

## Histopathological and Immunohistochemical Changes Induced by Contraceptive Pills in the Cervix of Female Rabbits

Gabri MS\*\*, Ibrahim MA\*\*, Abd El-kader DH\*, Hassan BN\*\*

\*Department of Histology, Faculty of Medicine, Cairo University

\*\*Department of Zoology and Entomology, Faculty of Science, Helwan University.

### Abstract

**Introduction:** oral contraceptive pills (OCPs) are the most popular form of hormonal contraception in young women.

**Aim of the work:** the present study focused on evaluating the effects of two different contraceptive pills including combined pills (estrogen and progesterone) and mini pills (progesterone only) on the cervix of female rabbit.

**Material and methods:** after three months of daily oral administration of these contraceptive pills the animals were sacrificed. The excised organs were dissected, processed and stained with H & E, PAS reaction and Masson's trichrome stain and orcein stain. This was followed by morphometric measurements and statistical study. **Results:** this study revealed that contraceptive pills administration - specially the combined one - caused marked alterations in the form of hyperplastic cervical mucosal cells and hypertrophied muscular layer. Also, there was a significant increase in collagenous and elastic fibres content in the muscular layer of the cervix. There was also a statistically significant increase in PAS positive materials in the lumina of the mucosal glands of the cervix. All these changes were less marked in the case of mini pill treatment

**Conclusion:** pills of progesterone only showed marked histopathological change, in the cervix as a contraceptive mean, but in less manner than the combined pills (estrogen and progesterone).

**Key words:** combined pills – mini pills – cervix – hyperplasia .

### Introduction

The introduction of the birth – control pill as an effective, coitally-independent method of contraception was a public health milestone of the last century.<sup>[1]</sup>

The oral contraceptive pill (OCP) brought to market over

50 years ago was designed around a 28-day cycle that included

a 7-day hormone-free interval to induce a withdrawal bleed.<sup>[2]</sup>

The conventional dosing scheme was developed to mimic the physiological event of monthly menstruation in non – pregnant women and to provide the illusion of natural menstrual cyclicality.

The hormone free interval reportedly has no physiological benefit, but was initially included to increase user acceptability.<sup>[3]</sup>

A majority of oral contraceptive failures are primarily because of one of two types of compliance problems: (1) taking pills incorrectly or (2) improper transition from one pill package to the next. About 20–30% of women taking oral contraceptives miss at least one pill each month and that adolescents miss an average of three pills a month.<sup>[4]</sup>

Combined oral contraceptive pills were developed to prevent ovulation by suppressing the release of gonadotropins. Estrogen negative feedback on the anterior pituitary greatly decreases the release of FSH, which inhibits follicular development and helps to prevent ovulation.<sup>[5]</sup>

Progestagen negative feedback and the lack of estrogen positive feedback on LH release prevent a mid-cycle LH surge. Inhibition of follicular development and the absence of LH surge prevent ovulation.<sup>[6]</sup> It is unclear whether ovulation suppression occurs at the level of the hypothalamus, the pituitary or both.<sup>[7]</sup>

Progesterone only pills primarily rely on the induction of viscous cervical mucus, which hampers the penetration of sperm into the female genital tract and cause changes in the endometrium, making it less suitable for implantation.<sup>[8]</sup>

For many women the pill provides a highly reliable and acceptable contraceptive, together with many benefits to generate health. Indeed it is often prescribed for its non – contraceptive benefits. These many advantages are accompanied by some disadvantages, which are small with a little

significance for most women. Notably, the risks are greater with combined contraceptive pills, containing both ethinyl estradiol and progesterone than progesterone only pills (pops).<sup>[9]</sup>

Combined oral contraceptive pills (COC) use is associated with an increased risk of developing venous and arterial thromboembolic events. Combined oral contraceptive pills produce a hypercoagulable state, which could be responsible for a two- to six-times greater risk of venous thromboembolism (VTE) for COC users than for those not using COCs. However, it is not known whether COCs cause any damage to the vessel walls, which could explain the increased risk of arterial thrombosis in COC users.<sup>[10]</sup>

The present study aimed to elicit and compare the histological and histochemical changes induced in the rabbit cervix due to the use of two different types of contraceptive pills.

#### Material and methods:

##### I - Material:-

###### 1- The drugs:

The drugs used in this work were mini contraceptive pills (progesterone only) for group of animals and combined pills (progesterone and estrogen) for another group as a daily dose for three months. They were purchased in tablets form that were crushed to powder and dissolved in distilled water and their doses were calculated according to the interspecies dosage conversion scheme<sup>[11]</sup>. The mini pills tablets contained 0.5 mg lynestrenol (Organon Oss Holland, Holland). Combined pills tablet contained 0.105 mg combined hormones (Kahira Pharm, Cairo, Egypt). Drugs were given orally using special blunt-tipped needle fixed on an ordinary syringe.

###### 2 -The animals

Fourteen adult female *Oryctolagus cuniculus* rabbits with an average body weight of about 1.5 kg were obtained from the farm of the Egyptian Organization of Biological Products and Vaccines in Helwan, Cairo. They were kept under good hygienic conditions and fed ad libitum and allowed free water supply.

The animals were divided into the following groups:

###### A- Control group:

Consisted of two animals that did not receive any drugs.

###### B- Mini pills group:

Consisted of six animals that received a daily dose of 0.0035 mg/day in 0.2 ml distilled water. This dose is equivalent to the daily therapeutic dose of human 0.5 mg/day.

###### C- Combined pills group:

Consisted of six animals that received a daily dose of 0.00735 mg/day in 0.2 ml distilled water and was equivalent to therapeutic dose of human 0.105 mg/day

#### II-Methods:-

Twenty four hours after the last dose, all the animals were sacrificed by decapitation and then dissected.

##### 1- For histological and histochemical examination:

The excised organs (cervix) were fixed in Carnoy's solution for about four hours, cleared in xylene and impregnated in parablax for blocking. Sections of 5 um thickness were prepared and stained with hematoxylin and eosin ,periodic acid Schiff's reaction , Masson's trichrome and orcein.<sup>[12]</sup>

##### 2-Morphometric measurements:

The mean area percent was measured for elastic fibres content and for collagen content. Also the optical density for PAS reaction in cervical tissues was done using the "Leica Quin 500C" image analyzer computer system (Leica Imaging System Ltd., Cambridge, England). All measurements were done within 10 non-overlapping fields/section for each animal, at 400 magnification, in a standard frame.<sup>[13]</sup>

##### 3-Statistical analysis:

The morphometric results were expressed as mean  $\pm$ SD. Statistical analysis was carried out using the "SPSS 9.0 for Windows" statistical software. Comparison between different groups was done using oneway analysis of variance (ANOVA) followed by post hoc (tucky) test. The results were considered statistically significant when "P" value was < 0.05.<sup>[14]</sup>

#### RESULTS

##### - Cervix Histological Results

###### I- Control group

The cervical mucosa contains prominent branched and simple glands, lined with simple columnar epithelium. The underlayer lamina propria is rich in blood vessels (Fig.1).

The muscularis externa consists of inner circular and outer longitudinal smooth muscle layers (Fig. 2).

Periodic Schiff's reaction shows positive reaction in the mucosal glands at the upper borders of lining epithelial cells and in its basement membrane (Fig. 3).

Normal elastic fibres content are seen in between the muscular layer (Fig. 4).

Collagenous bundles were observed in cervical mucosa, lamina propria and between the smooth muscles of the muscular layer (Fig. 5&6).

#### II- Mini group

Markedly proliferated and branched cervical glands were present with highly mitotic figures of its lining epithelium, also lamina propria was rich with blood vessels (Fig. 7).

Strong positive PAS reaction was observed in the apical cup of the cytoplasm of most of the lining epithelium of the cervical glands and in the basement membrane. Lamina propria fibres as well as the walls of the blood vessels showed moderate staining affinity (Fig.8).

An increased elastic fibres were observed in all layers of the cervix like, serosa and muscular layer underneath especially in between the longitudinal smooth muscles (Fig. 9).

Also, the muscular layer showed an increased elastic fibres in between the longitudinal smooth muscles fibres. The elastic fibres appeared in groups and fibres lay parallel to each other (Fig. 10).

The collagen showed a marked increase in both lamina propria and in between the cervical glands (Fig. 11).

The muscular layer also showed increased collagenous bundles in between the smooth muscles (Fig. 12).

#### III- Combined pills group

Estrogen combined with progesterone showed a great effect on the cervical glands. These effects make the glands be more highly branched and proliferated. Hypertrophied

muscles in muscular layer were seen (Fig. 13).

The glands of cervical mucosa were highly branched and lamina propria appeared highly vascularized (Fig. 14).

Extensively positive PAS reaction was shown in the whole cytoplasm of the lining epithelium of the cervical glands (Fig. 15).

Highly elastic fibres were present. These fibres aggregated as thick wavy bundles in between the muscular layers (Fig. 16).

The collagenous fibres were markedly increased in the mucosal layer, lamina propria and between the muscular layer (Fig.17, Fig.18).

#### Cervix morphometric analysis:

Application of ANOVA test showed that there was a significant increase in mucin content; this result was obtained on measuring the area percent of total mucin content of combined and mini pills -treated rabbits compared to the untreated animals (Fig. 19, table 1). The same results were obtained on measuring the area percent of collagen content of combined and mini pills - treated rabbits compared to the untreated animals (Fig. 20, table 1) and also obtained on measuring the area percent of elastic fibres content (Fig. 21, table 1).

#### DISCUSSION

Contraceptive pill treatment induced some histological changes in the cervical tissue, where clear cellular hyperplasia was seen in the cervical glands and increased mucus content was very clear in both combined and mini pill treatments. These results were supported by **Schnare**<sup>[15]</sup> who reported that the primary mechanism of the contraceptive pills is by thickening cervical mucus and impeding the progress of spermatozoa, thus preventing fertilization.

Cervical cells have hormonal receptors and undergo histological changes with the use of contraceptive pill according to **D'Souza**<sup>[9]</sup>.

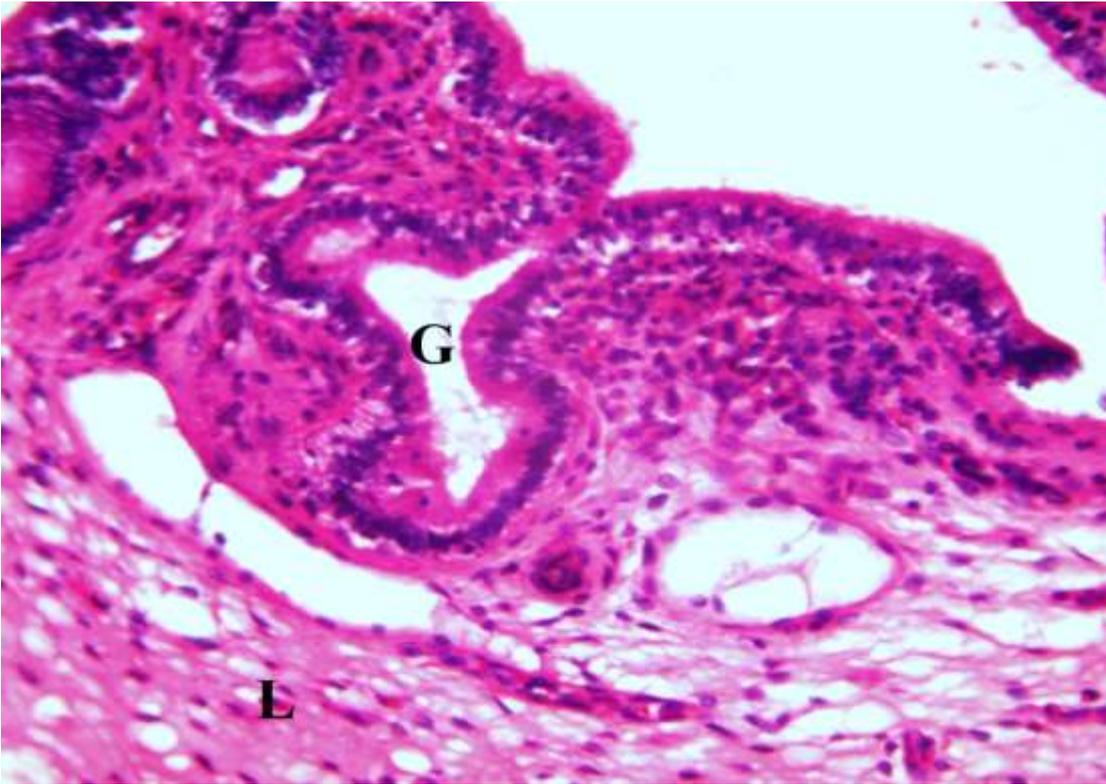
**Franceschi**<sup>[16]</sup> revealed that long-term use of contraceptive pills (5 or more years) may be associated with an increased risk of cancer of the cervix.

**Irwin**<sup>[17]</sup> suggested that using of contraceptive pills caused cervical cancer due to the increased susceptibility to sexual transmitted infections by enlarging the area of cervical ectopy, changing the cervical mucus, or altering the immune response, so steroids have been shown to promote the development of cervical cancer in animals.

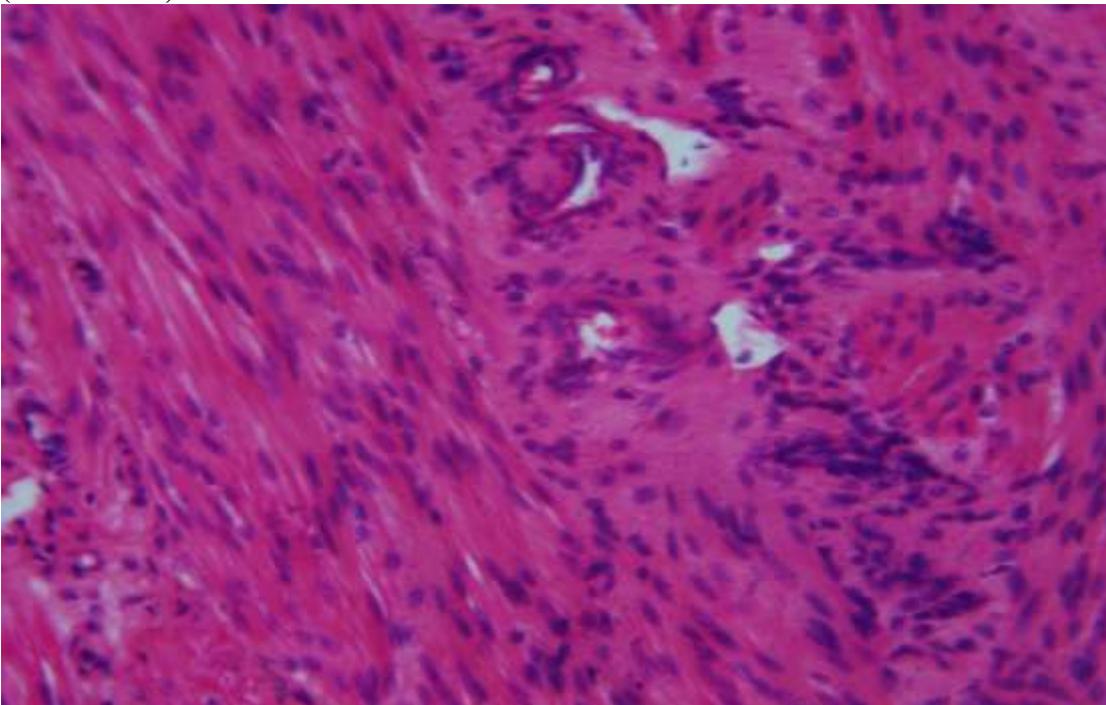
Also, a marked increase in the collagenous and elastic fibres content was seen in the lamina propria and myometrium of cervix either in mini pill treatment or in combined pill treatment by the action of contraceptive pills. This may be a sign of fibrosis which may be followed by cancer appearance. The present results are in agreement with those of **Chiaffarino<sup>[18]</sup>** and **Millas<sup>[19]</sup>** who reported that contraceptive pills directly related to uterine fibroids and they affect fibroblasts which in turn responsible for elastic fibres production.

### References

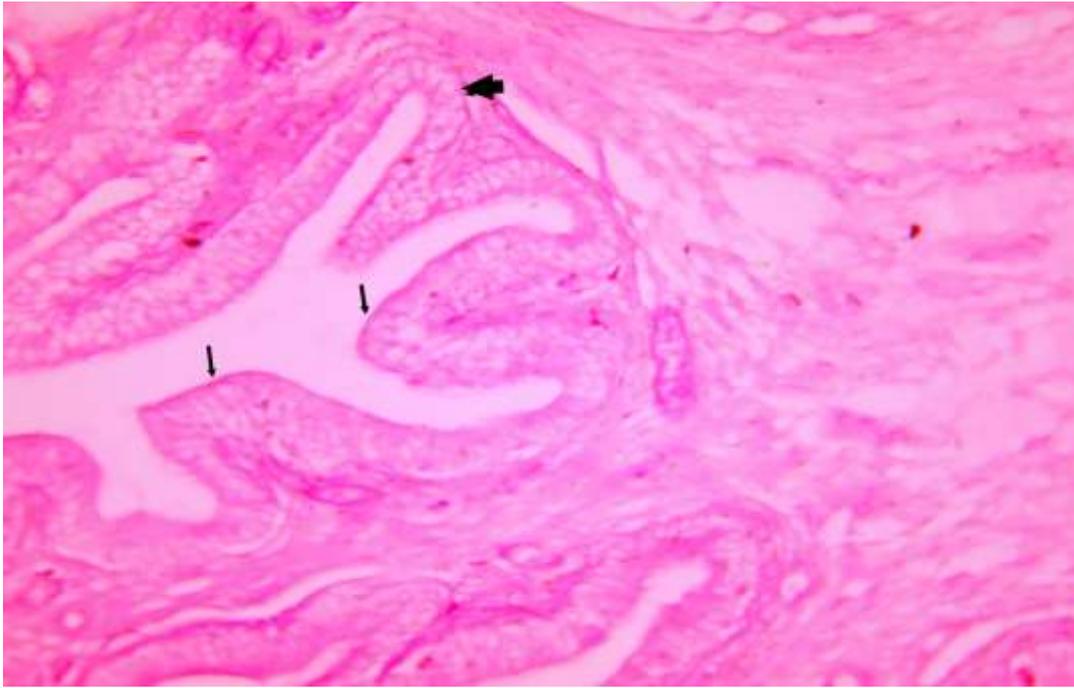
- 1-Monica V D (2014):** The combined oral contraceptive pill- recent developments, risks and benefits. *J. Best Pract. Res. Clin. Obst. Gyn.*, 28(6): 825–834.
- 2-Kaneshiroa B, Edelman A, Carlsons N E, Nichols M, Forbes M and Jensen J ( 2012):** A randomized controlled trial of subantimicrobial-dose doxycycline to prevent unscheduled bleeding with continuous oral contraceptive pill use . *J. Contracep.*, 86(1):22-27.
- 3-Rebecca L, Olufemi A and Roger A (2006) :** Ovarian follicular dynamics during conventional vs. continuous oral contraceptive use. *J. Contracep.* , 73:235– 243.
- 4- Mitchel D C, Joel S L, Scott E D, Amy J G and William O (2002):** The effect of extending the pill-free interval on follicular activity: triphasic norgestimate/35 µg ethinyl estradiol versus monophasic levonorgestrel/20 µg ethinyl estradiol. *J. Contracep.*,66(3):147-152 .
- 5-Hatcher R A, Trussell J, Nelson A L, Cates W, Stewart F H and Kowal D (2007):** *Contraceptive technology: 9<sup>th</sup> edn.* Ardent Media, New York, NY, pp.19–47.
- 6-Speroff L and Darney D B ( 2005):** *A Clinical Guide for Contraception,5<sup>th</sup> edn.* Lippincott, Williams & Wilkins,Philadelphia,USA.
- 7-Hemrika D J, Slaats E H, Kennedy J C, Robles-Korsen T J, and Schoemaker J (1993):** Pulsatile luteinizing hormone secretion during the first and the fourth cycle on two different oral contraceptives containing gestodene.*J. Acta Endocrinol. (Copenh)* ,129:229–236.
- 8-Korvera T, Klipping C, Heger-Mahnc D, Duijkers I, van Ostad G and Thom D (2005):** Maintenance of ovulation inhibition with the 75-µg desogestrel-only contraceptive pill (CerazetteR) after scheduled 12-h delays in tablet intake. *J. Contracep.*, 71 : 8 – 13.
- 9-D'Souza R E and Guillebaud J (2002):** Risks and benefits of oral contraceptive pills .*J. Best Pract. Res. Clin. Obstet. Gynaecol.*, 16 (2) :133-154.
- 10- Lizarelli P M, Martins W P, Vieira C S, Soares G M and Antônio S (2009):** Both a combined oral contraceptive and depot medroxyprogesterone acetate impair endothelial function in young women.*J. Contracep.*,79(1):35-4.
- 11-Paget G E and Barnes J M (1964):** *Evaluation of drug activities.* 2<sup>nd</sup> edn. Laurence and Bacharach,Academic Press,New York, P. 161.
- 12-Bancroft J and Gamble M (2002):** *Theory and Practice of Histological Techniques.* 5<sup>th</sup> edn., Churchill Livingstone,London.p 231.
- 13-Poggi p, Marchetti c and Scelsi R (2005):** Automatic morphometric analysis of skeletal muscle fibers in the aging man. *J. Anat. Rec.*, 217: 30–34.
- 14-Petrie A and Sabin C ( 2005) :** *Medical Statistics at a Glance.* 2<sup>nd</sup> edn., Sugden M. and Moore K., Blackwell Publishing Ltd. USA. .
- 15-Schnare M S ( 2002):** Progestin contraceptive. *J. Midwif. and Women's Health*, 47( 3):1145-1165.
- 16-Franceschi S (2005):** The IARC commitment to cancer prevention: The example of papillomavirus and cervical cancer. *J. Cancer Res.*, 166:277–297.
- 17-Irwin K L (1996):** The association between oral contraceptive use and neoplasia of the cervix, vagina and vulva. In: *Evidence-guided Prescribing of the Pill.* Parthenon publishing group, London: pp 145-156.
- 18- Chiaffarino F, Parazzini F, La Vecchia C, Ricci E and Crosignani G ( 1998):** Oral contraceptive use and benign gynecologic conditions. *J. contracept.*, 57:11-18.
- 19- Millas I, Liquidato B M, Buck H S, Barros M D, Paes R A and Dolci J E (2010):** Evaluation of estrogenic receptors in the nasal mucosa of women taking oral contraceptives. *J. Contracep.*, 83(6):571-7.



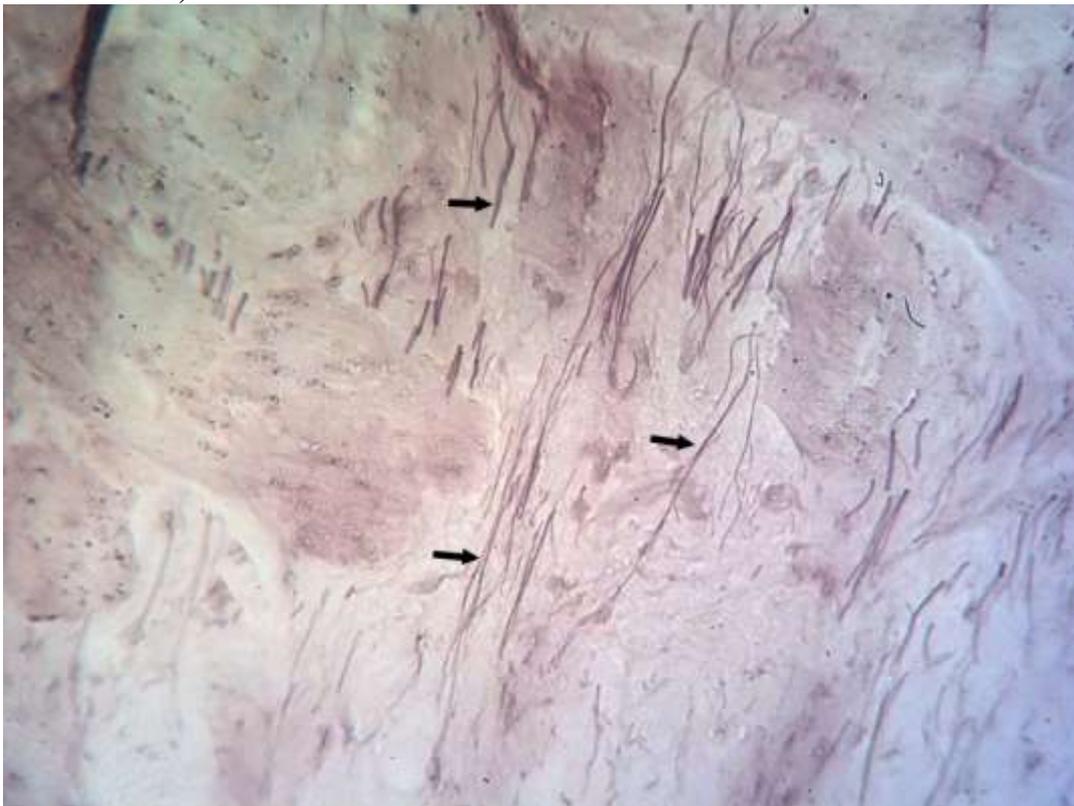
**Fig. 1** A photomicrograph of cervix section of the control rabbit showing mucosa with branched glands (G) lined with simple columnar epithelium and the underlying lamina propria (L) (H&E X400)



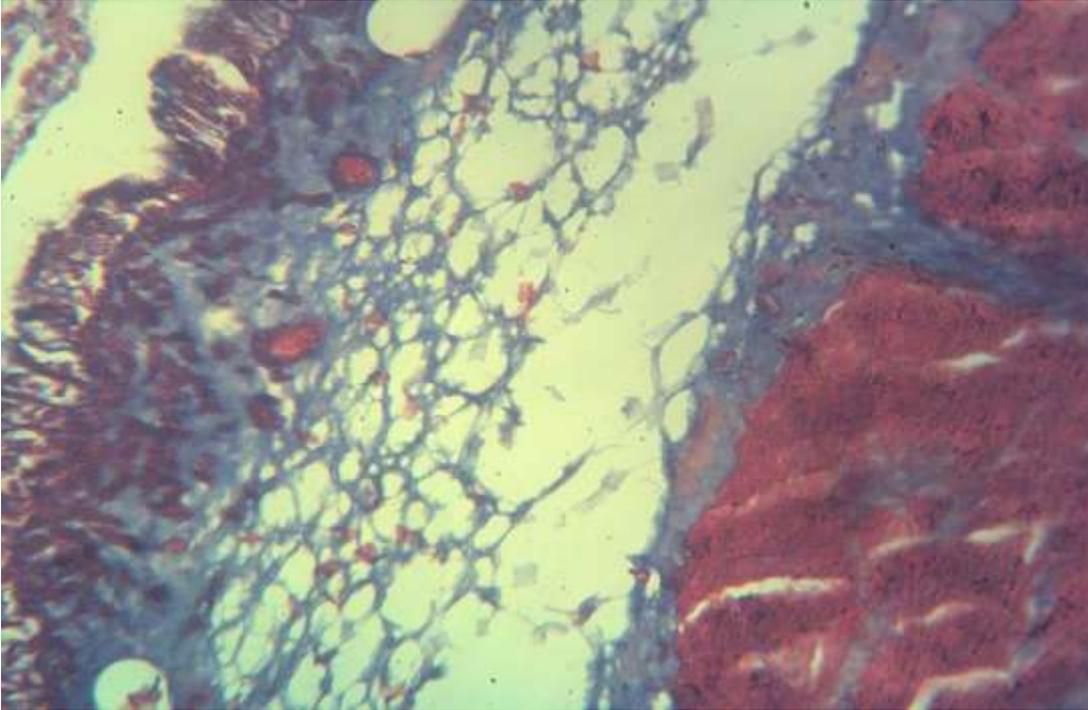
**Fig. 2** A photomicrograph of cervix section of the control rabbit showing the muscular layer (H&E X400)



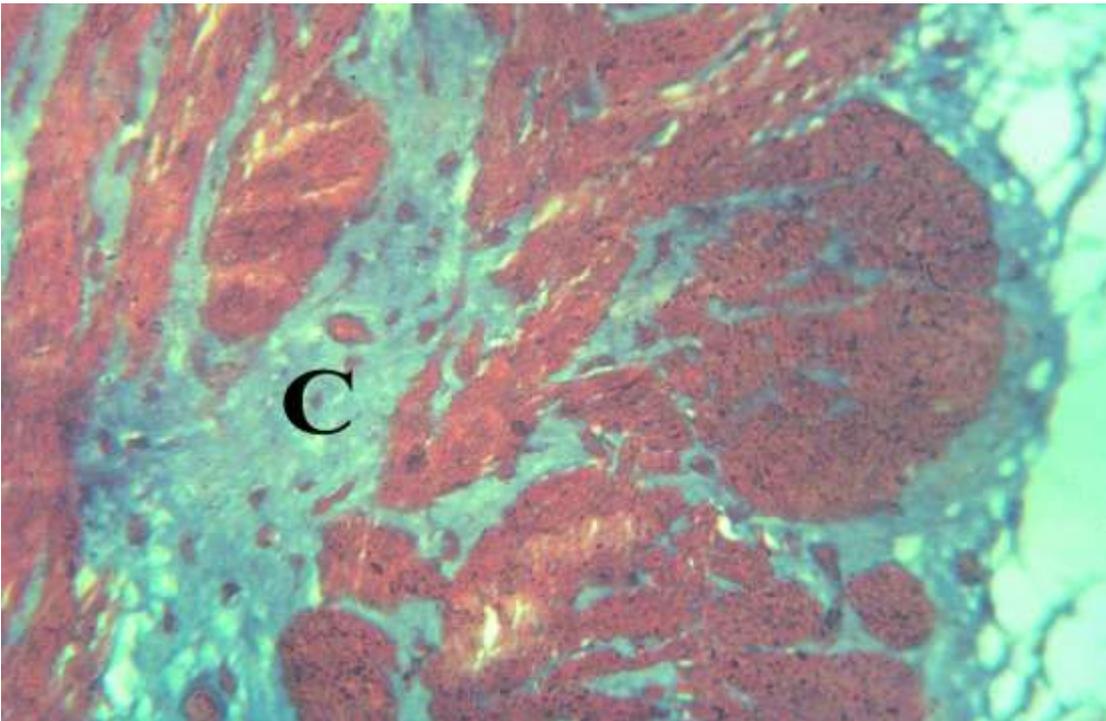
**Fig. 3** A photomicrograph of cervix section of the control rabbit showing the mucosal glands with positive PAS reaction at the upper border of lining epithelium(↓) and its basement membrane(←) . (PAS reaction X400)



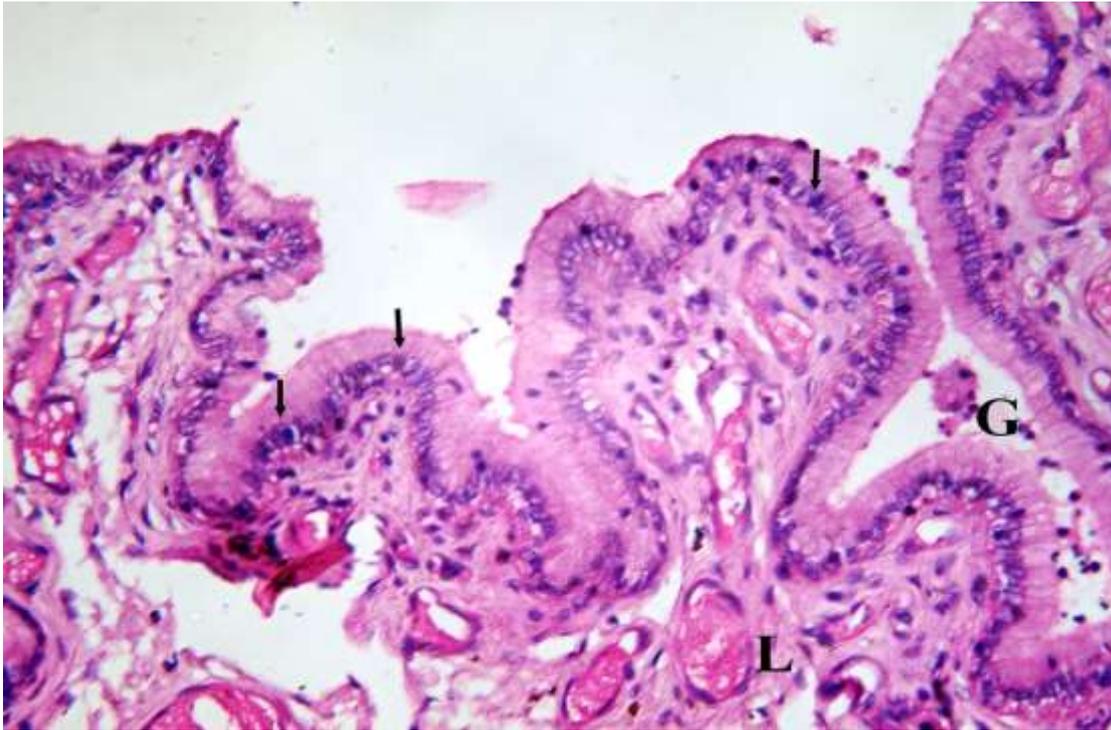
**Fig. 4** A photomicrograph of cervix section of the control rabbit showing thin elastic fibres (→) in between smooth muscles of musculosa . (Orcein stain X400).



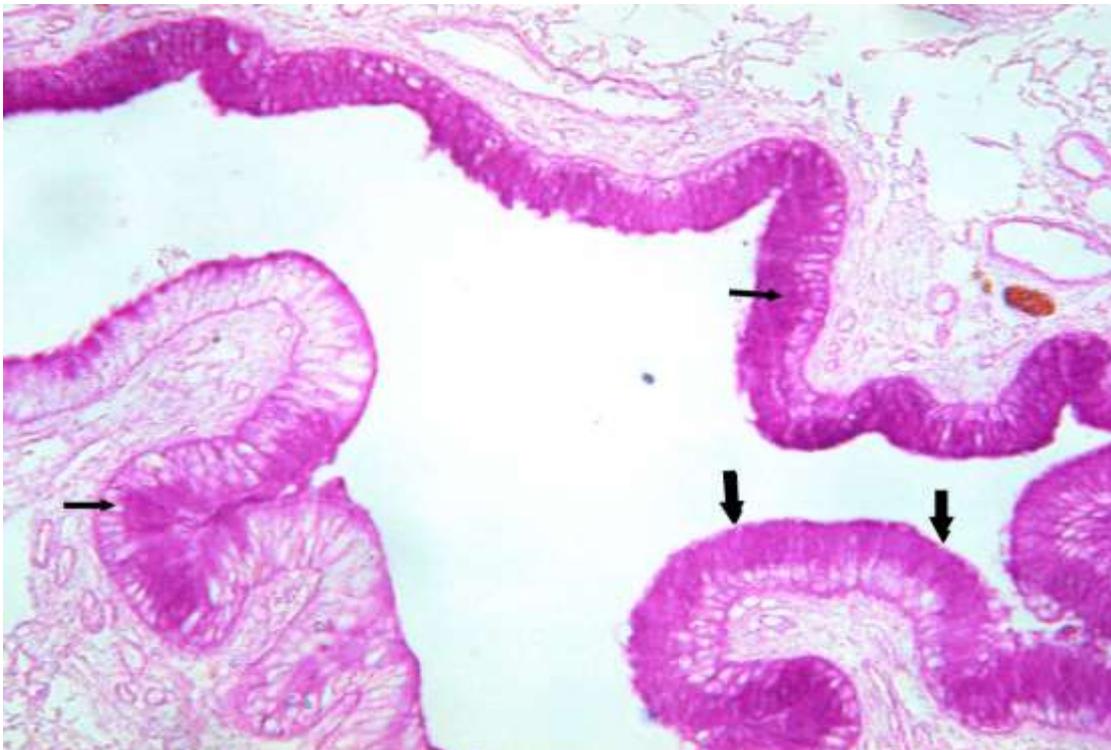
**Fig. 5** A photomicrograph of cervix section of control rabbit showing collagenous bundles ( C ) in the cervical mucosal layer. (Masson's trichrome stain X400)



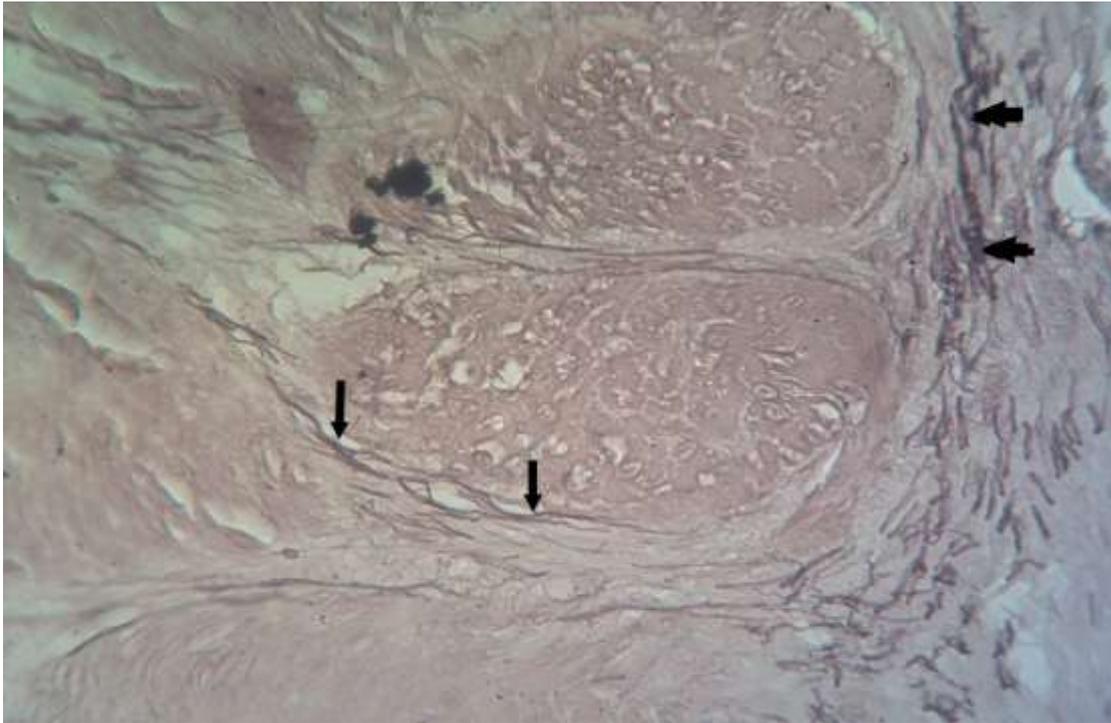
**Fig. 6** A photomicrograph of cervix section of control rabbit showing collagenous bundles ( C ) in between the smooth muscles. (Masson's trichrome stain X400)



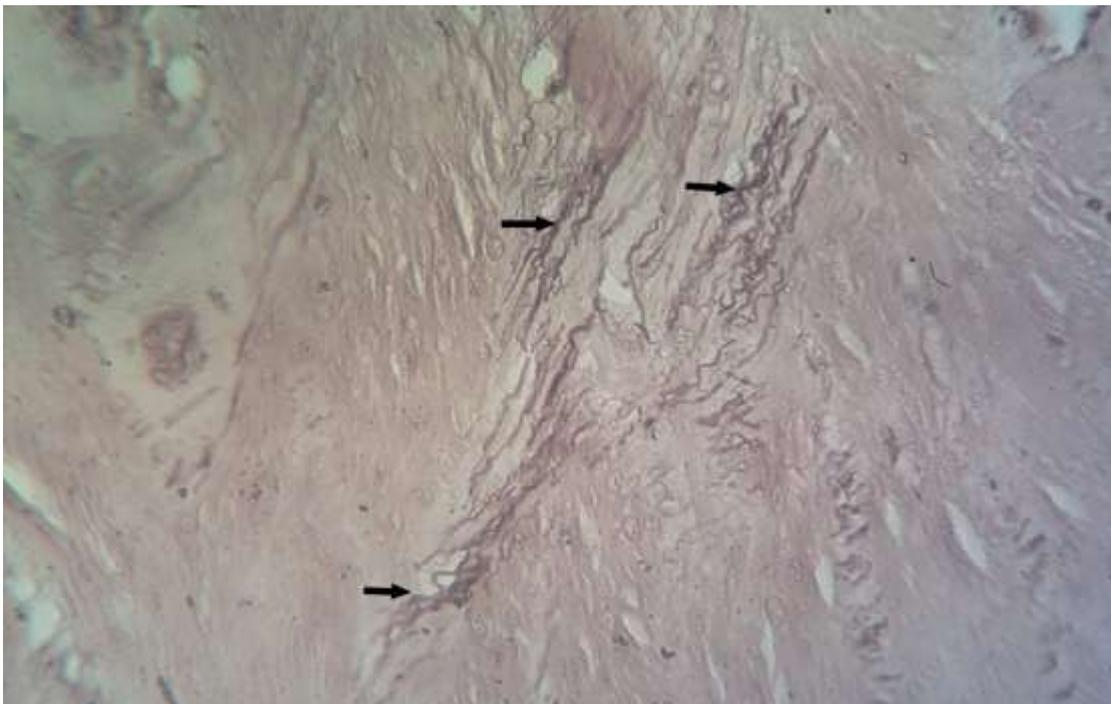
**Fig. 7** A photomicrograph of cervix section of mini pill treated rabbit showing markedly branched cervical glands (G), proliferated epithelial cell lining(↓), also lamina propria (L) is rich with blood supply . ( H&E X400)



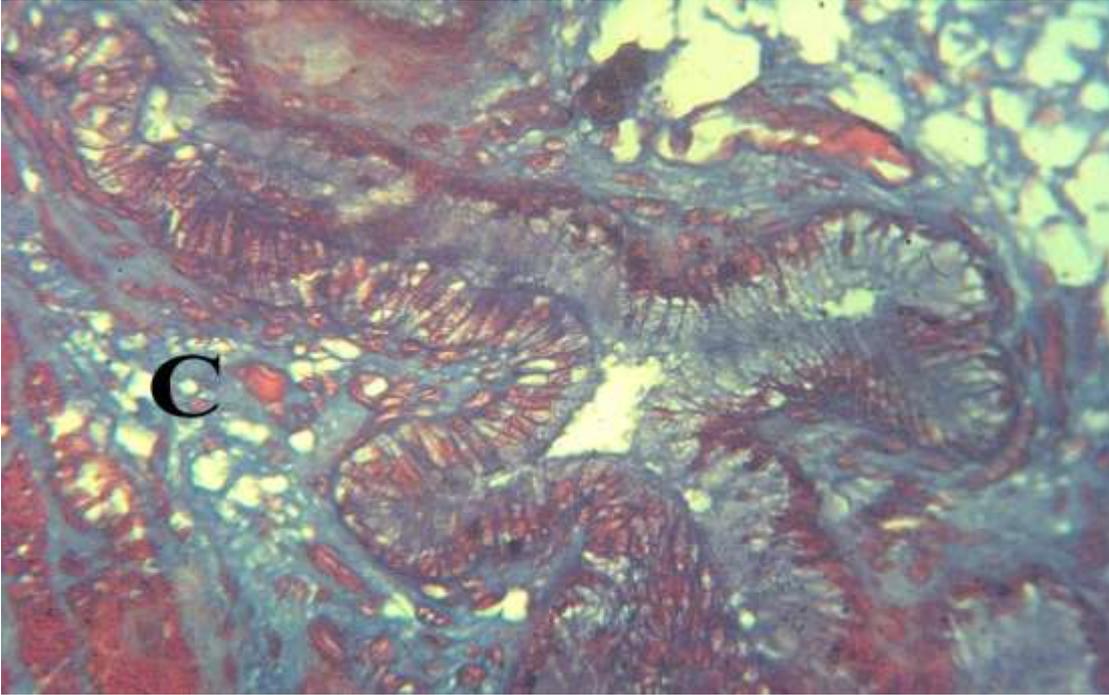
**Fig.8** A photomicrograph of cervix section of mini pill treated rabbit showing strong positive PAS reaction at the border(↓) and the whole cytoplasm (→) of cervical glands lining epithelium . Also, walls of blood vessels and fibres of the lamina propria are moderately positive to the reaction. ( PAS reaction X400)



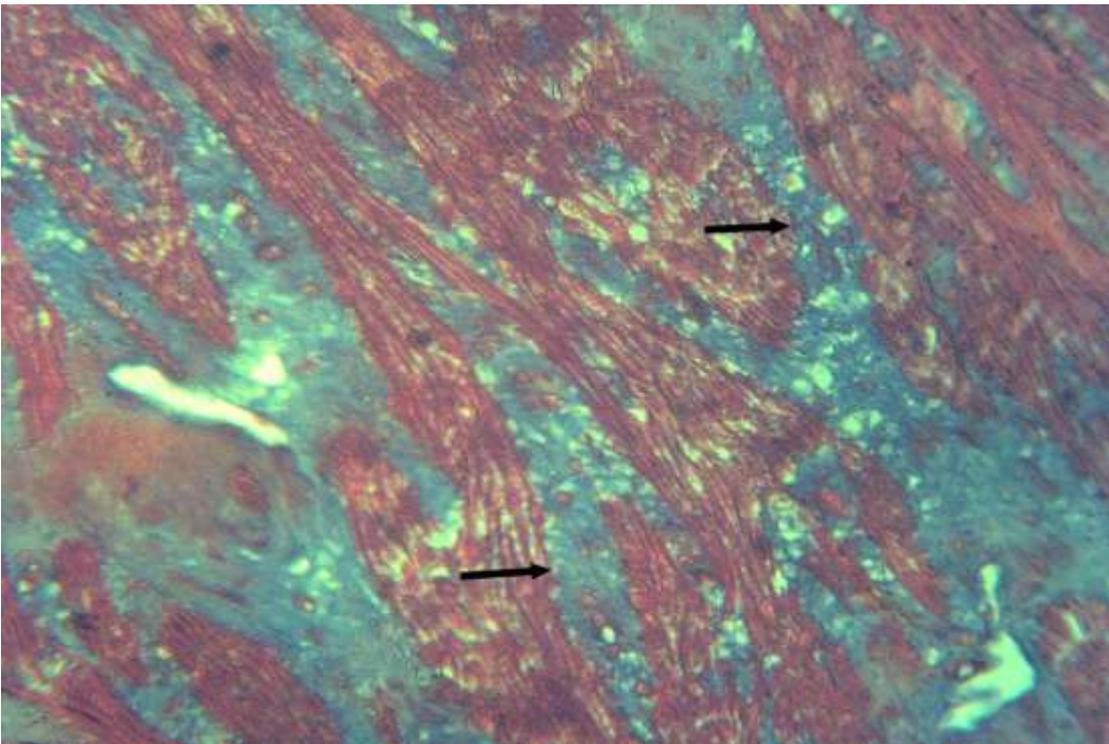
**Fig.9** A photomicrograph of cervix section of mini pill treated rabbit showing increased elastic fibres in serosa (←) and muscular layer underneath (↓) . (Orcein stain X400)



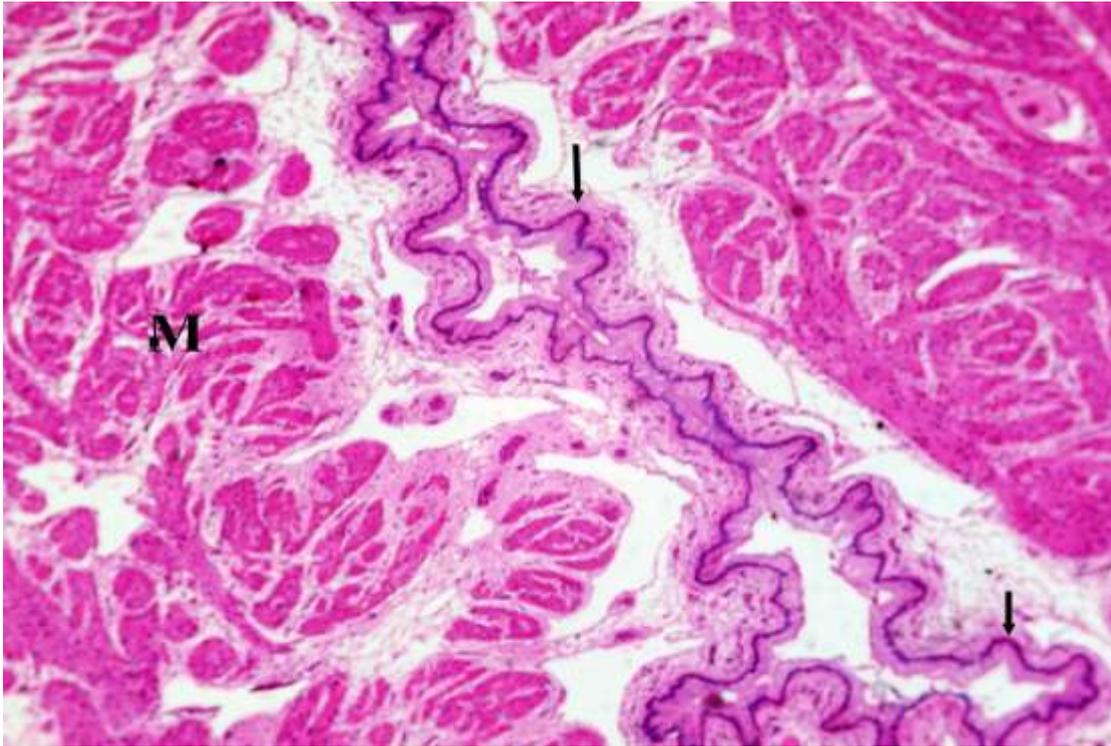
**Fig.10** A photomicrograph of cervix section of mini pill treated rabbit showing increased elastic fibres (→) in the muscular layer (Orcein stain X400)



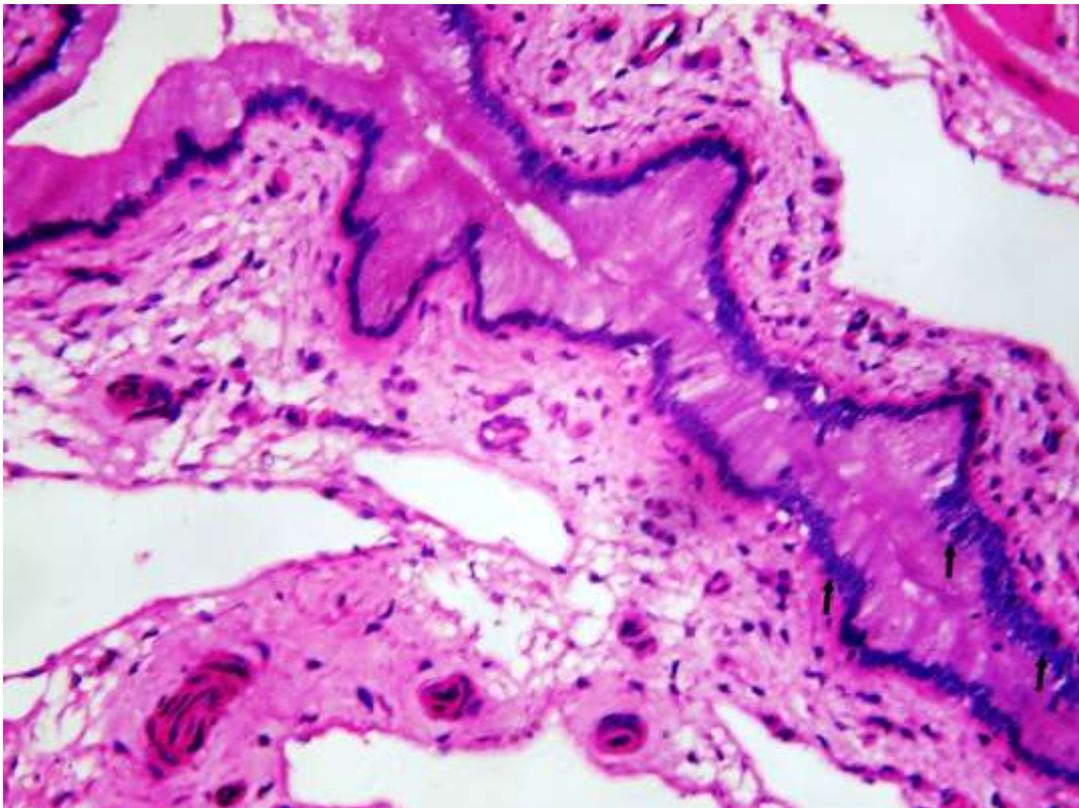
**Fig. 11** A photomicrograph of cervix section of mini pill treated rabbit showing increased collagenous bundles in between cervical glands(C) and in lamina propria .  
( Masson's trichrome stain X400)



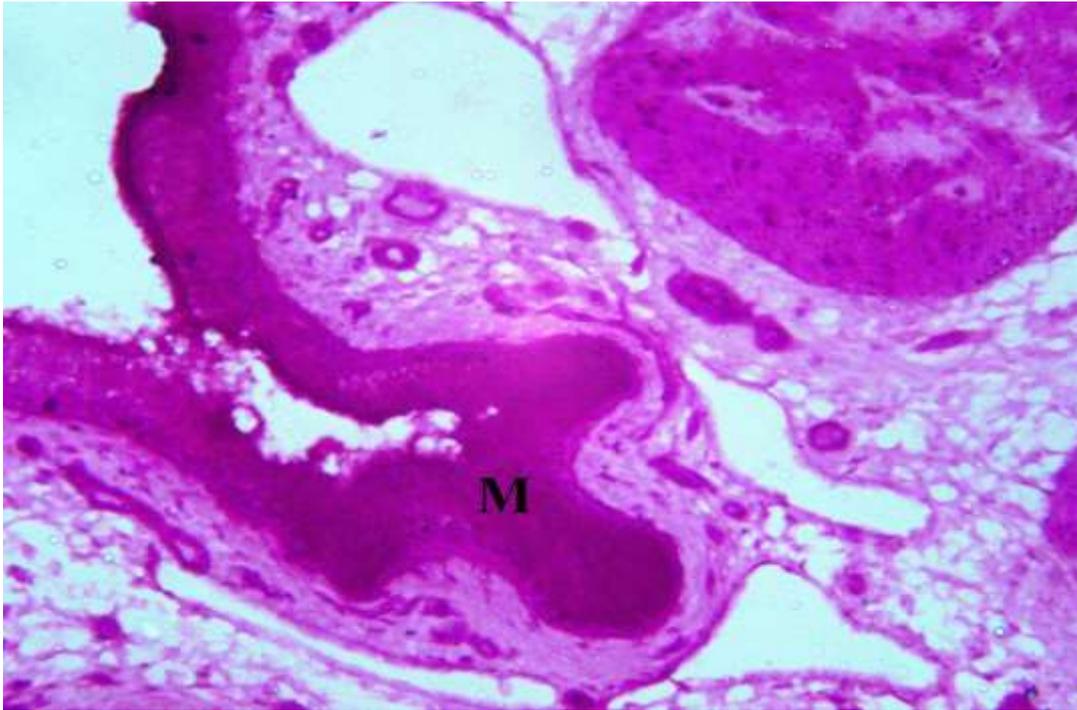
**Fig. 12** A photomicrograph of cervix section of mini pill treated rabbit showing increased collagenous bundles in muscosa (→) .  
( Masson's trichrome X 400)



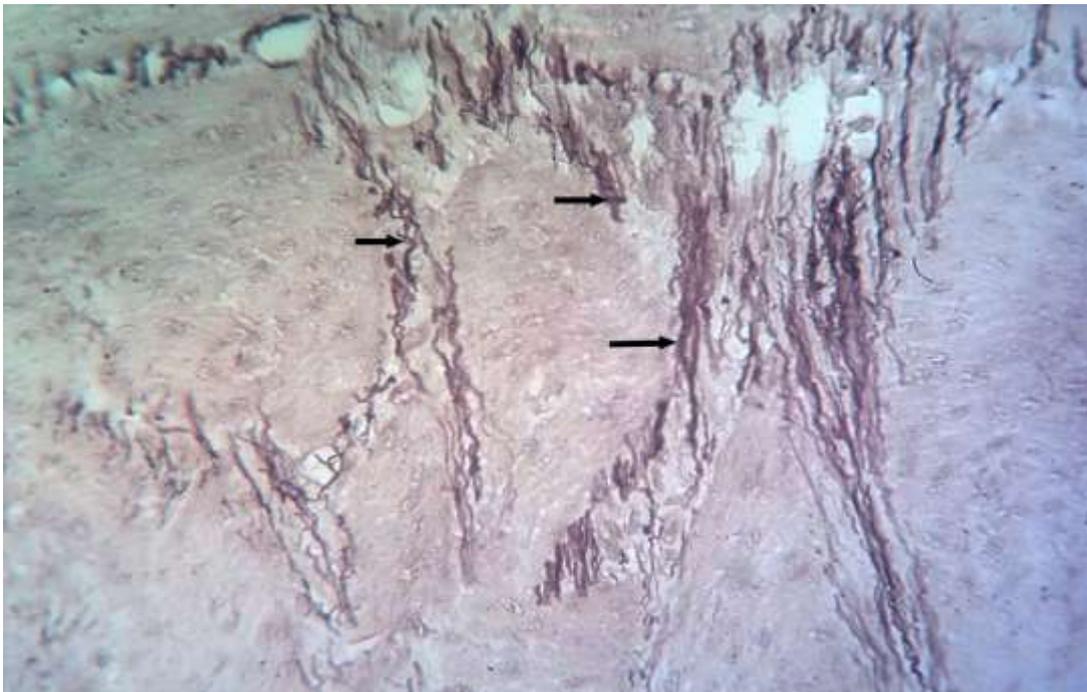
**Fig. 13** A photomicrograph of cervix section of combined pill treated rabbit showing mucosa with highly branched glands (↓) and hypertrophied muscles (L). (H&E X 100)



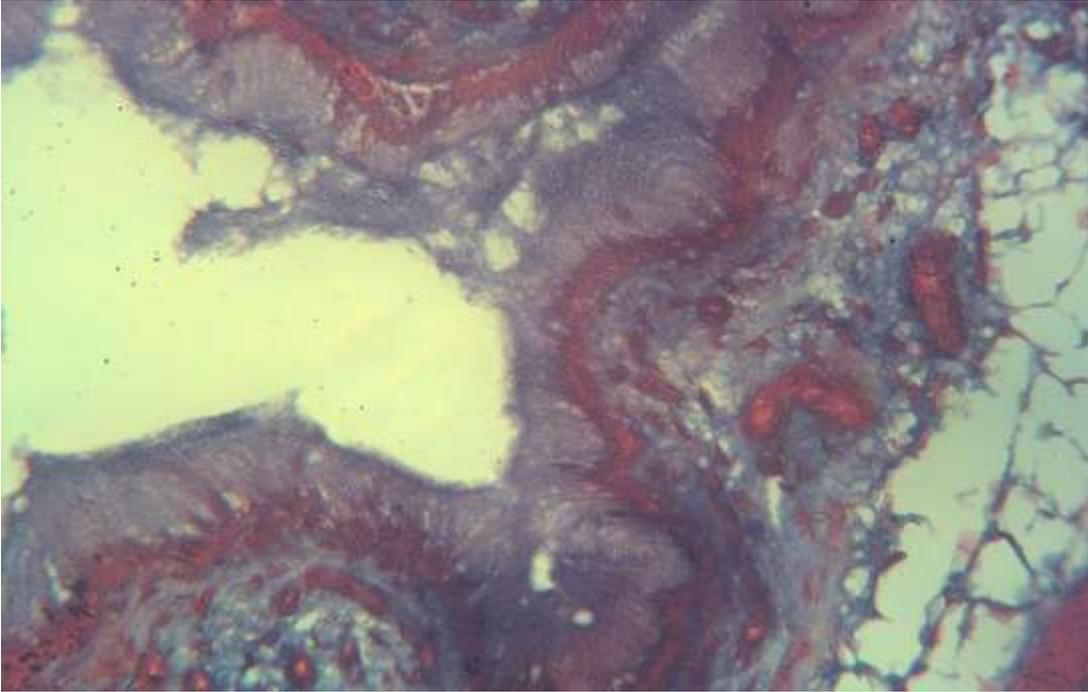
**Fig. 14** A photomicrograph of cervix section of combined pill treated rabbit showing mucosal glands with hyperplastic cells (↑). (H&E X 400)



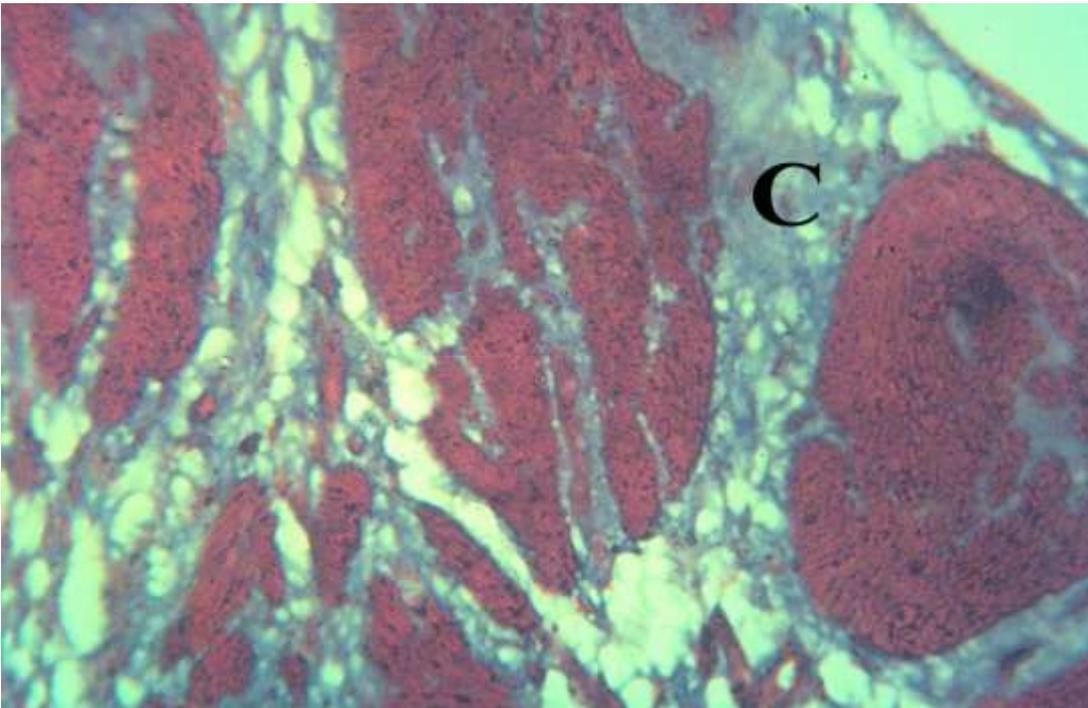
**Fig. 15** A photomicrograph of cervix section of combined pill treated rabbit showing great intensity of positive PAS reaction in the epithelial cell lining the glands (M) .  
( PAS reaction X 400)



**Fig. 16** A photomicrograph of cervix section of combined pill treated rabbit showing highly increased elastic fibres which are aggregated into thick wavy bundles in between the smooth muscles(→) ( Orcein stain X 400)



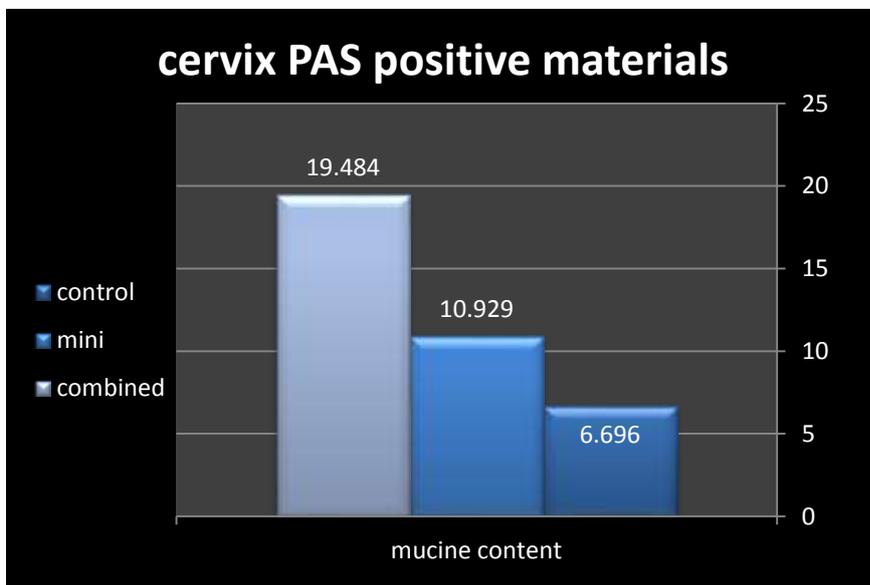
**Fig. 17** A photomicrograph of cervix section of combined pill treated rabbit showing highly increased in collagenous fibres in cervical mucosa and in lamina propria(C) .  
( Masson's trichrom stain X 400



**Fig. 18** A photomicrograph of cervix section of combined pill treated rabbit showing highly increased in collagenous fibres in between the subserosal smooth muscles of the macular layer (C).  
( Masson's trichrom stain X 400)

Parameters	Control	Mini pills	Combined pills
Mean area percent of mucin content±SD	6.6960±0.7002	10.9290±0.9464	19.4840±1.3044
Mean area percent of elastic fibres content±SD	6.023±.8237	9.094±.6091	12.8210±.9961
Mean area percent of collagen content±SD	17.6320±1.6014	22.7530±1.3739	26.2740±1.9984

**Table 1 Significant increase as compared to the different groups.**



**Fig.19 A histogram showing the mean area percent of PAS positive materials**

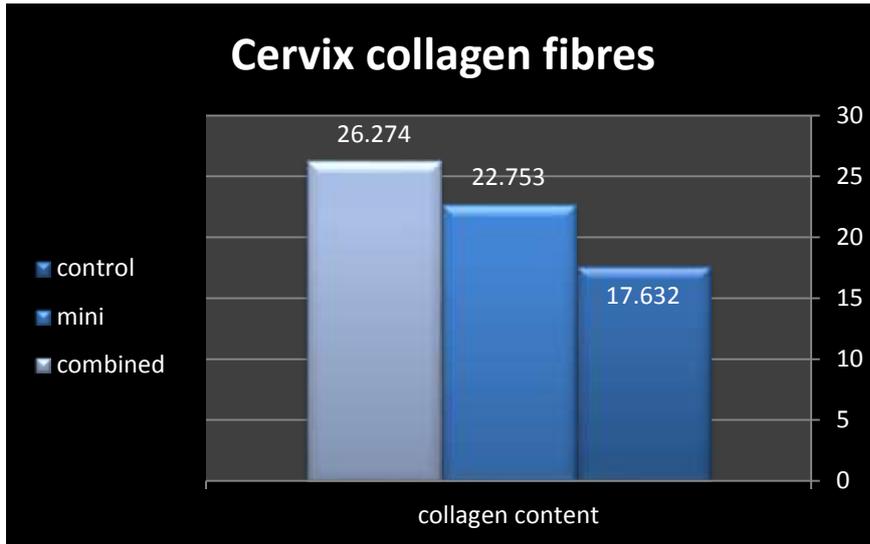


Fig.20 A histogram showing the mean area percent of collagen fibers

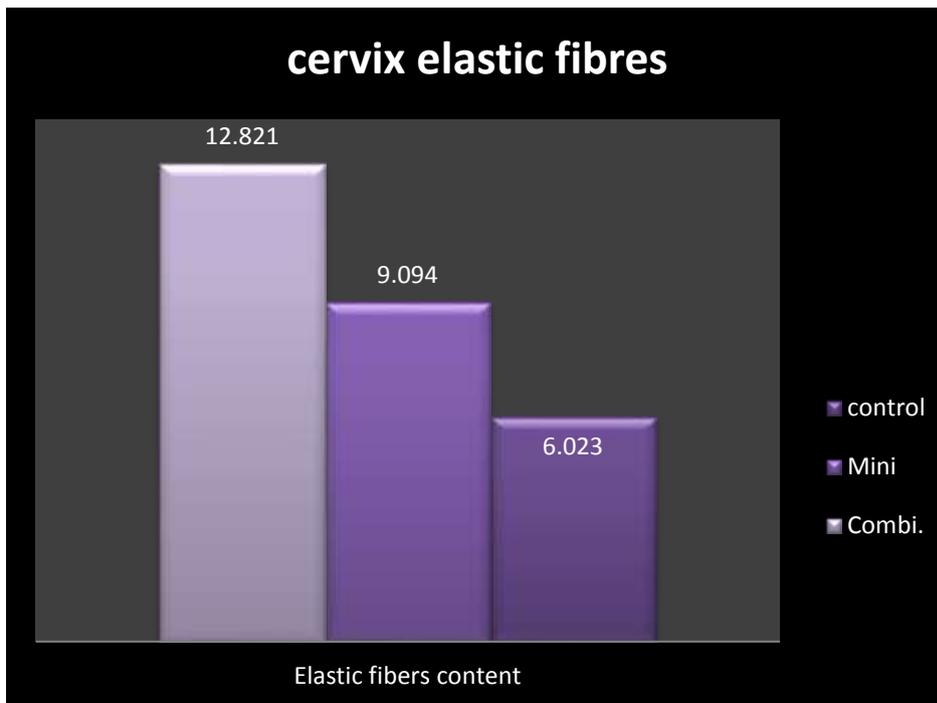


Fig. 21 A - histogram showing the mean area percent of elastic fibres