Effects of natural progesterone on endometriosis in an experimental rat model: is it effective?

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Summary

Purpose: To assess the effects of the natural progesterone on the endometriosis in a rat model. *Materials and Methods:* Endometriosis was surgically induced in 20 rats by transplanting an autologous fragment of endometrial tissue onto the inner surface of the abdominal wall. Rats in control group had no medication but 2.5mg/kg/weekly natural progesterone was administered to rats in study group for four weeks. After that, all rats were sacrificed and dimensions of endometriosis were measured and they were evaluated morphologically and histologically. Scoring systems were used to evaluate preservation of epithelia. *Results:* Two rats in the study group and one rat in the control group died of complications related to surgery. At the end of the treatment, there was a reduction in the size of the endometriotic lesions in the study group ($p < 0.01$). According to histological evaluation, the study group had lower score than control goup which was statistically significant ($p = 0.014$). *Conclusions:* Natural progesterone is effective against endometriosis in rat model.

Key words: Natural progesterone; Endometriosis.

Introduction

Endometriosis is defined as the presence of endometrial gland and stroma outside the uterus. Despite many studies to elucidate its pathogenesis and treatment, it still remains one of the most serious problems in gynecology because of its frequency, bothersome symptomatology, associated infertility, and potential for invasion of adjacent organs such as the gastrointestinal or urinary tract. It affects up to 10% of women of reproductive age and as many as 30% to 50% of all infertile women [1, 2].

Exact etiology of endometriosis is not well known but several factors are involved in the initiation and spread of endometriosis, including retrograde menstruation, coelomic metaplasia, immunologic changes, and genetic predisposition. Therefore, there are many hypotheses regarding its pathophysiology. Among them, retrograde menstruation theory of Sampson is widely accepted to explain the possible mechanism for endometriosis formation [3]. Endometrial tissue which is normally shed at the time of menstruation, is viable and capable of growth in vivo or in vitro. It was shown that almost 90% of women with patent fallopian tubes have reflux menstruation into the peritoneal cavity [4].

Although it is a histologically benign pathology, endometriosis shares many characteristics of malignancy such as local invasion and widely dissemination [5]. Therefore it may cause bothersome complaints and should be treated. It is known that it is a estrogen-dependent gynecological disease. In patients with endometriosis, estradiol can be synthesized locally in the endometriotic lesions from inactive precursors of adrenal or ovarian origin, via the aromatase pathway. These increased estradiol levels stimulate proliferation of endometriotic tissue. Interestingly, cyclic hormones tend to induce its growth, but continuous hormonal exposure, especially at high doses, generally induces significant regression. The synthetic progestins (e.g medroxyprogesterone acetate, dydrogesterone, and dienogest) have been used in the therapy of endometriosis for more than 40 years but their pharmacological action is still not understood in detail.

In this study, the authors aimed to show the effects of injectable natural progesterone, which has been used in clinic practice recently, on endometriosis foci in rat model.

Materials and Methods

Twenty female nonpregnant healthy Wistar albino rats weighting between 250 and 300 grams were used as a model for experimental induction of endometriosis. The rats were caged individually in a controlled environment with 12-hour light/dark cycles and were fed ad libitum.

Endometriosis was surgically induced in rats by transplanting an autologous fragment of uterine tissue onto the inner surface of the abdominal wall and arterial cascades of the small intestines adjacent to mesenteric blood vessels, as proposed by Vernon and Wilson [6] with modifications by Lebovic *et al.* [7]. Before the

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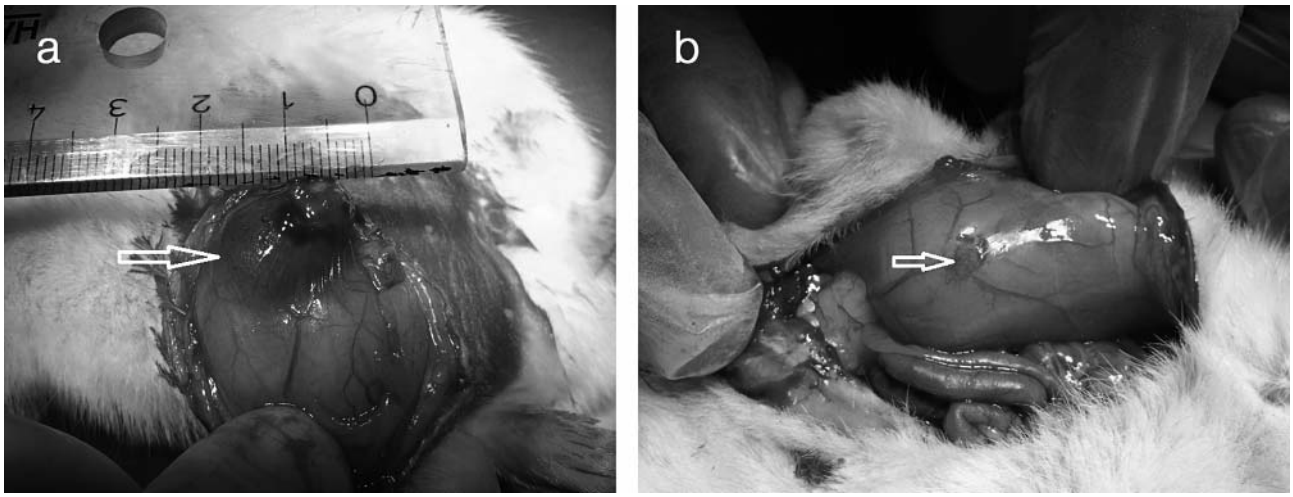

Figure 1. — Gross morphologic appearance of implanted uterine autograft (a) in control and (b) in natural progesterone-treated group.

operation, each rat was anesthetized with an intramuscular injection of ketamine. After that an abdominal skin was shaved and antisepsis was obtained by 10% povidone iodine solution. Using sterile techniques, a five-cm vertical midline incision was made and both uterine horns were exposed. A distal segment, one-cm in length, was resected from the right uterine horn. The segment was placed in phosphate-buffered saline at 37^{0}C and split longitudinally, and a 5.5 mm piece was sectioned. This piece of uterine tissue was transplanted without removing the myometrium on to the inner surface of the right abdominal wall with the serosal surface apposed and secured with single nonabsorbable 5-0 polypropylene suture at the middle to the abdominal wall. Before closure of the abdominal wall, two ml of saline was administered into the abdominal cavity to prevent drying and minimize adhesion formation. The abdominal incision was closed in two layers with the use of a simple interrupted 4-0 polyglactin suture for the peritoneum-fascia and for the skin.

After operation, the rats were randomized into control and study groups and all rats in both group were fed with top water and ad libitum (same protocol). After two months, all of the 20 rats in both groups underwent abdominal incision to see and measure the endometriotic tissue volume which was calculated by ellipsoid formula; volume (mm^3) = 0.52 x width x length x height. Development of endometriosis was seen in all rats. After that, the rats in control and study groups were treated with intramuscular two ml saline and 2.5 mg/kg/week natural progesterone, respectively. The treatment of the groups were continued for four weeks.

At the end of four weeks treatment, all rats in both control and study groups were sacrificed and the volume of the each ectopic uterine tissue was measured by ellipsoid formula (Figure 1). After excision, all samples were fixed in 10% buffered formalin solution for 24 hours. Then, routine tissue processing procedure was performed and then sampled tissues were embedded in paraffin. Paraffin wax blocks were cut in four µm thickness. Prepared sections were stained with hematoxylin-eosin (HE).

The histologic diagnosis of endometriosis was based on the morphologic identification of endometrial glandular tissue and stroma; glands and stroma of the endometrial type, with epithelial lining and luminal formation. In microscopic examination, the preservation of endometrial tissue was evaluated according to a semiquantitative scoring method as described by Keenan *et al.* [8]. Accordingly a well-preserved epithelial layer = score 3, a moderately preserved epithelium with leukocyte infiltrate = score 2, a poorly preserved epithelium (occasional epithelial cells only) = score 1, and no epithelium = score 0. All histological evaluations were performed by two different histologists who were blinded to the groups.

In present study, the mean volume of the endometriosis and endometrial tissue scores were compared between control and study groups before and after treatment. Mann-Whitney *U* test and Wilcoxon Rank tests were applied for statistical analysis by using SPSS program. Any *p* value less than 0.05 was considered as significant at 95% confidence level.

Results

Two rats in the study group and one rat in the control group died during follow up after randomization due to surgery related complications. Therefore, nine in control and eight rats in study groups were included in this experimental study. The standardized surgical procedures and the administration of the protocols were well tolerated by the remaining animals (n = 17). All laparotomy sites were intact and none of the animals had an incisional hernia. No side effects related to medication were observed in the treatment group.

The mean weight of rats in control and study groups was not statistically different from each other (293 ± 10.1 g vs 301 ± 11.8 g, respectively, $p = 0.65$). It was seen that all rats in both groups developed endometriosis at the end of the second month. The mean endometriosis volume before treatment was 62.96 ± 4.97 and 63.71± 5.66 mm^3 in control and study groups, respectively. The mean endometriosis volume before treatment in both groups didn't differ significantly ($p = 0.792$).

At the end of the study, the mean volume of the endometriosis in control group rats became enlarged and was calculated as 78.01 mm^3. This increase in control group was statistically different than its prior measurement (62.96 ± 4.97 vs 78.01 ± 7.23, $p = 0.01$). However, the mean vol-

ume of endometriosis in study group was lower than the value measured before treated with natural progesterone. The difference in study group before and after treatment was significant (63.71± 5.66 vs 6.24±1.9, respectively, $p < 0.001$). Also, the mean value between control and study groups after treatment differed significantly (78.01 ± 7.23 vs 6.24 ± 1.9, $p < 0.001$) (Figure 1, Table 1).

According to histological examination of specimens, scoring in natural progesterone group was lower than the control group. The difference between mean scores of the groups was statistically significant (1.50 ± 1.30 vs 2.55 ± 0.88 respectively, $p = 0.014$) (Figures 2-3).

Table 1. — *Comparison of the pre- and post-treatment volumes (mm³) in the control and study groups.*

	Volume of endometriosis Before treatment	Volume of endometriosis After treatment	*p* value
Control group (n = 9)	62.96 ± 4.97	78.01 ± 7.23	0.04
Study group (n = 8)	63.71 ± 5.66	6.24 ± 1.9	<0.001

Discussion

Endometriosis is a common health problem among women in reproductive age and exact underlying pathophysiology is currently not well understood. It is mostly located at genital system but theoretically can occur anywhere in the body. Although it is sometimes asymptomatic, it may often result in serious symptomatology according to its location and volume. Therefore, gynecologists tend to treat endometriosis and its related symptoms.

Investigations on endometriosis were done in both clinical and experimental studies. In preclinical studies, sur-

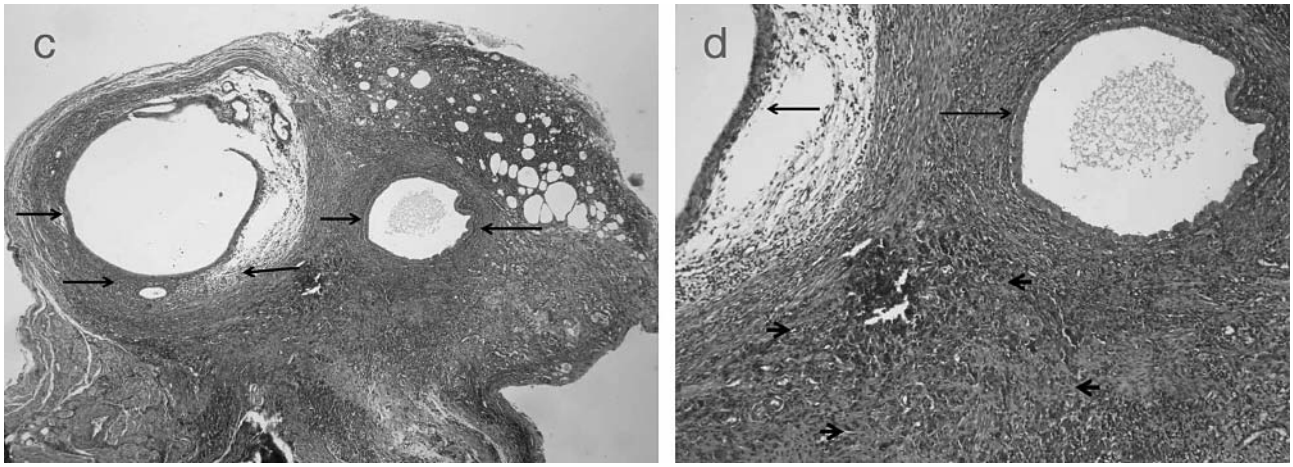

Figure 2. — Histology of the ectopic endometrium in control group. (c) Arrows show gland-stroma areas of endometrium (HE x40) (d) Endometrial glandular epithelium (long arrows) and hemosiderin-laden macrophages (short arrows) (HE x100) (Score = 3).

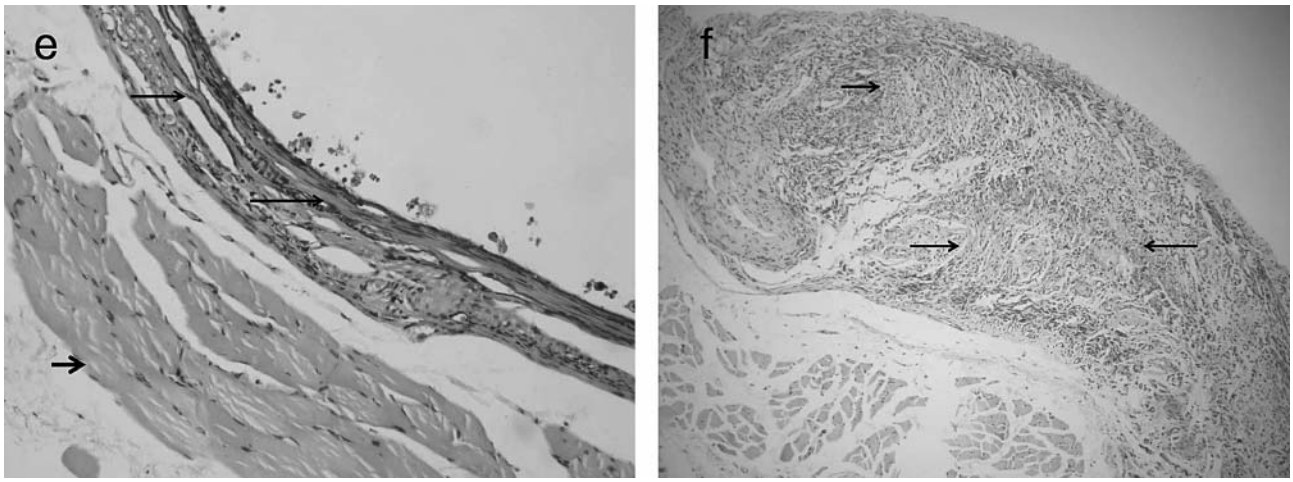

Figure 3. — Flattened glandular epithelium and decreased stromal structure in natural progesterone group. (e) The compressed endometrial glandular epithelium and reduction in the stroma (long arrows),stripped muscle tissue (short arrow) can be seen. (f) Hemosiderin laden macrophages (HE x200) (Score = 1)

gically induced endometriosis in rat models is useful in the attempt to understand the effect of possible therapeutic agents on the proliferation of endometrial tissue outside the uterine cavity [6, 9]. In the present study, the authors used the rat model of surgically induced endometriosis to test the efficacy of natural progesterone on endometriosis tissue.

Today, the effect of progesterones on endometriosis is well established. They inhibit endometriotic tissue growth by causing decidualization initially, and then atrophy. Additionally, they also inhibit pituitary gonadotropin secretion and ovarian hormone production. Therefore, the effectiveness of progesterones for treating endometriosis is not just linked to its growth inhibiting actions, but also to its induction of anovulation, inhibition of blood vessel growth, and anti-inflammatory actions.

Progesterones are divided into two groups, one is synthetics, and the other is natural progesterones. Synthetic analogues of progesterone which is more potent than its natural form have been developed to make the hormone available orally and to produce longer lasting effects [10]. They have been widely used and have almost similar bioactivity as progesterone. However, they are synthetically made in the laboratory and have been slightly altered biochemically to mimic natural progesterone [10]. The first synthetic progestin, norethindrone, was invented in 1951 and used in the first oral birth control pills. The most common progestins are medroxyprogesterone acetate and megestrol acetate. Synthetic progesterone-based therapies are well-established in the treatment of endometriosis since they lead to a regression of the disease and reduction of pain [11].

When a hormone is described as natural or bioidentical it signifies that its molecular structure is identical to the form naturally produced in the human body. In 1998, an oral natural (bioidentical) progesterone received FDA approval. Besides the administration through a variety of routes including oral, transdermal, vaginal, rectal, sublingual/buccal, and intrauterine there is also an injectable form of natural progesterone which was used for this study. Plasma levels of progesterone are most reliable and consistent when the hormone is given as an intramuscular injection of progesterone [9]. It is rapidly absorbed, and a 100-mg injection produces plasma concentrations of 40 to 50 ng/ml in two to eight hours [12]. In this study, the authors also prefered intramuscular route to be sure to give the exact dosage of progesterone.

Although they may have side-effects, progesterones are generally safe medications in treatment of endometriosis. In a review literature, Rudel and Kincl noted that no toxicities were reported up to now after long-term oral or parenteral administration [13]. Their only finding was an increase in the body and liver weights of female rats receiving parenteral progesterone. However some authors reported quiet frequently such complaints as drowsiness, headache, dizziness, nausea, etc after oral or parenteral progesterone administration [12, 14]. Also, intravenous administration induces sleep at doses of 250 to 500 mg [12]. On the other hand, synthetic progestins often cause androgenic side-effects such as acne, body and facial hair, depression, and weight gain.

Natural progesterone is a recent subject of gynecology which is chemically identical to human physiologic progesterone. There is an ongoing debate between the superiority of natural progesterone on its synthetic identical in gynecologic disease treatment. Although there is limited number of comprehensive studies, it is logic to expect no clinical difference regarding the effect of them on gynecologic disease such as osteoporosis prevention, endometriosis, premenstrual syndrome, etc. However some authors stated fewer side-effects among women on natural progesterone treatment. Also, secondary benefit as lipid profile enhancement of natural progesterone usage were noted [15]. In the present study, the authors did not compare natural progesterone with synthetic counterpart. They attempted to demonstrate the potential effect of natural progesterone on endometriotic tissue.

According to the authors' knowledge, there was no study to investigate the effects of natural progesterone on endometriosis in literature. Hence they designed an animal model of endometriosis in 20 rats and divided them into two groups, one was control and the other was study group that were administered natural progesterone weekly via intramuscular route. At the end of the study, lesions size measured and histological staging were performed and compared with control. They demonstrated that the mean endometriotic lesions' diameter was smaller in the study group than controls. In addition, reducing the size of the endometrial explants is clearly supported by the lower histological score in study group which had statistically significant.

The main limitation of this experimental study is that the authors did not compare the effect of synthetic progesterone with its natural counterparts due to limited number of rats. Secondly small number of rats in the groups was another limitation. However, the present study is the first study to show the efficacy of natural progesterone on ectopic uterine tissue in rat model. The result give an idea regarding the natural progesterone effect on endometriosis.

Conclusion

As a conclusion, use of natural progesterone was effective on rat model endometriosis. It may be logic to use natural progesterone because of its non-toxicity, fewer side- effects, and less is expense than synthetic progestins. However further experimental and clinical studies are required to compare its efficacy with the synthetic counterpart.

References

[1] Strathy J.H., Molgaard C.A., Coulman C.B.: "Endometriosis and infertility: A laparoscopic study of endometriosis among fertile and infertile women". *Fertil. Steril.*, 1982, *38*, 667.

[2] Rice V.M.: "Conventional medical therapies for endometriosis". *Ann. N. Y. Acad. Sci.*, 2002, *955*, 343.

[3] Sampson J.: "Peritoneal endometriosis is due to the menstrual dissemination of endometrial tissue into the peritoneal cavity". *Am. J. Obstet. Gynecol.*, 1927, *14*, 433.

[4] Halme J., Hammond M.G., Hulka J.F., Raj S.G., Talbert L.M.: "Retrograde menstruation in healthy women and in patients with endometriosis". *Obstet. Gynecol.*, 1984, *64*, 151.

[5] Starzinski-Powitz A., Gaetje R., Zeitvogel A., Kotzian S., Handrow-Metzmacher H., Herrmann G., *et al.*: "Tracing cellular and molecular mechanisms involved in endometriosis". *Hum. Reprod. Update*, 1998, *4*, 724.

[6] Vernon M.W., Wilson E.A.: "Studies on the surgical induction of endometriosis in the rat". *Fertil. Steril.*, 1985, *44*, 684.

[7] Lebovic D.I., Kir M., Casey C.L.: "Peroxisome proliferator–activated receptor-gamma induces regression of endometrial explants in a rat model of endometriosis". *Fertil. Steril.*, 2004, *82*, 1008.

[8] Keenan J.A., Williams-Boyce P.K., Massey P.J. Chen T.T., Caudle M.R., Bukovsky A.: "Regression of endometrial explants in a rat model of endometriosis treated with the immune modulators loxoribine and levamisole". *Fertil. Steril.*, 1999, *72*, 135.

[9] Jones R.C.: "The effect of a luteinizing hormone-releasing hormone antagonist on experimental endometriosis in the rat". *Acta Endocrinol.*, 1987, *114*, 379.

[10] Murray J.L.: "Natural progesterone: what role in women's healtcare?" *Women's Health in Primary Care,* 1998, *1*, 671.

[11] Olive D.L., Pritts E.A.*: "*The treatment of endometriosis: a review of the evidence". *Ann. N. Y. Acad. Sci.*, 2002, *955*, 360.

[12] Maxson W.S.: "The use of progesterone in the treatment of PMS". *Clin. Obstet. Gynecol.*, 1987, *30*, 465.

[13] Rudel H.W., Kincl F.A.: "The toxicity of progesterone". In: Tausk M., (ed.), *International encyclopedia of pharmacology and therapeutics*. New York, NY: Pergamon Press, 1971, 405.

[14] Ferre F., Uzan M., Janssens Y., Tanguy G., Jolivet A., Breuiller M., et al.: "Oral administration of micronized natural progesterone in late human pregnancy. Effects on progesterone and estrogen concentrations in the plasma, placenta, and myometrium". *Am. J. Obstet. Gynecol.,*1984, *148*, 26.

[15] The Writing Group for the PEPI Trial: "Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women". *JAMA*, 1995, *273*, 199.

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