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RESEARCH ARTICLE

Histpathological and Immunohistochemical Changes Induced by Contraceptive Pills in Female Rabbits Uterus

Gabri MS[2], Abdel kader DH[1], Ibrahim MA[2], Hassan BN[2]

1.Department of histology, Faculty of Medicine,Cairo university,Egypt.

2.Department of zoology and entomology, Faculty of Science, Helwan University,Egypt.

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Corresponding Author

Gabri MS

Abstract

The present study focuses on evaluating the effect of two different contraceptive pills including combined pills (estrogen and progesterone) and mini pills (progesterone only) on the uterus of female rabbit .

After three month of daily oral administration of these contraceptive pills the animals were sacrificed. The excised organs were dissected, processed and stained with H & E, PAS and Masson's trichrome, orcen stain and anti alpha smooth muscle actin immunohistochemical reaction. This was followed by morphometric measurements and statistical study. This study revealed that contraceptive pills administration specially the combined one caused marked alterations in the form of hyperplasia in uterine mucosal cells and hypertrophied muscles in muscular layers. Also, a statistically significant increase in collagenous and elastic fibers content in myometrium layer in the uterus . There was also a statistically significant increase in the PAS reaction in the lumen of the mucosal glands in uterus.an intensive asma immunohistochemical reaction was noticed also in the myometrium smooth muscle. All these changes were less marked after mini pill treatment .

Conclusion: Progesterone only pills could be safer on uterus as a contraceptive mean when compared to combined pills.

INTRODUCTION

The oral contraceptive pill (OCP) brought to market over 50 years ago was designed around a 28-day cycle that includeda 7-day hormone-free interval to induce a withdrawal bleed(Kaneshiro *et al*.,2014).

Initiation of contraception during the postpartum period is important to prevent unintended pregnancy and short birth intervals, which can lead to negative health outcomes for mother and infant. (Tepper *et a*l., 2015)

The conventional dosing scheme was developed to mimic the physiological event of monthly menstruation in nonpregnant women and to provide the illusion of natural menstrual cyclicity. The HFI reportedly has no physiological benefit but was initially included to increase user acceptability (Rebecca *et al*.,2006) .

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.Some studies demonstrate that 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 Creinin *et al*.,2002) . . (

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 prevent ovulation(Hatcher *et al*., 2007). .

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 a LH surge prevent ovulation (Speroff and Darney ,2005) .

ovulation suppression, thinning of the endometrium and cervical mucus thickening is due mostly to progestin and are dose-dependent. It is unclear whether ovulation suppression occurs at the level of the hypothalamus, the pituitary or both (Hemrika ,1993).

Progesteron only pills primarily rely on the induction of viscous cervical mucus, which hampers the penetration of sperm into the female genital tract, and on changes in the endometrium, making it less suitable for implantation (Korver *et al*.,2005).

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 and of a little significance for most women.Notably the risks are greater with combined contraceptive pills (cocs),containing both ethinyleoestradiol and a progesterone,than progesterone only pills(pops)(D'Souza and Guillebaud,2002). .

COC use is associated with an increased risk of developing venous and arterial thromboembolic events. COCs 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 wall, which could explain the increased risk of arterial thrombosis in COC users(Lizarelli *et al*.,2008).

AIM OF THE WORK:

The aim of the present study was to elicite and compare the histological and histochemical changes induced in the rabbit uterus due to the use of two different types of contracaptive pills.

Material and methods:

I - Material:-

1- The drugs:

The drugs used in this work were mini contraceptive pills (progesterone only pills) contained 30 tablet for group of animals during lactation , and combined pills(progesterone and estrogen) for another group contained 21 tablets of the hormone and seven hormone free tablet which represents the hormonal free interval and were given to mature non lactating females. The tablets in both types were crushed to powder and dissolved in distilled water and their doses were calculated according to the interspecies dosage conversion scheme of Goush (Paget and Barnes,1964).The mini pills tablets contained 0.5 mg lynestrenol(Organon Oss Holland , Holland). While each Combined pills tablet contained 0.105 mg combined hormones (Kahira pharm,Cairo,Egypt) They were given oraly using special blunt-tipped needle fixed on an ordinary syringe, as a daily dose for three months.

2 -The animals

Fourteen adult female 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 exiperimental animals were divided into the following groups:

A- Control group:

Animals did not receive any drug or vehicle.

B- Mini pills group:

Consisted of six animals each received a daily dose of 0.0035 mg/day of progesterone only pills in 0.2 ml distilled water .This dose equivalent to the daily therapeutic dose for human(0.5 mg /day).

C- Combined pills group:

Consisted of six animals that received a daily dose of 0.00735 mg/day; of combined pills each dose was calculated to be in 0.2 ml distilled water and was equivalent to therapeutic dose for human(0.105 mg/day).

II-Methods:-

Twenty four hours after the last dose all animals were sacrificed by decapitation and then dissected and subjected to the following:

1- For histological and histochemical examination

The excised organs (uterus) were fixed in Carnoy solution for about four hours, cleared in xylene and impregnated in parablast for blocking , sections of 5 micron thick were prepared and stained with Hematoxylin and Eosin ,Periodic acid Schiff's , Masson's trichrome and orcen (Bancroft and Gamble,2002).also anti ASMA immunohistochemical reaction has been done for both uterus and cervix .

2-.Morphometric measurements:

The mean area percent was measured for elastic fibers content ,and for collagen content,also for anti-ASMA immune histochemical reaction and the optical density for PAS reaction in uterin 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 .(Paola et al.,2011) .

4-Statistical analysis:

The morphometric results were expressed as mean ±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 (Petrie and Sabin, 2005).

RESULTS

-Uterus Histological Results

I- Control Group:

The endometrium is lined by simple columnar epithelium(the mucosa),which is supported with a lamina propria very cellular connective tissue rich in blood vessels. Also lamina propria contains tubular glands which open on the mucosal surface(fig.1).

The epithelium lining the uterin glands is simple columnar and containing both secretory and nonsecretory ciliated cells(fig.2).

`Both the luminal surface of the mucosa and the interlacing fibers of the lamina propria showed moderate +ve PAS reaction (fig.3).

Orcen stain showed the lamina propria containing moderat amount of elastic fiber (fig.4).

The same picture is present in thickened middle layer of myometrium showing moderat elastic fibers content in between the smooth musle (fig.5).

endometrium and lamina propria showed moderat collagenous fibers content specially around the endometrial glands. (fig.6),also myometrium showed moderate collagenous fibers content in between the smooth muscles (fig.7).A mild positive immune histochemical reaction is observed for the anti smooth muscle actin in the smooth muscle of the endometrium.fig.7a

2- Mini bills treated Group:

After mini bills treatment to female rabbits and under the effect of progesterone a clear histological changes were observed where the lining epithelium of the uterine glands as well as the mucosal cells appeared mostly to be shorter than normal while very few cells still looks like normal in its hight.Also lamina propria became more compact and less vascularized (fig.8).

Few parts of the uterin glands showed cell proliferation and some parts of them showed hyperplasia (fig.9).

With high magnification the nuclei are deeply stained ,rounded and also peripheral chromatin appear in most cases and some signs of proliferation of the lining cells of uterin glands were observed (fig.10).

A moderately increase in positive PAS rection was seen in the apical border and the basement membrane of the uterin glanduler cells as well as the lamina propria (fig.11).

The elastic fibers content also increased in between uterin muscles (fig.12) .

A marked increase in the collagenous content were noticed inbetween the endometrial glands (fig.13).

These increase in the collagenous content were also seen in between the uterin muscles (fig.14). A moderate positive immune histochemical reaction is observed for the anti smooth muscle actin in the smooth muscle of the endometrium.(fig.14 a)

3- Combined bills treated Group:

After both estrogen and progesterone administration an intensive histological and pathological changes were observed ,A great number of undulating glands present,their lining epitheluim increased in number and hight which may be hyperplastic phenomena,also condensed lamina propria was seen(fig.15).

Also, hypertrophied muscles appeared in myometrium (fig.16).

With high magnification A highly branched gland with hyperplastic cells and much more glanduler secretion were seen(fig.17).

A strong and granular positive PAS reaction in the apical cytoplasm of the epithelial cells lining the uterin glands (fig.18).

An intensive increase in elastic fiber content were observed in all uterin layer (fig.19).

Orcen stain showed a huge quantity of elastic bundles in between the hypertrophied myometrial muscles (fig.20).

Also, these intensive increase in elastic fibers were seen in the form of bundles in the middle thickened muscular layer of myometrium. (fig.21).

The collagenous content ,also, showed agreat increase inbetween the endometrial glands and in lamina propria.(fig.22) and (fig.23).

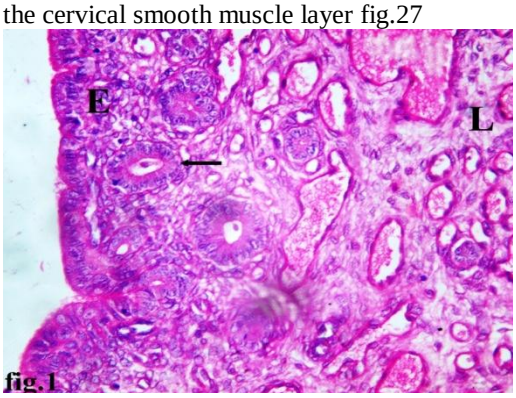
A great increase in the collagenous content were seen in between the myometrium smooth muscle (fig.24). An intensive positive immune histochemical reaction was observed for the anti smooth muscle actin in the smooth muscle of the endometrium.fig.24a

For cervix immune histochemical results

Control group showing a mild positive immune histochemical reaction for the anti smooth muscle actin in the cervical smooth muscle layer. (fig 25)

Mini pills group showed moderat positive immune histochemical reaction the anti smooth muscle actin in the cervical smooth muscle layer fig.26

Combined pills group showed an intensive positive immune histochemical reaction the anti smooth muscle actin in the cervical smooth muscle layer fig.27



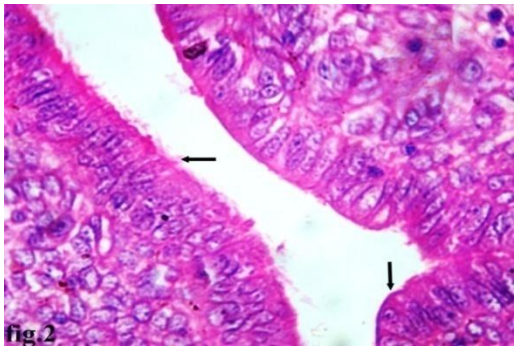
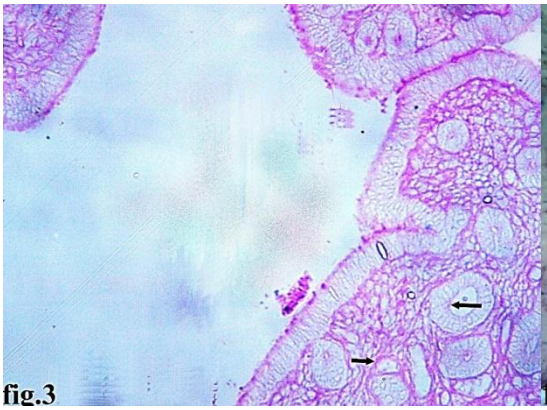


Fig.1 A photomocrograph of uterus section of control rabbit showing endometrium(E) with simple tubuler uterin glands (←)and lamina propria(L) rich in blood vessels H&E X400) .

Fig.2. A photomocrograph of uterus section of control rabbit showing normal partialy ciliated(←) and partially secretory simple columnar epithelium(↓) lining uterin glands. H&EX1000) .



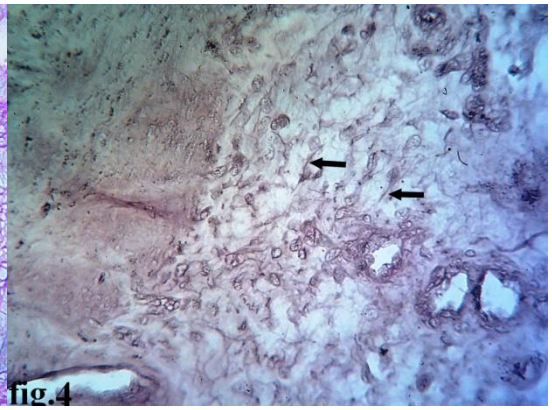


Fig.3 A photomocrograph of uterus section of control rabbit showing +ve PAS reaction in the apical border(←) of the epithelial cells lining the uterin glands and their basement membrane(→) ,as well as the interlacing fibers of the lamina propria. PAS X 400

Fig.4 A photomocrograph of uterus section of control rabbit showing normal elastic fiber content(←) in lamina propria orcenx400

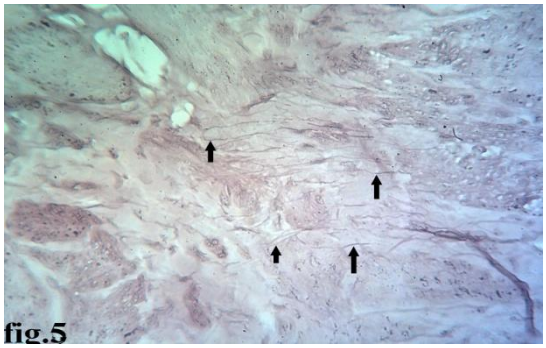


fig.5

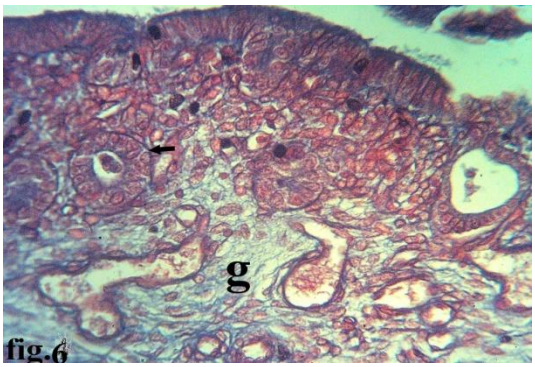


fig.6

Fig.5 A photomocrograph of uterus section of control rabbit showing normal elastic fibers content in middle part of myometrium in between the smooth muscle(↑) Orcen X400
Fig.6 A photomocrograph of uterus section of control rabbit showing normal collagenous fiber content in lamina propria which is highly vascularized (g) and around the uterin glands(←) Masson's trichromX400

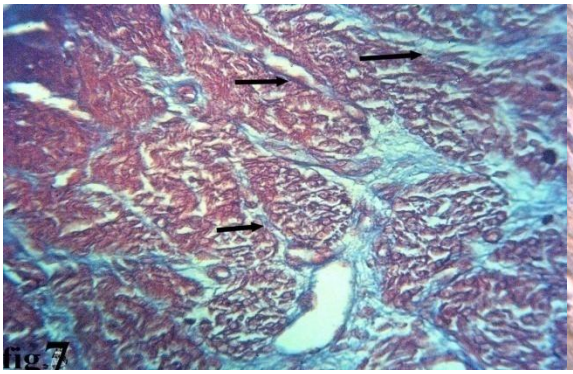


fig.7

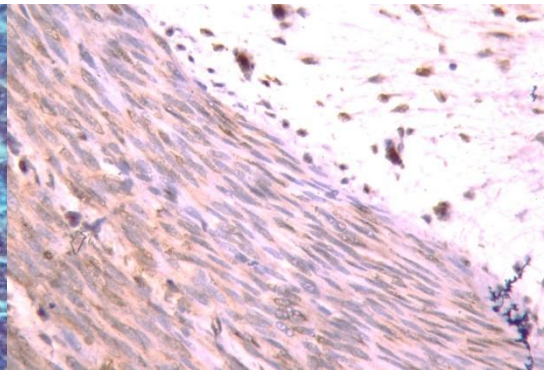


fig.7a

Fig.7 A photomocrograph of uterus section of control rabbit showing normal collagenous fiber in between the smooth muscle bundles of myometrium (→) Masson trichromX400)
Fig.7a A photomocrograph of uterus section of control rabbit showing mild positive immunoreactivity in myometrium and lamina propria (Anti – α- SMA X 400)

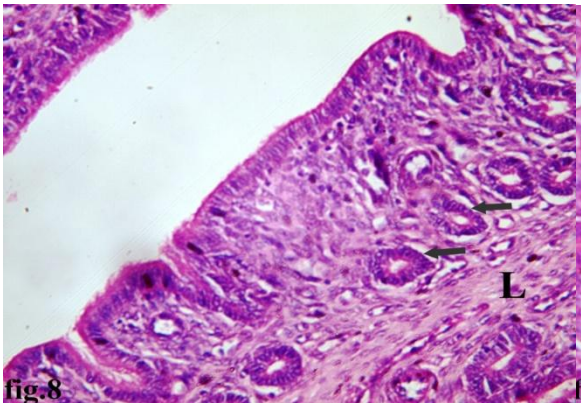


fig.8

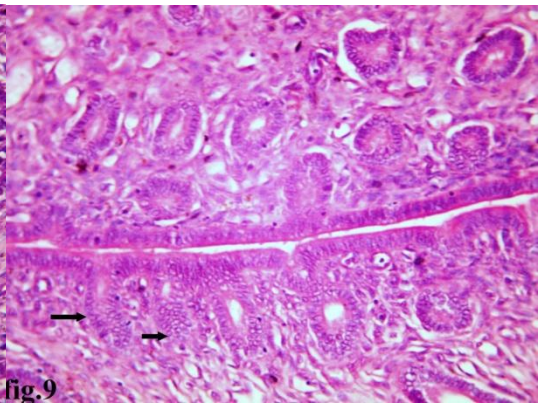


fig.9

Fig.8 A photomocrograph of uterus section of mini pill treated rabbit showing small size and less hight uterin glandular cells(←) ,lamina propria become more compact and less vascularized than normal one. H&E X 400).
Fig.9 A photomocrograph of uterus section of mini pill treated rabbit showing some uterin glands with proliferated (→) lining epithelium and some others with small size and less hight cells(H&E X 400).

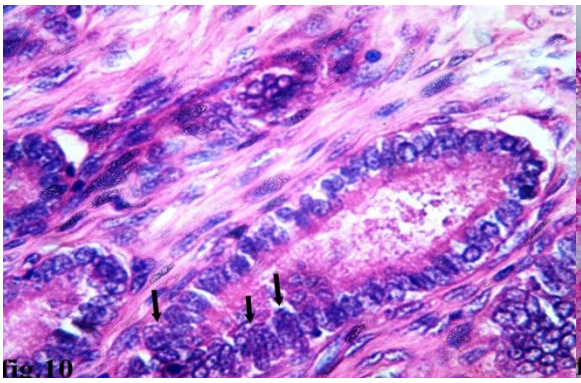


fig.10

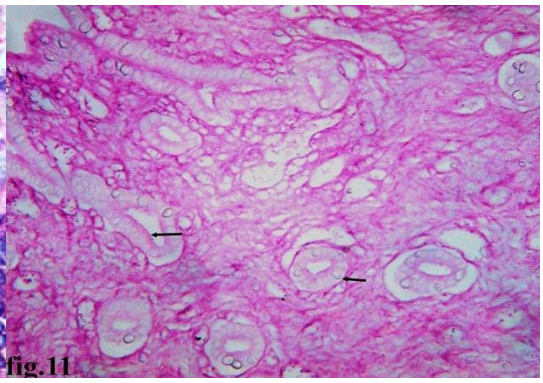


fig.11

Fig.10 A photomocrograph of uterus section of mini pill treated rabbit showing uterin glands with cell proliferation in some parts of lining epithelium(↓) ,and some other parts of shrunken cells with darkly stained ,rounded nucleus and peripheral chromatin(←). notice the increased number and size of the myoepithelial cells around the glands as well as fibroplasts(arrow head) in lamina propria (M) H&E X1000)

Fig.11 A photomocrograph of uterus section of mini pill treated rabbit showing a moderatly positive PAS reaction in basement membrane of epithelial lining and apical border of its cells(←) PAS X400

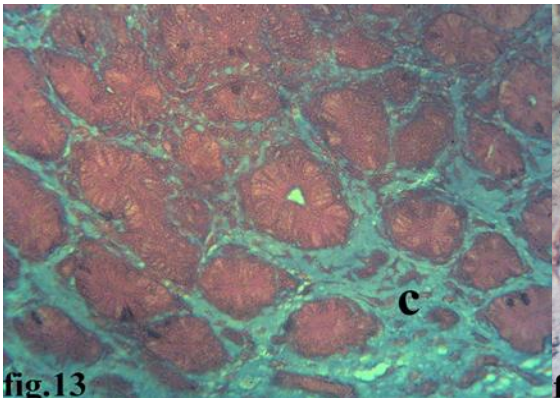


fig.13

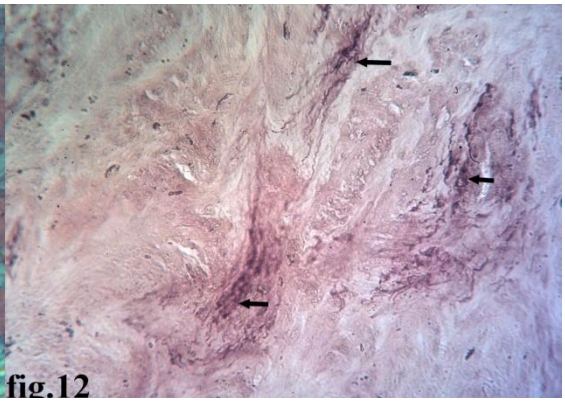


fig.12

Fig.12 A photomocrograph of uterus section of mini pill treated rabbit increased elastic fibers content in myometrium in between the smooth muscles(←) orcen x400

Fig.13 A photomocrograph of uterus section of mini pill treated rabbit showing a marked increase in collagenous fibers content in between uterin glands (c) of the endometrium (Masson's trichromx400)

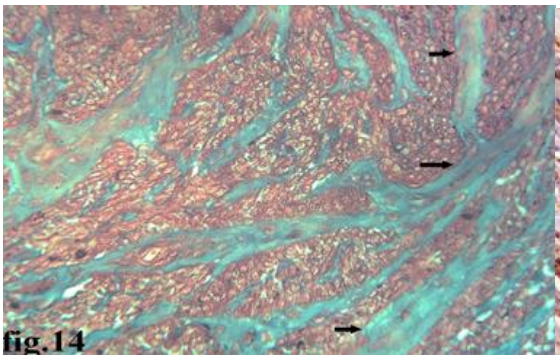


fig.14

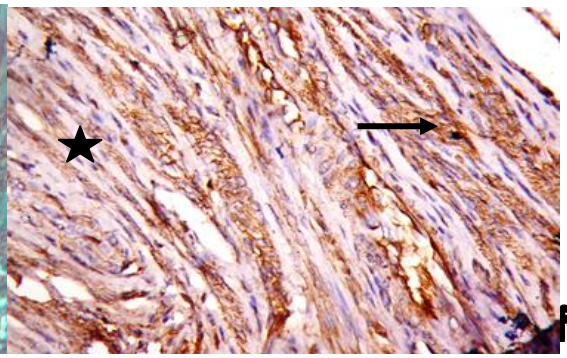


fig.14 a

Fig.14 A photomocrograph of uterus section of mini pill treated rabbit showing great increase in collagenous content in between the uterin muscles(→) of myometrium (Masson trichromx400)

Fig.14 a A photomocrograph of uterus section of mini pill treated rabbit showing moderatly positive immunoreactivity in myometrium(→) and lamina propria* (Anti – α- SMA X 400)

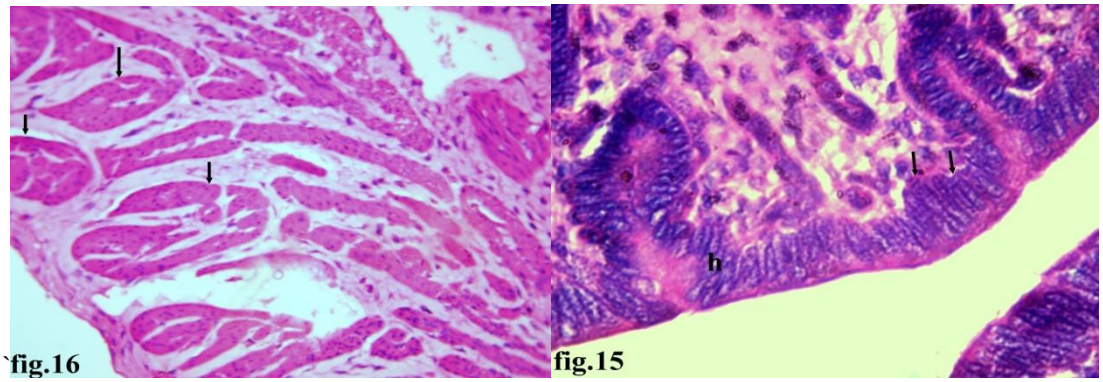


Fig.15 A photomocrograph of uterus section of combined pill treated rabbit showing endometrial glands with hyperplasia of epithelial cells lining the uterin glands (h) and increased hight of these cells(↓),also condensed lamina propria. H&E x400

Fig.16 A photomocrograph of uterus section of combined pill treated rabbit showing hypertrophied myometrial muscle (↓) (H&E x400)

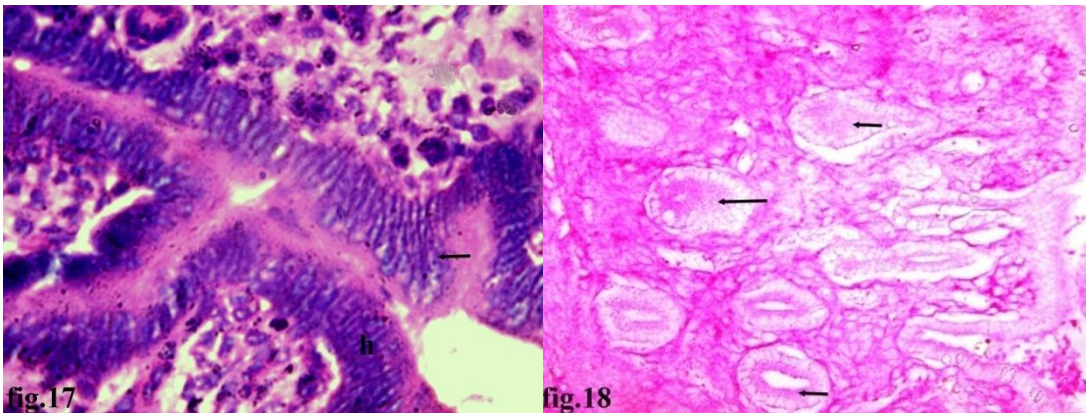


Fig.17 A photomocrograph of uterus section of combined pill treated rabbit showing hyperplasia (h) and increased hight epithelial cells(←) of uterin glands,notic the luminal secretions of endometrial glands H&E x 1000

Fig.18 A photomocrograph of uterus section of combined pill treated rabbit showing strong and glanduler positive PAS reaction in the apical cytoplasm of the epithelial cells(←) lining the uterin glands (PAS x 400)

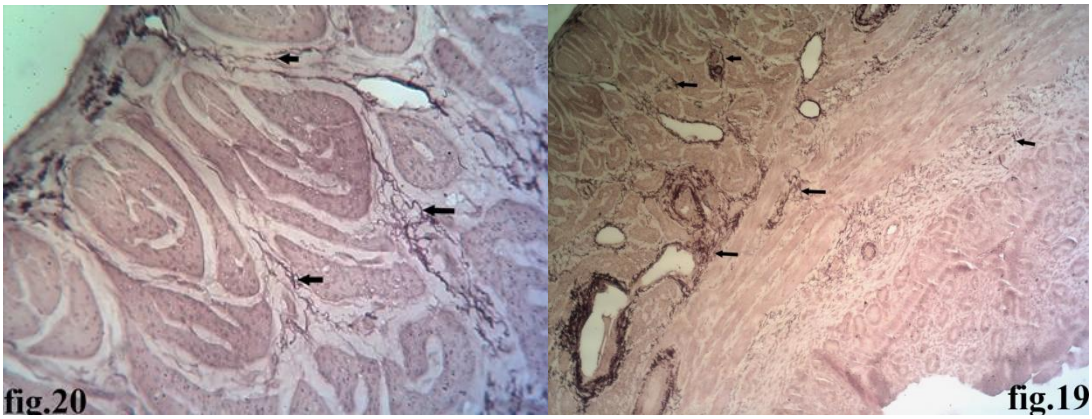


Fig.19 A photomocrograph of uterus section of combined pill treated rabbit showing great increase in elastic fibers content in uterin layers(←) . Orcen X100

Fig.20 A photomocrograph of uterus section of combined pill treated rabbit showing great increase in elastic fibers content insubserosal uterin myometrium(←),and in middle myometrial part(→). Orcen X400

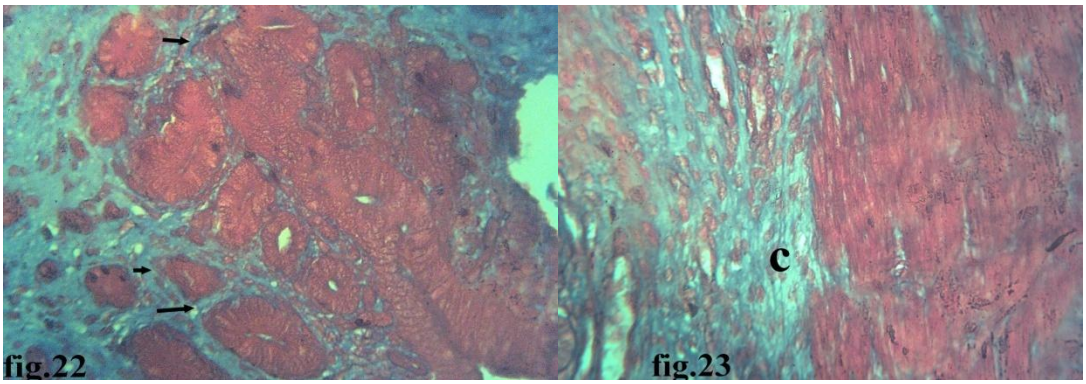


fig.22 fig.23

Fig.21 A photomocrograph of uterus section of combined pill treated rabbit showing numerous elastic fibers forming bundles in between the muscles of myometrium(↓). Orcen X400

Fig.22 A photomocrograph of uterus section of combined pill treated rabbit showing great increasing in collagenous content in endometrium around uterin glands(→) Masson's trichromX400

Fig.23 A photomocrograph of uterus section of combined pill treated rabbit showing great increase in collagenous content in lamina propria (c) Masson's trichromX400

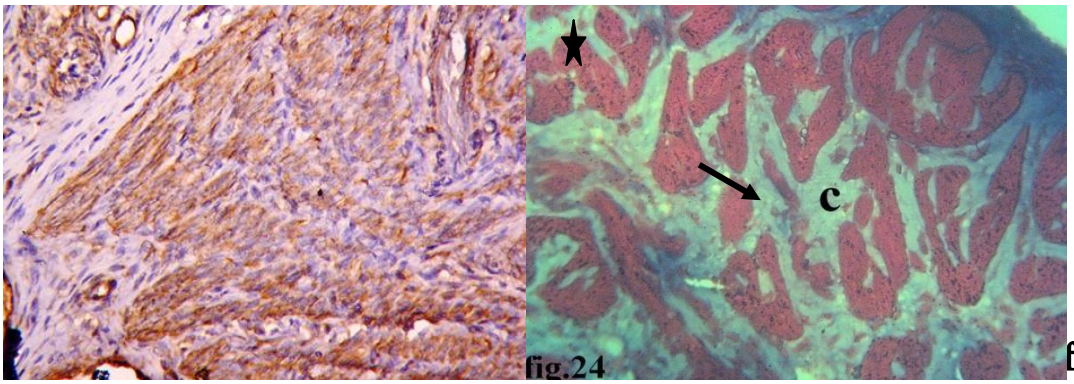


fig.24 fig.24 a

Fig.24 A photomocrograph of uterus section of combined pill treated rabbit showing great increase in collagenous content in between the smooth muscles(c) of myometrium Masson trichromX400 . Fig.24 a A photomocrograph of uterus section of combined pill treated rabbit showing intensive positive immunoreactivity in myometrium(→) and lamina propria* (Anti – α- SMA X 400)

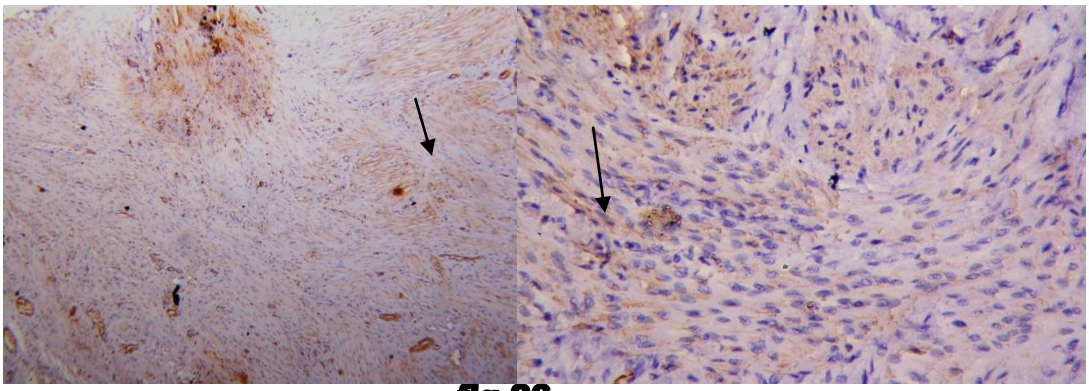


fig.25 fig.26

Fig.25 A photomocrograph of cervix section of control rabbit showing mild positive immunoreactivity in cervical muscle layer (↓) (Anti – α- SMA X 400)

Fig.26 A photomocrograph of cervix section of mini pill treated rabbit showing moderatly positive immunoreactivity cervical muscle layer (↓) (Anti – α- SMA X 400)

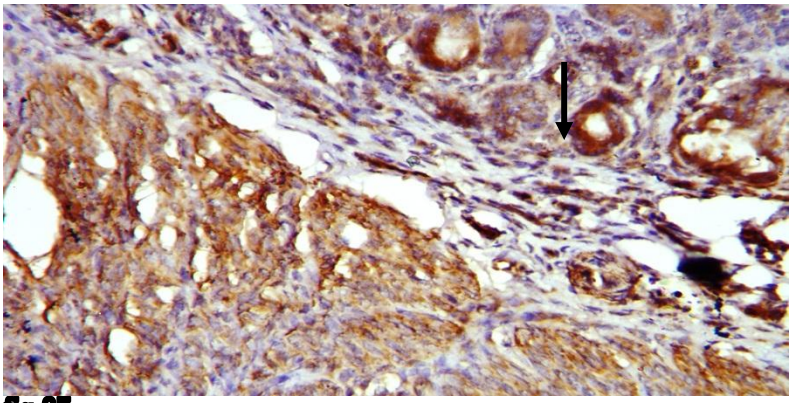


fig.27

Fig.27 A photomocrograph of cervix section of combined pill treated rabbit showing intensive positive immunoreactivity in cervical muscle layer (↓)

Uterus morphometric results :

parameters	control	mini pills	combined pills
Mean area percent of glycogen content±SD	6.55±0.8820	9.67±0.5549	18.326±1.6739
Mean area percent of elastic fiber	2.0660± .2976	3.7520± .3974	6.3560±.8409

content±SD			
Mean area percent of collagen content±SD	10.8700±1.0915	14.6300±2,1137	21.6640±1.1253

Application of ANOVA test showed that there was significant increase on measurement of the area percent of PAS reaction in mini and combined pills treated rabbit compared with untreated one(Fig.25,Table.1).The same results would be obtained on measurements the area percent of collagen content of mini and combined pills treated rabbit compared with untreated One (Fig.26,Table.1) and also obtained on measurment the area percent of elastic fibers content(Fig.27 table)

Significant increase as compared to the different groupe Table 1.

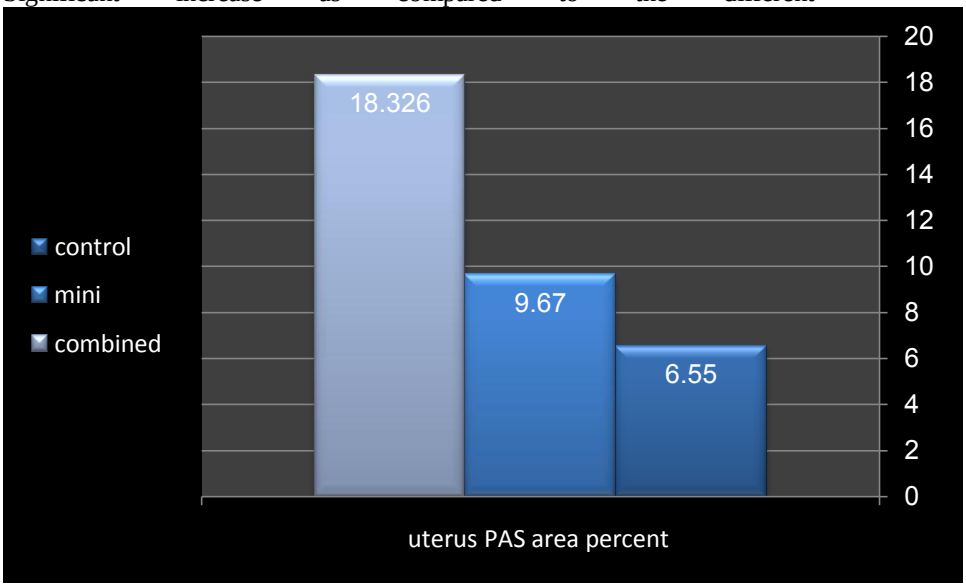


Fig.25 A histogram showing the mean area percent of PAS

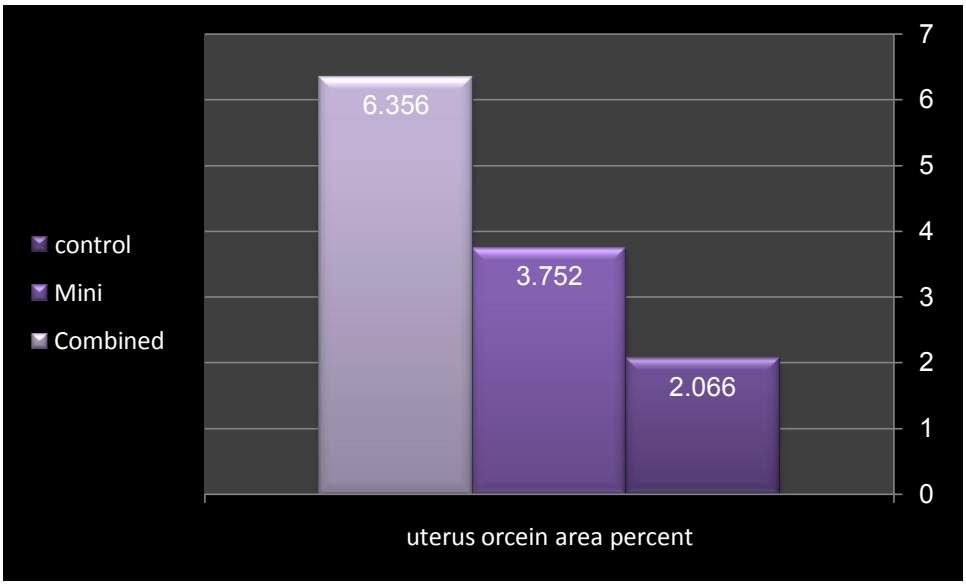


Fig.26 A histogram showing the mean area percent of orcen

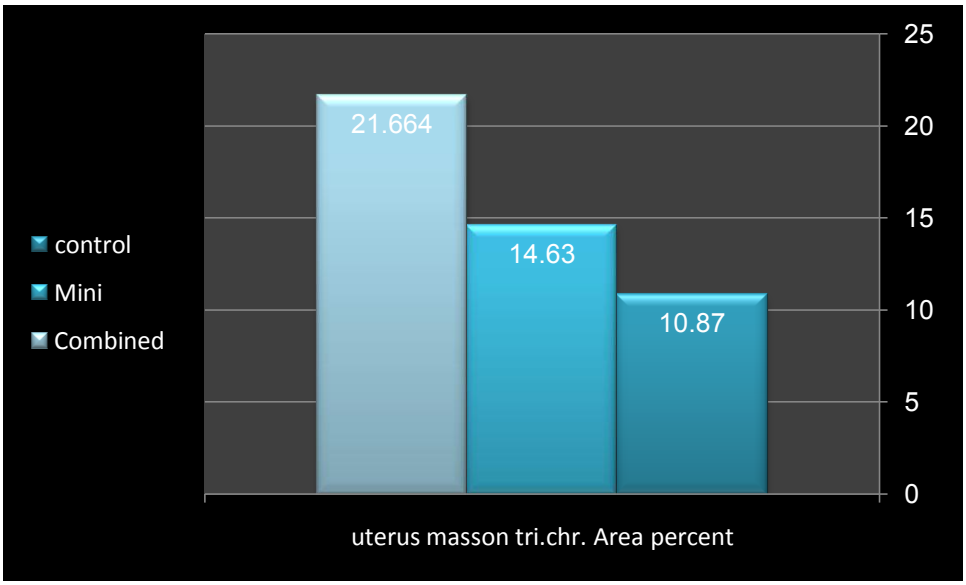


Fig.27 A histogram showing the mean area percent of masson's trichrom

DISCUSSION

The histological observations of the uterus of mini contraceptive pills treated rabbit

progesterone showed histological and histochemical changes in the uterin glands and myometrium and also the collagenous and elastic fiber content

These changes were detected as small size and decreased hight of glandular lining epitheliale cells less vascularized and compact lamina propria.this may leading to signs of atrophied endometrium. These results may be similar to the suggestion of Schnare 2002 who reported that progestins cause atrophic changes to the endometrial lining, inhibiting implantation or nidation if fertilization occurs.

The same results found by Korvera et al .,2005 who stated that mini pills relay on changes in the endometrium, making it less suitable for implantation

PAS reaction shows that secretions of endometrial glands slightly increased after mini pills treatment(kuehnel ,2003 recorded that glycogen synthesis in the uterin glands is stimulated by progesteron

A slight increase in the collagenous content in the uterus was seen after mini pills administration .Halmes & Woessner (1975) found that large doses of progesterone (150 mg) alone increased collagen synthesis in the rat uterus.

Millas et al., 2011 Also, found that contraceptive pills effects fibroblasts which inturn responsible for elastic fibers production that's may explain the increasing in fibrous content in uterin myometrium

The histological observations of the uterus of combined contraceptive pills treated rabbit

Cellular hyperplasia was noticed in the cells of uterin glands in a great manner and these gland were full with secretory granules also hypertrophied muscles in the myometrium.

William et al., 1989 reported that exogenous estrogen can alter uterine gland genesis induced temporary hypertrophy, hyperplasia, and cellular degeneration in the luminal epithelium during each of the dosing periods without corresponding changes in the stroma or myometrium.

The increased content of collagenous fibers was increased due to estrogen and progesterone. This result is supported by Smok & Rojas , 2010 Who reported that estrogens can be related to collagen III predominance in ovarian stroma

An intensive increasing in elastic fibers appeared as bundles in between myometrium smooth muscles may be related to contnous use of combined pills .This result could be similar to results of Chiaffarino et al .,1998 who recorded that risk of uterine fibroids has been either inversely or directly related to OC.

Our study recorded that a moderat to intensive anti alpha smooth muscle actin reaction in smooth muscles of the myometrium of uterus and of the muscle layer of cervix

These may be in accordance with **Christensen et al 1995** who found that the expression of αsma increased in stromal fibroblasts of baboon uterus by long term treatment of steroid hormons like estrogen and progesterone .he explained that αSMA appears to be important for decidual transformation and the production of specific proteins.

(Edan, 2011) stated that Healthy fibroblasts convert to myofibroblasts in the presence of tumour cells and scanty amount of collagen around it. Myofibroblasts are the most abundant cell types found in cancer. They have features of both myoblasts(expressing actin) and fibroblasts. Myofibroblasts originate from interstitial and perivascular fibroblasts and venous smooth muscle cells.

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