

Histological Changes of Cervix in Ovariectomized Indigenous Rabbits

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Abstract:

Histological changes which occurred in the cervix of the ovariectomized rabbits without hormone and with hormones injection were investigated.

A total of 45 female rabbits were divided into three groups, the first group was used as a control, while, the second and third groups were used for experiments.

In control group the tunica mucosa thrown into branched folds which lined with simple cuboidal to columnar epithelium, and a lamina propria was well vascular dense irregular collagenous connective tissue and a tunica muscularis displayed double layers of smooth muscle fibers. In ovariectomized group without hormones injection, the tunica mucosa showed delicate mucosal folds which lined with low simple cuboidal epithelium and the tunica muscularis was very thin layer, and a significant decreased in the height and thickness of mucosal folds, thickness of lamina propria and tunica muscularis. In ovariectomized groups with hormones injection, the cervix was very thick walled organ and their mucosal folds were the tallest, pyramidal shaped and their epithelium turned into simple columnar epithelium. The thickness of tunica muscularis was increased due to their two layers those intermingled with much of collagen bundles. Statistically, a significant ($P<0.05$) increased in the values of all parameters.

The present study concluded that that cervix in doe has a narrower lumen with huge collagenous connective tissue content that increased in response to steroid hormones, in addition to hyperplasia and hypertrophy, consequently for keeping intrauterine during the preimplantation stage and pregnancy.

Key words: cervix, ovariectomy, hormones.

التغيرات النسجية لعنق الرحم بعد إزالة المبايض جراحياً في الأرانب المحلية

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الخلاصة:

تمت دراسة التغيرات النسجية في عنق الرحم للأرانب التي أزيلت مبايضها جراحياً، من دون حقن الهرمونات ومع حقن

الهرمونات الستيرويدية.

تم تقسيم ما مجموعه (٤٥) من إناث الأرناب إلى ثلاث مجاميع، أستخدمت المجموعة الأولى كمجموعة سيطرة، في حين أستخدمت المجموعتين الثانية والثالثة للتجارب.

بدأت الغلالة المخاطية في مجموعة السيطرة بشكل طيات مقسمة ومغطاة بظهارة مكعبة بسيطة الى عمودية بسيطة. الصفيحة الأساسية كانت مكونة من نسيج ضام كثيف غير منتظم غني بالأوعية الدموية وألياف الكولاجين. الغلالة العضلية تكونت من طبقتين من ألياف العضل الأملس. في المجموعة المستأصلة المبايض دون حقن الهرمونات، بدأ الغشاء المخاطي للغلالة المخاطية بشكل طيات رقيقة وظهرت مغطاة بظهارة بسيطة مكعبة واطئة وكانت الغلالة العضلية بشكل طبقة رقيقة جداً، وأظهرت النتائج الإحصائية إنخفاض كبير في إرتفاع وسك الطيات المخاطية، سمك الصفيحة الأساسية والغلالة العضلية. أمل في المجموعة المستأصلة المبايض مع حقن الهرمونات، فقد ظهر عنق الرحم سميك الجدران وكانت الطيات المخاطية أطول وهرمية الشكل، كانت الظهارة من النوع العمودي البسيط، كما لوحظ زيادة سمك الغلالة العضلية بسبب إحتواء طبقتيها على الكثير من حزم الكولاجين. إحصائياً سجلت زيادة معنوية في قيم جميع القياسات.

وخلصت الدراسة إلى أن عنق الرحم في الأرناب يمتاز بتجويف أضيق ويحتوي على محتوى كبير من النسيج الضام الكولاجيني كأستجابة لحقن الهرمونات الستيرويدية، فضلاً عن فرط الضخامة الخلوية وفرط التنسج وبالتالي حفظ البيض داخل الرحم خلال المرحلة السابقة للانغراس ومرحلة الحمل.

الكلمات المفتاحية: عنق الرحم، إزالة المبايض، الهرمونات.

Introduction:

The cervix is a fibrous connective tissue conserve intrauterine embryon during the implantation stages and considerable main site of sperm passage through into uterus^[1,2]. The cervix is controlling the concept in the uterine lumen when the fertilized eggs reach the uterine cavity and lay few days before implantation in addition to that large numbers of eggs were expelled into the outer environment when the super ovulated rabbit at early times after mating^[3].

Estrogens and progesterone are controlling structure, functioning and maintenance of the female genital system and regulate its growth, proliferation and secretion^[4].

The female rabbit has a bicornuate duplex uterus which shows two separate uterine horns and no uterine body and each horn has its own cervix, and the two cervixes open into a single vagina^[5].

This study has intended to describe histological changes in normal, bilateral ovariectomized doe without hormonal injection and bilateral ovariectomized doe

with injection of progesterone and estrogen hormones^[6].

Materials and Methods:

Twelve indigenous healthy does with four buck aged about one year old were housing in floor pattern and fed on standard pellets, green vegetables and tap water ad libitum until parturition to obtain the required ages of this study, this method according to^[6,7]. After parturition a number of 45 doe have been distributed into three and four months old and these ages have divided into two subgroup:

First group: (control) contained (15 does), the animals of this group have divided into two subgroups (five each) according to their ages (three and four months).

Second group (Treated): Contained (20 does), this group was subdivided into two subgroups:

Ovariectomy without hormonal injection: Involved (10 does). These animals have subjected to ovariectomy at age of one month. The first ten does have killed at the 60th day after the operation while the

remaining rabbits have killed at the 90th day after the operation.

Ovariectomy with hormonal injection: Involved (20 does) have subjected to ovariectomy at age of one month and treated with exogenous hormones at least for 19 days before taking the samples. Treatment with exogenous hormones was applied for ovariectomized group at three and four months of age. Estrogen was given as repeated doses of 0.25 mg/kg B.W., (two doses at 48hrs. interval). The other hormone was progesterone which administered after 24 hours of estrogen injection in doses of 2-2.5mg/kg B.W. Finally after the completion of progesterone injection, the does were injected with estrogen one dose daily for 10 consecutive days via IM route. Tissue samples have obtained on (90 and 120) days. The rabbits of three groups were sacrificed by injection of an overdose of sodium pentobarbital and their cervix samples were removed, trimmed and fixed in Bouin's solution for 10 days then prepared for paraffin embedding and sectioned at 6 μ m, the tissue sections have stained with hematoxylin and eosin stain, Masson trichrome stain [8].

Results and Discussion:

Control:

At three and four months old, the cervix showed slightly thick wall and its tunica mucosa has displayed tall and narrow branched mucosal folds (figure-1). These folds have lined low ciliated and non-ciliated simple columnar epithelium which formed epithelial crypts; this result is similar with result of [9]. Lamina propria has composed of well vascular dense irregular connective tissue (figure-2). Tunica muscularis composed of two layers: thin inner circular and thick outer longitudinal smooth muscle fibers (fig.3). The histometrical results of three months showed that, the mucosal folds height and thickness were (870.6 \pm 10.2) and

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(137.5 \pm 8.5) μ m respectively. The thickness of lamina propria and tunica muscularis were (98.5 \pm 2.3) and (750 \pm 10.9) μ m respectively, while in four months the mucosal folds height and thickness were (1000.12 \pm 7.3) and (142.1 \pm 6.1) μ m respectively. The thickness of lamina propria and tunica muscularis were (101.9 \pm 8) and (890.4 \pm 5.1) μ m respectively.

The statistical analysis revealed significant at (P<0.05) differences between both ages (figures-4, 5).

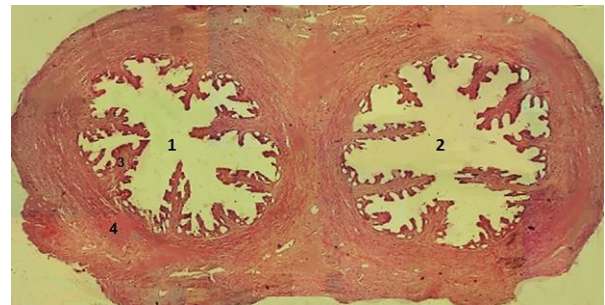


Figure-1: Cervix of four months does shows: left cervix (1), right cervix (2), mucosal fold (3) and tunica muscularis (4). H&E stain, 40x.

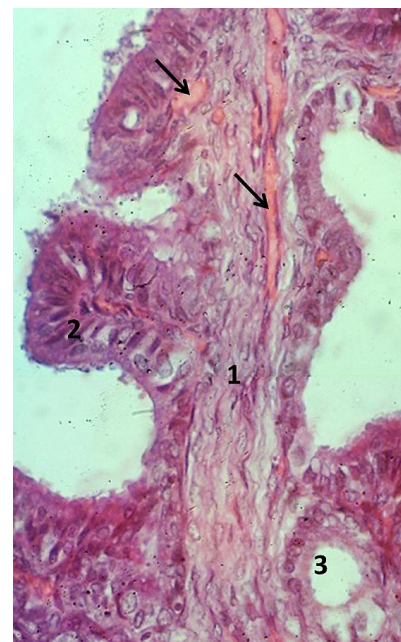


Figure-2: Mucosal fold of four months does cervix shows: lamina propria (1), epithelium (2), crypt (3) and blood vessels (Arrows). H&E stain 400x.

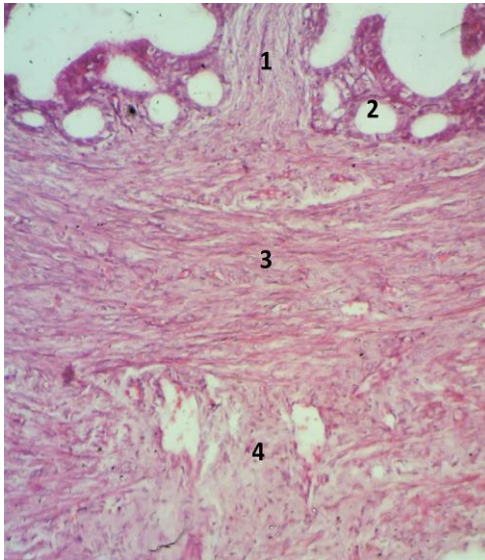


Figure-3:Wall of four months does cervix shows: mucosal fold (1), epithelium crypt (2), inner circular layer of tunica muscularis (3) and outer longitudinal layer of tunica muscularis (4). H&E stain 100x.

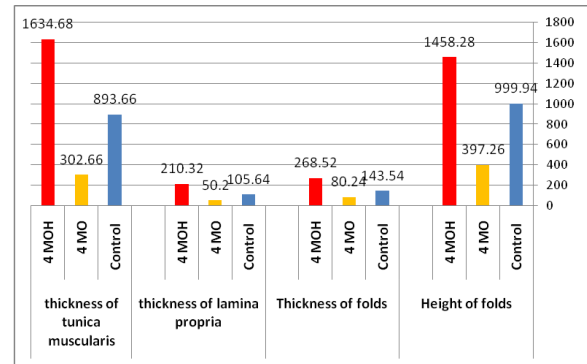


Figure-5:Shows the means of height and thickness of mucosal folds, thickness of lamina propria and tunica muscularis at four months overctomized does. (n=5), the colors differences represent significant at (P<0.05%).

*4MOH = 4months ovaroectomy with hormones injection.

*4MO = 4months ovaroectomy without hormones injection.

Ovariectomy without hormones injection:

In both (three and four months old) ovariectomy without hormones injection, the wall of cervix appeared thin and tunica mucosa has displayed delicate branched mucosal folds which were irregular shaped and were more interconnected in complicated fashion, showing a labyrinthine structure and its core was more densely packed irregular connective tissue. These folds were lined with low ciliated and non-ciliated simple cuboidal epithelium which displayed no secretory activities (figures- 6, 7). Tunica muscularis was thin and composed of two layers: inner circular and outer longitudinal smooth muscle fibers.

The statistical results revealed significant (P<0.05) decreased in the means values of all parameters (height and thickness of mucosal folds, thickness of lamina propria and tunica muscularis) (figure-4), this result suggests that the prolonged ovariectomy had given an effect similar to that of immature status due to absent of effects of FSH and LH, even the presence of other silent growth factors which

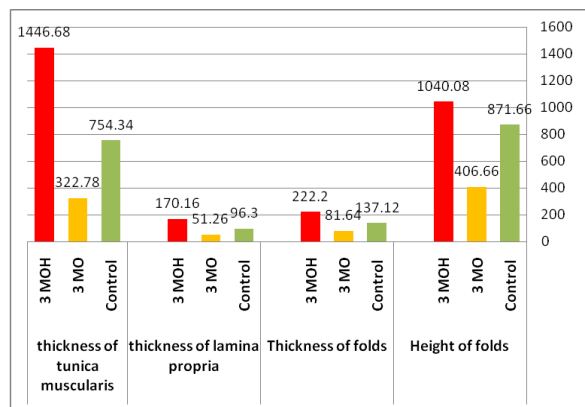


Figure-4:Shows the means of height and thickness of mucosal folds, thickness of lamina propria and tunica muscularis at three months overctomized does. (n=5), the colors differences represent significant at (P<0.05%).

*3MOH = 3months ovaroectomy with hormones injection.

*3MO = 3months ovaroectomy without hormones injection.

in general could act on the genital tract for a period could name pre pubertal stages, this suggestion is compatible with^[10,11] who referred for stromal growth factor like (FGF-7) which stimulated cells proliferation and differentiation.



Figure-6: Cervix of four months ovariectomized doe without hormones injection shows: Inter cervical septum (1), left cervix (2), right cervix (3), tunica muscularis (4) and adventia (5) H&E stain.

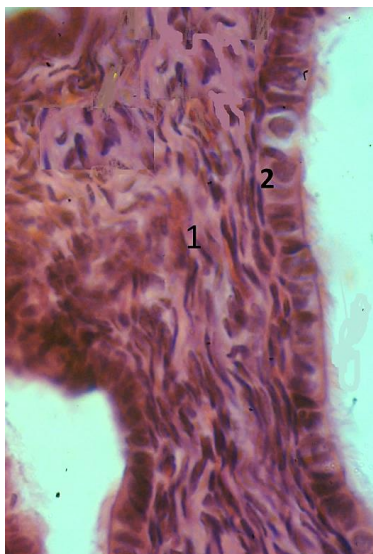


Figure-7: Mucosal fold of four months ovariectomized doe without hormones injection shows: Lamina propria (1) and epithelium (2). H&E stain 400x.

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Ovariectomy with hormones injection:

In three and four month's old ovariectomy with hormones injection, the wall of cervix was very thick. Tunica mucosa showed furthermore of branched mucosal folds which appeared tall pyramidal shape (figure-8). The folds were lined with simple to pseudo stratified columnar epithelium contained goblet cells with multinucleated cells (figure-9), the changing of epithelium was observed by^[12], this suggested that the cells division was induced by hormones.

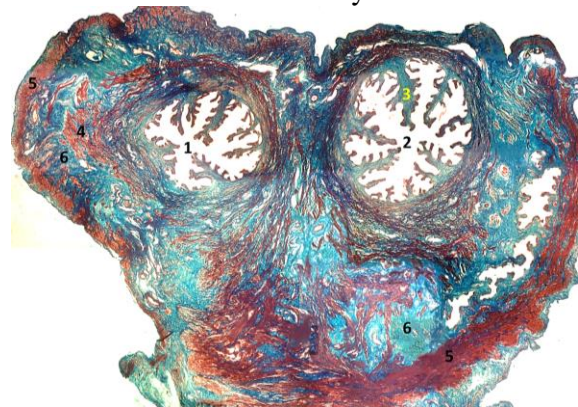


Figure-8: Cervix of three months ovariectomized doe with hormone injection shows: Left cervix (1), right cervix (2), mucosal fold (3), inner circular layer of tunica muscularis (4), outer longitudinal layer of tunica muscularis and collagen bundles (6). Masson's trichrome stain 40x.

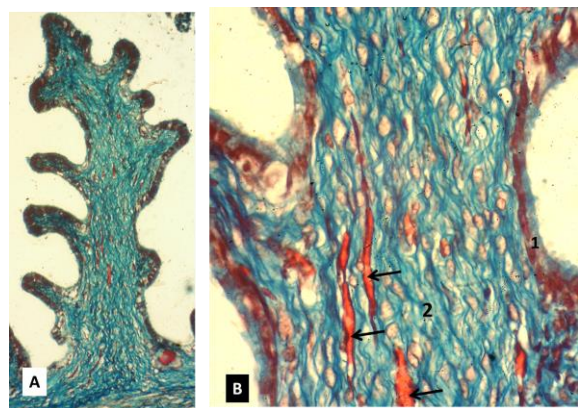


Figure-9: (A) Mucosal fold of three months ovariectomized doe with hormones injection. (B) Shows: epithelium (1), lamina propria composed of collagen fivers (2), and blood vessels (arrows). Masson trichrome stain 10 & 400x .

The present result showed secretory cells exhibiting apocrine secretion projections on their apical surfaces (figure-10), such result has recorded by^[13], who referred to more distinct changes occurred in the production and secretion of mucus, in the shape of cells in the number of different secretory cells of the cervix.

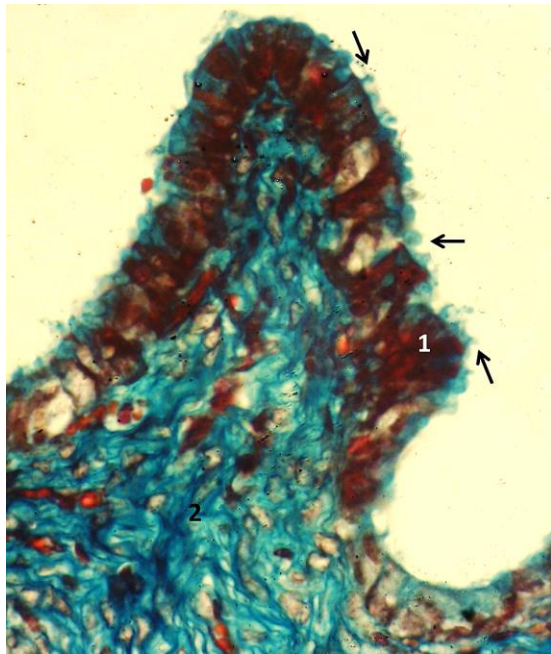


Figure-10: Mucosal fold of three months ovariectomized doe with hormones injection shows: epithelium (1), lamina propria composed of collagen fibers (2), and secretory activities (Arrows). Masson trichrome stain. 400x.

The present result suggested that, the secretory activity of the epithelium reach their peaks during period of hormone injection indicating that estrogens cause an increase in the size of cells and secretory activity of cervical epithelium, these observations suggest that the production of secretory granules in the cervical epithelial cells is regulated by hormonal events^[14,15,16] who stated that the estrogen led to hypertrophy, activate ciliation and increased the secretion in ovariectomized animals.

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The present results revealed extensive proliferation and the branching of mucosal folds with injection of hormones in long term (four months) furthermore epithelia crypts formation, this result agree with result of^[17,18,19] whom mentioned that estradiol administration stimulated cells growth, proliferation and hyperemia also the estrogen and progesterone have gave different affections on the uterine glands after ovariectomy and caused an increase in the height of surface epithelial of endometria^[20].

The main histological feature in mucosal folds was the increasing the connective tissue and cellular core content which manifested by much of collagen bundles (figure-9) also the wall of cervix was very thick represented by a tunica muscularis which composed of two layers: inner circular and outer longitudinal smooth muscle fibers those separated by very thick layer of collagen bundles housed blood vessels (figure-8), this suggested that the cervical ripening by hormones injection is important for cervical normal labour, that represented an events occur in the cervical ground matrix as collagen content, such observation has recorded chemically by^[21], who recorded an increasing of dermatan sulphate and hyaluronic acid, those responsible to bind tightly to collagen fibres, consequently give the cervix its rigidity, also^[22], has find an increase in collagen content of human cervix treated by PGEj.

The present results revealed significant ($P < 0.05$) increased in the values of all parameters (height and thickness of mucosal folds, thickness of lamina propria and tunica muscularis) (figure-5), this increases were due to increase in amount of collagen bundles, that contrary to^[16,23], who mentioned that both estrogen and progesterone after ovariectomy in ewes could affect the uterine size, which increased by hypertrophy rather than by tissue hyperplasia.

References:

- 1 - Suzuki H, Tsutsumi YO. Morphological changes of uterus and cervical epithelium during early pregnancy in rabbits. *J Fac Agr. Hokkaido Univ.* 1981; 60(1): 47-61.
- 2 - Hafez ESE. Sperm transport in the human and mammalian cervix. In: *The Cervix*, W.B. Saunders Company. London. 1976: 164-75.
- 3 - Tsutsumi Y, Terami Y, Takeda T, Suzuki H, Matsui S. In vivo egg recovery from the vaginae and the pattern of egg distribution in super ovulated rabbits. *Jap J Anim Rep.* 1980; 26(1): 6-14.
- 4 - Vinci A, Bacci B, Benazzi C, Caldin M, Sarli G. Progesterone receptor expression and proliferative activity in uterine tumours of pet rabbits. *J Comp Path.* 2010; 142(4): 323-7.
- 5 - Shively M.J. *Veterinary anatomy basic, comparative and clinical.* Texas A and M University Press. College Station. 1987: 519-25.
- 6 - Oner H, Oner J, Kukner A, Ozan E. Effects of estrogen and/ or progesterone on the changes occurring in the uterine luminal epithelium of ovariectomized rats. *Acta Veterinaria.* 2002; 52(2-3): 97-106.
- 7 - Blesson CS, Masironi B, Sahlin L. Effects of selective estrogen receptor agonists on estrogen receptor expression in the uterus of ovariectomized rats. *Open J of Molecular and Integrative Physiology.* 2012; 2(2): 35-43.
- 8 - Bancroft JD, Marilyn G. *Theory and practice of histological techniques.* 6th Ed. London, Elsevier Limited. 2008: 168-73.
- 9 - Tyler KT. Histological changes in the cervix of the rabbit after coitus. *J Reprod Fert.* 1977; 49(2): 341-5.
- 10 - Rubin P, Constine LS, Fajardo LF, Phillips TL, Wasserman TH. Late effects of normal tissues scoring system. *Int J Radiat Oncol Biol Physics.* 1995; 31(5): 1041-42.
- 11 - Bartol FF, Wiley AA, Spencer TE, Vallet JL, Christensen RK. Early uterine development in pigs. *J Reprod Fertil Suppl.* 1993; 48: 99-116.
- 12 - Hilliard J, Eaton LW. Estradiol-17 β , progesterone and 20 α -hydroxypregn-4-en-3-one in rabbit ovarian venous plasma. II. from mating through implantation. *Endocrinol.* 1971; 89(2): 522-27.
- 13 - Hafez ESE, El-Banna AA, Yamashita T. Histochemical characteristics of cervical epithelia in rabbits and cattle. *Acta Histochem.* 1971; 39(2): 195-205.
- 14 - Jansen RPS, Bajpai K. Oviduct acid mucus glycoproteins in the estrous rabbit: ultrastructure and histochemistry. *Biol. Reprod.* 1982; 26(1): 155-68.
- 15 - Verhage HG, Brenner MO. Estradiol-induced differentiation of the oviductal epithelium in ovariectomized cats. *Biol Reprod.* 1975; 13(1): 104-11.
- 16 - Abe H, Oikawa T. Effects of estradiol and progesterone on the cyto-differentiation of epithelial cells in the newborn golden hamster. *Anat Rec.* 1993; 235(3): 390-8.
- 17 - Lawrence PR, James DK, Kim CK, Darlene LK, Wendy JM, Dale AR. Time-course of the uterine response to estradiol-17 β in ovariectomized ewes: uterine growth and microvascular development. *Biol Reprod.* 1998; 59(3): 606-12.
- 18 - Boreham MK, Wai CY, Miller RT, Schaffer JJ, Word RA. Morphometric analysis of smooth muscle in the anterior vaginal wall of women with pelvic organ prolapse. *Am J Obstet Gynecol.* 2002; 187(10): 56-63.
- 19 - Pessina MA, Hoyt RFJ, Goldstein I, Traish AM. Differential effects of estradiol, progesterone, and testosterone

- on vaginal structural integrity. *Endocrinol.* 2006; 147(1): 61-9.
- 20 - Sritulasi K, Gopalakrishnan CR, Vanithakumari G. Anti estrogenic and anti progestational activity of methotrexate and its effect on uterine histoarchitecture of ovariectomized albino rats. *BioResearch Bulletin.* 2010; 1(3): 292-300.
- 21 - Uldbjerg N, Ekman G, Malrenstrom AG, Ulnsten U, Wingenerup L. Biochemical changes in cervical connective tissue after local application prostaglandin E2. *Gynecol Obstet Invest.* 1983; 15(5): 291-9.
- Date of acceptance: 8-5-2016**
- 22 - Ekman G, Uldbjerg N, Malmstrttm A, Ulnsten U. Increased postpartum collagenolytic activity in cervical connective tissue from women treated with prostaglandin E2. *CynecoL Obstet. Invest.* 1983; 16(5): 292-8.
- 23 - Johnson KL, Cummings AM, Birnbaum LS. Promotion of endometriosis in mice by polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls. *Environ Health prospect.* 1997; 105(7): 750-5.