cut on positively charged slides for immunohistochemistry study using IgG rabbit monoclonal antibody against Human integrin αvβ3. Integrin αvβ3 integrin expression in endometrial sample was graded and calculated according to an HSCORE-score (H-score) which depended on two things: staining intensity and percentage of cells staining at each intensity. When the cell cytoplasm and membrane stain brownish, it means that the cell expresses integrin and gives positive results. The intensity of this staining was scored using a four-point semi-quantitative scoring system (0–3) and as follows: (−) = no staining, (+) weak or focal staining, (++) moderate staining and (+++) strong staining. The percentage of cells stained at each intensity scored as follows: (−) = 0–5%, (+) = 5–25%, (++) = 25–50%, (+++) = > 50%. Statistical analysis analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft Office 2007, USA. Statistical parameters including frequency, range, mean and standard deviation were measured to describe the data. The groups were compared by applying independent sample t-test and chi square. The degree of association between continuous variables was calculated by Pearson's correlation coefficient (r). The cut-off value, sensitivity and specificity were calculated by applying Receiver operative characteristics (ROC) curve and the results were considered statistically significant when *p* value was less than 0.05.

## Results

Eighty females were enrolled in this case control study; forty women with unexplained infertility (Case group) vs. forty fertile females (Control group). The comparisons of patients mean age and mean body mass index in both groups as illustrated in Table 1 showed no statistically difference. Type and duration of infertility in case group are shown in Table 1 as well.

**Table 1. Clinical and demographic features comparison between the two study groups**

| Parameters | | Case group | Control group | *p* value |
|---|---|---|---|---|
| **Age (years)** | Mean ± SD | 28.30 ± 4.12 | 29.65 ± 3.95 | 0.513 |
| | Range | 18 - 34 | 22 – 36 | |

| Parameters | | Case group | Control group | *p* value |
|---|---|---|---|---|
| **BMI (Kg/m²)** | Mean ± SD | 27.13 ± 4.32 | 26.87 ± 5.34 | 0.362 |
| | Range | 23 - 35 | 21 – 33 | |
| **Duration of infertility (years)** | Mean ± SD | 4.00 ± 1.26 | -------- | ------- |
| | Range | 2 - 6 | -------- | |
| **Type of infertility** | Primary | 26 | -------- | ------- |
| | Secondary | 14 | -------- | |

There was significantly lower endometrial thickness and higher resistance index in unexplained infertility patients ($p < 0.001$) as seen in Table 2.

**Table 2. Comparison of endometrial thickness and resistance index between the two groups**

| Parameter | Case group | Control group | *p* value |
|---|---|---|---|
| **Endometrial thickness (mm) (Mean ± SD)** | 5.76 ± 1.12 | 11.13 ± 1.35 | < 0.001* |
| **Resistance index (Mean ± SD)** | 0.68 ± 0.06 | 0.53 ± 0.04 | < 0.001* |

***: *P.* value < 0.05 (significant)**

Integrin αvβ3 expression according to an HSCORE-score (H-score) ranged in unexplained infertility and control groups 0-2 and 1-3, respectively with higher scores mean in control group (1.80 versus 0.43). The integrin scores were significantly different between the two groups with *P.*value less than 0.001 as seen in Table 3.

**Table 3. Comparison of integrin score between the study groups**

| Parameter | Statistics | Unexplained infertility group | Control group | *P* value |
|---|---|---|---|---|
| **Integrin score** | Mean ± SD | 0.43 ± 0.55 | 1.80 ± 0.56 | < 0.001* |
| | Range | 0 – 2 | 1 – 3 | |
| **Integrin score n. (%)** | 0 | 24 (60%) | 0 | < 0.001* |
| | 1 | 15 (37.5%) | 11 (27.5%) | |
| | 2 | 1 (2.5%) | 26 (65%) | |
| | 3 | 0 | 3 (7.5%) | |

***: *P.*value < 0.05 (significant)**

There was a positive significant correlation between integrin score and ET (r=-0.708 & $p<0.001$), on the contrary there was significant negative correlation between integrin score and subendometrial blood flow resistance index (r=-0.786 & $P<0.001$). There was also a negative correlation between ET and resistance index (r=-0.802 & $P<0.001$) as presented in Table 4.

**Table 4. Correlation between integrin score with endometrial thickness and resistance index in unexplained infertility and control groups**

| Parameters | | Endometrial thickness | Resistance index |
|---|---|---|---|
| **Integrin Score** | R | 0.708 | -0.786 |
| | *p* value | < 0.001 | < 0.001 |
| **Endometrial thickness** | R | 1 | -0.802 |
| | *p* value | < 0.001 | < 0.001 |

**r: Pearson's correlation coefficient; *: p value < 0.05 (significant)**

ROC curve was applied to calculate integrin score, endometrial thickness and resistance index cut-off values as a diagnostic predictor of unexplained infertility. According to the results the best cut-off value of integrin score was 0 with sensitivity=60%, specificity=100 % and area under curve =0.933.

Endometrial thickness ≤ 7.2 mm (sensitivity=92.5%, specificity=95% & area under curve= 0.992) and for the resistance index was ≥ 0.62, sensitivity=85 %, specificity=95 % and area under curve =0. 965.

The positive predictive values were 100%, 94.9% and 94.4% respectively while the negative predictive values were 71.4%, 92.7% and 86.4% respectively. The P value was high ($P$ <0.001) meaning that the three parameters cut-off values had significant ability to discriminate between unexplained infertility and control groups as seen in Table 5.

**Table 5. Receiver operative characteristics of integrin score, endometrial thickness and resistance index as a predictor of unexplained infertility**

| Characteristics | Integrin score | Endometrial thickness | Resistance index |
|---|---|---|---|
| **Cut off value** | 0 | ≤ 7.2 mm | ≥ 0.62 |
| **Sensitivity** | 60% | 92.5% | 85% |
| **Specificity** | 100% | 95% | 95% |
| **Accuracy** | 80% | 93.5 | 90% |
| **Positive predictive value** | 100% | 94.9% | 94.4 |
| **Negative predictive value** | 71.4% | 92.7 | 86.4 |
| **Area under curve (AUC)** | 0.933 | 0.992 | 0.965 |
| ***p* value** | *< 0.001** | *< 0.001** | *< 0.001** |

***: p value < 0.05 (significant)**

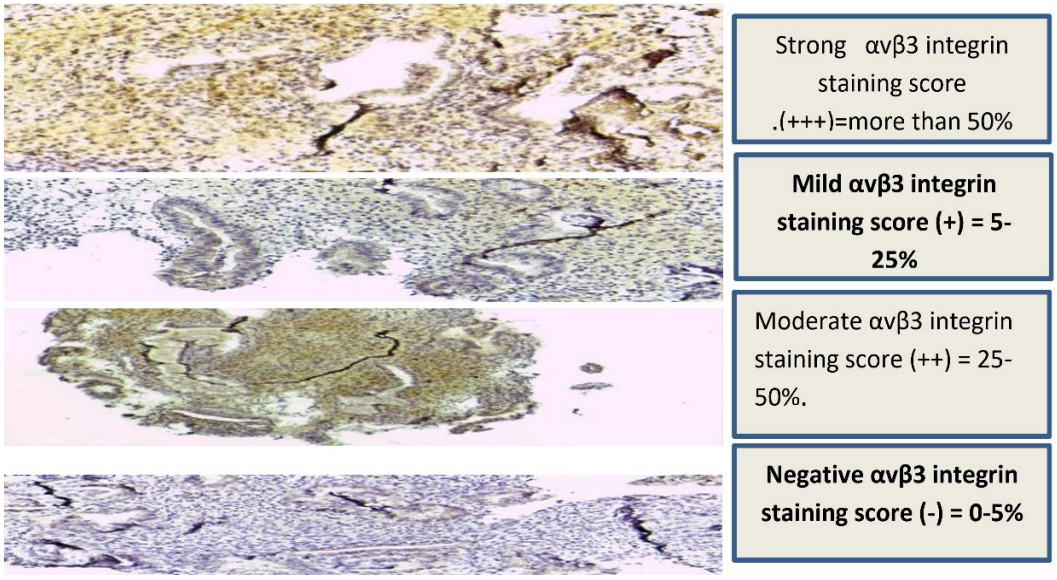


**Figure 1. Integrin αvβ3 expression according to an H-score**

## Discussion

In the current study, endometrial samples were obtained six days after LH surge and αv β3 integrin expression was assessed and quantified using H-scoring system. There was significantly less expression of αvβ3 integrin among unexplained infertile woman with a median score of zero and score one for control groups, with 60% sensitivity and 100% specificity when zero score was used as a cut-off value for the infertile group. These results go with Elnaggar et al. results that showed low expression of αv β3 integrin in unexplained infertile women with 74.3% sensitivity and 100% sensitivity as well **(12)**. Dorostghoal et al. studied the β3 integrin, calcitonin and plexin-B1 expression in the endometrium at the period of implantation in unexplained infertile woman and ended up in a conclusion that low β3 integrin and calcitonin expression separately or in combinations might be considered as potential markers of unexplained

infertility **(13)**. Wang et al. study used ultrasonic parameters and biomarkers to assess the receptivity of the endometrium during the period of implantation in women with unexplained infertility and the study showed that all the parameters declined in the study group and that Integrin αv β3was the best biomarkers to assess endometrial receptivity with 96.7% sensitivity and 89.5% **(14)**. Ceydeli et al. studied the αvβ3 integrin expression at different sites of the endometrium and it showed that this expression was not different in unexplained infertile and fertile women but the stromal αv β3 integrin was founded to be expressed significantly less than luminal & glandular αvβ3 integrin **(15)**. He et al. did a study on osteopontin and Integrin αvβ3 expression during the period of implantation in IVF cycle with high serum progesterone and oestradiol level and showed that the clinical value of assessing endometrial receptivity with osteopontin and integrin αvβ3 seems to be uncertain, but the comparison was between the infertile with different serum level of progesterone and estrogen **(16)**; however, Casals et al. study on osteopontin and alpha-v beta-3 integrin expression in the endometrium of infertile and fertile women showed no statistically significant difference between the two groups **(17)**, which differ from the current study results and this difference might be due to small sample size or using different scoring system for integrin expression. Regarding the ET, the current study showed that women in case group had lower E with a median of 5.76 ± 1.12 versus 11.13 ± 1.35 for fertile group. The cut-off value of endometrial thickness was ≤ 7.2 mm (sensitivity = 92.5%, specificity = 95% & area under curve = 0.992). This result was in agreement with Elnaggar et al. that showed the median endometrial thickness was 5 mm in infertile group versus 11 mm for fertile group with cut-off value of 7 mm and below for case group with 82.9% sensitivity and 97.1% specificity **(12)**. El-Zenneni et al. study showed that the ET was significantly less in women with unexplained infertility and with cut-off 9 mm and below would provide 74% sensitivity and specificity **(18)**. The assessment of subendometrial blood flow 2D color Doppler resistance index shows reduced blood flow in infertile group with elevated resistant index and the best cut-off value in unexplained infertility was ≥ 0.62 with sensitivity 85 %, specificity 95 % and area under curve = 0.965. El-Mazny et al. study using 3D–TVU showed Peri-implantation endometrial perfusion is impaired in women with unexplained infertility **(19-21)**. The current study showed that by using the three parameters together, alpha-v beta-3 integrin, endometrial thickness and subendometrial blood flow resistance index, they had significant ability to discriminate between unexplained infertility and control groups, and can better assess the endometrium in period of implantation and identify the woman at risk of failed implantation or miscarriage, but further studies with large sample size is recommended.

## Conclusion

Alpha-v beta-3 integrin and endometrial thickness are significantly low in unexplained infertility women while the subendometrial blood flow resistant index significantly increased, and using them together can possibly be used as a diagnostic predictor of unexplained infertility. To investigate the usefulness of utilizing these methods in clinical practice, we need to conduct larger studies with sufficient statistical power. Ideally, these studies should investigate more than one menstrual cycle in the same woman. This is done in order to get around the cyclical changes that occur between various menstrual cycles.

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## Conflict of Interest

The authors declare no conflict of interest.

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