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# The correlation between laparoscopic staging of endometriosis and nerve fiber density in eutopic endometrium: A prospective Observational Study

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## **Abstract**

**Objective:** Recent studies proved higher density of small nerve fibers in eutopic endometrium in endometriotic females which can be used for diagnosis of endometriosis. This study investigate this finding in a sample of Egyptian females and also asses if there is difference in the density of small nerve fibers in eutopic endometrium in different stages of endometriosis diagnosed by laparoscopy.

**Patients and Methods:** A prospective observational study comprised of Ninety-seven endometrial biopsies taken from women who underwent laparoscopy for infertility and/or pelvic pain. Forty seven biopsies were from non endometriotic women, mean -while fifty were from endometriotic ones. These biopsies stained with immunohistochemical stain using protein gene product 9.5 (PGP9.5) as a marker for nerve fibers.

**Results:** Nerve fiber density in eutopic endometrium was significantly higher in endometriotic group than non-endometriotic group ( $9.70 \pm 2.29$  vs.  $1.53 \pm 0.78$ , p value 0.001). In the endometriotic group nerve fiber density significantly differs in different laparoscopic stages (F15.611) ( $p < 0.001$ ).

**Conclusions:** Detection of nerve fiber density through endometrial biopsy can be used as a semi-invasive method for diagnosis of endometriosis and may be helpful for staging.

**Keywords:** endometriosis diagnosis, immunohistochemistry, endometrial biopsy, nerve fibers.

## **Introduction**

Endometriosis is defined by the ectopic presence of endometrial glands and stroma [1]. It affects about one third of infertile women as well as those suffering from chronic pelvic pain [2,3]. It occurs mostly in women aged 20-50 years and mostly encountered inside the pelvis [4]. Laparoscopy still the gold standard for the diagnosis and considered ideal when combined with histo-pathological confirmation [5, 6]. There is deficiency of non-invasive diagnostic methods for diagnosis of endometriosis and this leads to a period of lag between symptoms and laparoscopic diagnosis of endometriosis. This delay in the diagnosis and consequently the treatment may aggravate the condition [7]. Therefore thinking in a simple non-invasive method was suggested to reduce this delay especially for minimal and mild forms of the disease [8]. Attempts to develop non-invasive radiological imaging techniques as diagnostic tools for endometriosis have been compromised by low specificity (8,9). Magnetic Resonance Imaging (MRI) is the best imaging modality for diagnosis of endometriosis but its cost is high (10). Many researches had been done to develop a serum marker as CA125 for diagnosis and follow up of endometriosis but proved to be relatively non specific (11). Eutopic endometrium in endometriotic females is biologically different from that of women with a normal pelvis. One of these differences is a higher density of small unmyelinated nerve fibers in endometriotic than non endometriotic females [9,10]. This can

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be used as a semi-invasive method for diagnosis of endometriosis [12,13]. Nerve fibers in the functional layer of the endometrium can be identified through immunohistochemical analysis of various neural transmitters using markers as protein gene product 9.5 (PGP9.5).

This prospective observational study, aim to investigate this finding in a sample of Egyptian females and also investigate if there is difference in nerve fiber density in eutopic endometrium in different laparoscopic stages in endometriotic females.

## **Patients and Methods**

This prospective observational study was conducted at Obstetrics & Gynecology Department and Pathology Department, Mansoura University Hospitals, Egypt, during the period from January 2014 to December 2014. Ninety seven patients were selected from those seeking advice for infertility treatment or suffering from chronic pelvic pain and coming for laparoscopy as a part of their management. The idea of the study was approved by Obstetrics & Gynecology as well as Pathology Departments and by the local institutional ethical committee at Mansoura University Hospitals. A written consent was taken from all patients before being involved and after receiving detailed written and verbal information about the research idea.

All selected females were in the reproductive age group with no history of any hormonal therapy or uterine curettage at least 3 months before. All patients were admitted in the early postmenstrual period. After full history taking, systematic examination, ultrasound evaluation and full laboratory investigations, laparoscopy was decided. For all, laparoscopy was done by same surgeon under general anesthesia using double or triple puncture techniques. Laparoscopic diagnosis and staging of endometriosis was done according to standard methods laid by American Fertility Society Revised Classification of Endometriosis (ASRM1996) [6]. Accordingly, scores (1-15) was assigned as minimal or mild disease, moderate (16-40), and severe (>40). Lastly, endometrial biopsies had been obtained using Novak's curette. Endometrial biopsies fixed in 10% neutral buffered formalin for 24 h then processed, embedded in paraffin blocks, sectioned at 4mm thickness and stained with hematoxylin and eosin (H&E). Immuno-histo-chemistry test (IHC) was done on prepared sections after performing heat antigen retrieval in citrate buffer pH 6 for 20 minutes at 100°C using anti-PGP9.5 antibody that is considered as a special pan neural marker. Sections then incubated over-night at 4°C. Images were taken using light

microscopy for detection of nerve fiber density in the endometrium (number/mm<sup>2</sup>). All histopathological examinations were done by the same pathologist who is not informed by the surgical result.

### ***Statistical analysis:***

Data were collected, tabulated and analyzed using SPSS (Statistical Package for Social Sciences) version 17. Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test. Quantitative data was presented as mean  $\pm$  SD. Student t-test was used to compare between two groups. F-test (One Way ANOVA test) was used to compare between more than two groups. P value was considered significant when  $< 0.05$

## **Results**

This study included 97 patients. As proved by laparoscopy, endometriosis was diagnosed in 50 cases, mean-while the rest (47) were non endometriotic. Patients' demographic criteria in both groups are shown in table [1]. There was no significant difference as regard the mean age (years) in endometriotic and non endometriotic groups ( $28.90 \pm 4.47$  vs  $28.75 \pm 4.58$ ;  $p 0.743$ ). There was a significant difference as regard the mean duration (years) of patients' complaint being ( $5.33 \pm 2.35$ ) in endometriotic versus ( $4.86 \pm 2.02$ ) in non-endometriotic group ( $p 0.037$ )

In table [2] nerve fiber density study appeared significantly much less in the non-endometriotic group compared to the endometriotic one ( $1.53 \pm 0.78$  vs  $9.70 \pm 2.29$ ;  $p < 0.001$ ). More over the mean for nerve fiber density vary significantly from one stage to the other in endometriotic patients as shown in table [3]. It was found ( $7.69 \pm 1.85$ ,  $9.44 \pm 0.53$ ,  $10.0 \pm 0.87$  and  $11.69 \pm 2.12$ ) for stages I, II, III and IV respectively ( $F_{15.611}$ ), ( $p < 0.001$ ).

When comparing patients stage by stage as regard nerve fiber density; there were significant difference between stage I vs. II ( $p < 0.001$ ), stage I vs. III ( $p < 0.001$ ), stage I vs. IV ( $p < 0.001$ ). Stage II vs. IV ( $p < 0.001$ ) stage III vs. IV ( $p < 0.001$ ). No significant difference when comparing stage II and III ( $p 0.145$ ). These are shown in table (4).

## **Discussion**

Laparoscopy is the most important procedure used to diagnose endometriosis as there are many other diseases can cause similar symptoms [5,6].

Here this study use a recently developed technique coinciding with laparoscopy for diagnosis of endometriosis by examining the endometrium via

immunohistochemical analysis. To our knowledge, this is the first study evaluating the difference in nerve fiber density in eutopic endometrium obtained from endometriotic and non endometriotic Egyptian females. Some strengths in this study included its prospective nature and reasonable number of cases included in relation to its relatively short time (one year study).

This study confirmed that the nerve fiber density in the non endometriotic group was ( $1.53 \pm 0.78$ ) compared to ( $9.70 \pm 2.29$ ) in the endometriotic patients. This appears statistically highly significant ( $p < 0.001$ ). Our results comes in correlation to findings by some others [12,14-19] who demonstrated that the mean density of nerve fibers in eutopic non endometriotic females differs from that obtained from endometriotic females.

Researches from Australia, Jordan and Belgium, where researchers at the University of Sydney and Mu'tah University in Karak, Jordan, found that endometrial biopsy from eutopic endometrium then testing for the presence of nerve fibres can diagnose endometriosis with nearly 100% accuracy. In this study, Professor Ian S. Fraser, head of the Queen Elizabeth II Research Institute for Mothers and Infants at the University of Sydney and Dr Moamar Al-Jefout, assistant professor in reproductive medicine at Mu'tah University found that testing endometrial biopsies for the presence of nerve fibres was able to diagnose endometriosis with 83% specificity and 98% sensitivity. This double blind study confirmed the results of a pilot study published in 2007 by the same group [12,14].

Dr Attila Bokor, a doctoral fellow at the University of Leuven, who did the study as part of his PhD project on another research group from Belgium and Hungary said; The density of the small nerve fibres was about 14 times higher in endometrium from patients with minimal to mild endometriosis when compared with women with a normal pelvis(18) Dr Bokor, and team of Prof D'Hooghe said that combination of three different neural markers increases the sensitivity, specificity and diagnostic accuracy of this method up to 95% sensitivity and 100% specificity(18).

Contrary to our result, some did not find a correlation between endometriosis and nerve fibre density as in study done by Maria Luisa Barcena de Arellano, et al. July 2012, who found that The nerve fibre density did not significantly differ between the adenomyosis and control group(20).

Again this study demonstrated that in endometriotic females nerve fiber density vary significantly from one stage to the other as we found that in stage I the mean estimated nerve fiber density was ( $7.69 \pm 1.85$ ),

( $9.44 \pm 0.53$ ) in stage II, ( $10.0 \pm 0.87$ ) in stage III and lastly in stage IV it was much higher ( $11.69 \pm 2.12$ ) ( $F_{15.611}$ ), ( $p < 0.001$ ). Selective comparison between nerve fiber density in different stages shown in table (4) demonstrated a significant difference between all stages ( $p < 0.001$ ) except between stage II and stage III. Up to our knowledge it is the first study tried to find if there is difference in nerve density in laparoscopically staged endometriosis. These findings can help in the diagnosis and to put staging through a semi-invasive method.

As a limitation in our study, it was a single center one. Multicenter study on a large scale of population will be more informative in reflecting the true association among laparoscopic and immunohistochemical findings.

## **Conclusion**

Density of the nerve fibers through endometrial biopsy can be used as semi-invasive method for diagnosis of endometriosis and possibly also for staging.

## **Acknowledgment**

We thank all persons and patients who participating in this study.

## **Disclosure**

Nothing to disclose.

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**Table (1)** Demographic criteria of the study groups.

	Non-endometriotic (mean±sd)	endometriotic (mean±sd)	P
Age(years)	28.75 ± 4.58	28.90 ± 4.47	0.743
Duration (years)	4.86 ± 2.02	5.33 ± 2.35	0.037

**Table(2):** nerve fiber density in endometriotic and non endometriotic patients

	Non-endometriotic (n = 47)	Endometriotic (n = 50)	P
Density n/mm <sup>2</sup>	1.53 ± 0.78	9.70 ± 2.29	< 0.001

**Table (3):** Nerve fiber density in different stages (n/mm<sup>2</sup>)

	Mean	SD	F	P
Stage I (n = 16)	7.69	1.85	15.611	< 0.001
Stage II (n = 9)	9.44	0.53		
Stage III (n = 9)	10.0	0.87		
Stage IV (n = 16)	11.69	2.12		

**Table (4):** Selective comparison between nerve fiber density in different stages

	P value
Stage I vs. Stage II	< 0.001
Stage I vs. Stage III	< 0.001
Stage I vs. Stage IV	< 0.001
Stage II vs. Stage III	0.145
Stage II vs. Stage IV	< 0.001
Stage III vs. Stage IV	< 0.001

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## NEWS AND VIEWS

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### **Realizing a desired family size: when should couples start?**

**J. Dik F. Habbema<sup>1,\*</sup>, Marinus J.C. Eijkemans<sup>2</sup>, Henri Leridon<sup>3</sup> and Egbert R. te Velde<sup>1</sup>**

*Human Reproduction Volume 30, Issue 9 Pp. 2215-2221*

In order to have a chance of at least 90% to realize a one-child family, couples should start trying to conceive when the female partner is 35 years of age or younger, in case IVF is an acceptable option. For two children, the latest starting age is 31 years, and for three children 28 years. Without IVF, couples should start no later than age 32 years for a one-child family, at 27 years for a two-child family, and at 23 years for three children. When couples accept 75% or lower chances of family completion, they can start 4–11 years later. The results appeared to be robust for plausible changes in model assumption.

Comment of the editor:

The study emphasizes the importance of age in female reproduction and the role of IVF in the current era. The unanswered question is when to start IVF in older age group and how long should they wait before trying.

### **Fruit and vegetable intake and their pesticide residues in relation to semen quality among men from a fertility clinic**

**Y.H. Chiu<sup>1</sup>, M.C. Afeiche<sup>2</sup>, A.J. Gaskins<sup>1,3</sup>, P.L. Williams<sup>3,4</sup>, J.C. Petrozza<sup>5</sup>, C. Tanrikut<sup>6</sup>, R. Hauser<sup>2,3</sup> and J.E. Chavarro<sup>1,3,7,\*</sup>**

*Human Reproduction Volume 30, Issue 6 Pp. 1342-1351*

Total fruit and vegetable intake was unrelated to semen quality parameters. High pesticide residue fruit and vegetable intake, however, was associated with poorer semen quality. On average, men in highest quartile of high pesticide residue fruit and vegetable intake ( $\geq 1.5$  servings/day) had 49% (95% confidence interval (CI): 31%, 63%) lower total sperm count and 32% (95% CI: 7%, 58%) lower percentage of morphologically normal sperm than men in the lowest quartile of intake ( $< 0.5$  servings/day) (P, trend = 0.003 and 0.02, respectively). Low-to-moderate pesticide residue fruit and vegetable intake was associated with a higher percentage of morphologically normal sperm (P, trend = 0.04).

Comment

This observational study demonstrates that consumption of fruits and vegetables with high levels of pesticide residues was associated with a lower total sperm count and a lower percentage of morphologically

normal sperm among men presenting to a fertility clinic.

### **CDC Issues Zika Travel Alert**

Pregnant women in any trimester should consider postponing travel to 14 countries and territories in South and Central America and the Caribbean where mosquitos are spreading the Zika virus, the Centers for Disease Control and Prevention (CDC) announced tonight. Viral infection in pregnant women has been associated with microcephaly in infants.

In what it calls a level 2 travel alert, the CDC also advises women who are thinking about becoming pregnant to consult with their physician before traveling to these areas, and if they do, follow strict precautions to avoid mosquito bites. Safeguards include wearing long-sleeve shirts and long pants and using insect repellent. The 14 countries and territories covered by the travel alert are Brazil, Colombia, El Salvador, French Guiana, Guatemala, Haiti, Honduras, Martinique, Mexico, Panama, Paraguay, Suriname, Venezuela, and the Commonwealth of Puerto Rico.

There's growing evidence of a link between Zika and microcephaly, we thought it was important to warn people as soon as possible. Problems associated with microcephaly, which include seizures, developmental delays, intellectual and motor disabilities, and hearing loss can range from mild to life-threatening.

### **Short-term Magnesium Sulfate Use Appropriate, ACOG, SMFM Say**

**Laurie Barclay, MD**

The new recommendations, which update the previous opinion from September 2013, are published in the January issue of *Obstetrics and Gynecology*.

Appropriate indications for magnesium sulfate use in obstetric care are:

the prevention and treatment of seizures in women with preeclampsia or eclampsia, fetal neuroprotection before anticipated early preterm (less than 32 weeks of gestation) delivery, and short-term prolongation of pregnancy (up to 48 hours) to allow antenatal corticosteroid administration to pregnant women at risk for preterm delivery within 7 days.

The U.S. Food and Drug Administration (FDA) advises against use of magnesium sulfate injection for more than 5–7 days to stop preterm labor in pregnant women. Based on this, the drug classification was changed from Category A to Category D, and the labeling was changed to include this new warning information.

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### **Cervical Pessary Does Not Reduce Early Preterm Birth**

*Kypros H. Nicolaides, N Engl J Med. 2016;374:1044-1052.*

A cervical pessary is a silicone device that is placed around the cervix transvaginally. It supports the cervix and reduces direct pressure from the uterus on the cervical canal by directing the cervix toward the sacrum.

The researchers randomly assigned 466 participants to the cervical pessary group and 469 to the expectant management group.

[A]mong girls and women with singleton pregnancies who had a cervical length of 25 mm or less at 20 to 24 weeks of gestation, placement of a cervical pessary did not result in a lower rate of spontaneous early preterm delivery than the rate with expectant management," conclude the researchers. "Pessary placement also did not affect the rates of perinatal death, adverse neonatal outcomes, or the need for neonatal special care.

### **Vaginal Progesterone Does Not Prevent Preterm Birth**

Society for Maternal-Fetal Medicine (SMFM) 2016 Pregnancy Meeting.

The double-blind, placebo-controlled, randomized OPPTIMUM study — the largest study to date of this

intervention — involved 1228 women with singleton pregnancies at risk for preterm birth because of a positive fetal fibronectin test, a history of spontaneous preterm birth at 34 weeks of gestation or earlier, or a cervical length 25 mm or less.

Women were randomized to use vaginal progesterone 200 mg daily from 22 to 24 weeks through to 34 weeks of gestation or placebo.

progesterone had no significant effect on either the obstetric or childhood outcomes. It also had no significant effect on any components of the primary outcome, including fetal death or live-born delivery before 34 weeks. Not only the study found no beneficial effect but it found differences in respiratory, renal, and gastrointestinal disabilities, which were significantly increased in the progesterone group.

The US Food and Drug Administration has not approved vaginal progesterone for the prevention of preterm birth in women with a short cervix.

Comment:

These 2 papers shows that we are still a long way from finding a proper prophylactic treatment for preterm birth, more over it shows how evidence can change from one side to the other all of us were using vaginal progesterone to prolong pregnancy however it seems a pause for vaginal progesterone is warranted .