Histological changes of female reproductive organs subjected to different jumping exercise intensities and honey supplementation in rats

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Abstract

Background: We assessed histopathological changes of ovaries and uterus in female rats subjected to different jumping exercise intensities combined with honey supplementation at 1 g/kg body weight/day.

Methods: Seventy-two rats were divided into 6 groups, 12 rats in each: control(C), 20 and 80 jumps (20E, 80E), honey (H), and 20 and 80 jump with honey (20EH, 80EH).

Results: The endometrium was significantly thicker in the rats in H, 20EH and 80EH groups compared to C, 20E and 80E. The myometrium thickness was significantly lower in 80E and significantly higher in 80EH compared to C, respectively. There was significantly higher myometrium thickness in 20EH and 80EH compared to 20E and 80E and H. The number of glands of the uterus in 20E and 80E was significantly lower than C. However, there was a significantly higher number of glands in H, 20EH and 80EH compared to 20E and 80E. The numbers of uterus vessels were significantly lower in 80E compared to 20E. However, the numbers of vessels were significantly higher in H, 20EH, and 80EH compared to 80E. The number of ovarian haemorregia was significantly lower in 20E, 80E, H, 20EH and 80EH compared to C. The number of corpora lutea was significantly lower in 80EH, H, 80E and 20E compared to C. However, the number of corpora lutea was significantly higher in 20EH compared to J20 and H.

Conclusion: This study suggested that jumping exercises in particularly high-intensity exercise may induce histopathological changes in uterus and ovary in rats, and honey supplementation may ameliorate these effects.

Introduction

Apart from the important health benefits earned by exercise and physical activity, high-intensity physical activity may accompany with female reproductive disorders. Low energy availability and stress induced by intense exercise may cause hypothalamic dysfunction such as disturbance of gonadotropin realizing hormone (GnRH) pulsatility and hypoestrogenism which in turn may result in menstrual disorder, infertility and osteoporosis (1–3). On the other side, high intensity and prolonged physical activity increase production of reactive oxygen species (ROS) by metabolic and physiological processes and cause cellular damage such as lipid damage, depletion of adenosine triphosphate and inhibition of protein synthesis (4). Furthermore, ROS may influence the biology of the female reproduction system at the levels of the ovary, follicle, and oocyte (5). The increased stress hormone concentrations such as cortisol reduce estradiol production by reducing the function of granulosa cell within the follicle, which lead to deteriorating oocyte quality (5). Recently we have shown that jumping exercise at high-intensity level could cause an increment in serum cortisol levels, and it was accompanied with lower levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and progesterone in adult female rats (6). It also has been found that an intense and exhaustive exercise programme is accompanied by the reduction in uterus thickness in female rats (7).

77 Several non-pharmacological therapies such as increased caloric intake reduced exercise energy
78 expenditure, and pharmacological treatment has been described to prevent and/ or treatment of
79 disorders caused by intense exercises (8). Honey is a natural complex of sugars contains
80 carbohydrates such as fructose, glucose, raffinose and sucrose, and flavonoids, enzymes,
81 antioxidants, minerals, organic acids, proteins, phenolic acids, phytochemicals and vitamins such
82 as vitamins C and E (9). Tualang honey is a wild multi-floral honey found in the Malaysian Rain
83 Forest with intermediate glycemic index as well as phenolic compounds and antioxidant activity
84 (10), and the flavonoids present in honey particularly kaempferol and quercetin have been
85 revealed to have estrogenic activity which may be beneficial for female reproductive health
86 (11,12). It has been reported that administration of 0.2g/ kg Tualang honey elicited beneficial
87 effects in the enhancement of uterus weight and thickness of uterus endometrium and vaginal
88 epithelium in ovariectomized rats (13). Furthermore, we have shown that administration of
89 Tualang honey at 1g/kg body weight could reduce the adverse effect induced by exercise on
90 female reproductive hormones in rats (6,14). To date, however, whether different intensities of
91 exercise may induce changes on histology of uterus and the possible protective effect of honey
92 supplementation have not yet been reported. Therefore, we aimed to investigate the effect of
93 different jumping exercise intensities on uterus histology and the possible protective effect of
94 honey supplementation in rats.
95

96 **Materials & Methods**
97 Seventy-two, 9-week old female Sprague- Dawley rats with no significant mean difference in
98 initial body weights (nearest 0.1 g) were entered in this study (7, 2). The experimental protocol
99 was approved by the Animal Ethics Committee, USM and have been described previously
100 (6,14).
101

102 To aim of standardization in hormonal phase, the rats were scrutinized to detect diestrus phase
103 two times; beginning and end of the experiment. Vaginal secretion of each rat was collected by
104 flushing the vagina with 10 µL of normal saline using a clean pipette. The unstained wet smear
105 was seen under a light microscope at 100x magnifications. The rats were assigned into 6
106 experimental groups (12 rats in each group) by block-randomization; control group with free
107 cage activity and no intervention (C), low intensity; 20 jumps per day at 5 days per week for 8
108 weeks (20E), high intensity; 80 jumps per day for 5 days per week for 8 weeks (80E), honey
109 supplementation for 7 days per week for 8 weeks (H), 20 jumps per day for 5 days per week
110 combined with honey supplementation for 8 weeks (20EH), and 80 jumps per day for 5 days per
111 week combined with honey supplementation for 8 weeks (80EH). Each rat in the training groups
112 was positioned at the bottom of a specially designed wooden box and jumping was commenced
113 by applying an electrical grid and after a few days of training, the rats jumped without electrical
114 stimulation. Each jump took 4 seconds. The rats in the control group (C) were also handled
115 during the duration of the study to imitate the stress induced by handling. At the end of the
116 experiment, the rats were anaesthetized using chloroform by lying for 2-3 minutes in a dried jar

117   containing chloroform-soaked gauze pad and then they were decapitated in diestrus phase
118   (Scientific Research Instrument, U. K)(6,14).
119
120   After decapitation, the uterus horns and ovaries were carefully removed and cleaned. The
121   required organs were fixed in 10% neutral buffered formalin. These specimens were stored in a
122   screw-capped specimen container prior to the next step procedures within a reasonable time.
123   Each tissue was subjected to tissue fixation, processing, paraffin wax embedding, section cutting,
124   and staining according to the previous study (15). The standard hematoxylin and eosin (H& E)
125   stained tissues of ovaries and uterine horns were observed for any changes under a light
126   microscope attached with an image analysis system. Objective lenses of 4X and 10X were used
127   to observe the qualitative and quantitative parameters of ovaries and uterine horns including the
128   thickness of endometrium and myometrium, as well as the number of uterine glands and vessels.
129   The mean of uterus thickness, including endometrium and myometrium of each rat was
130   measured based on the maximum and minimum sagittal section thickness in μm. The number of
131   uterine glands and vessels was counted twice for each rat and then the mean was calculated
132   (Figure 1). Regarding ovaries, the number of Graafian follicles, primary follicle, cyst,
133   haemorregia, corpora lutea, and blood vessels were counted twice for each rat and then the mean
134   was calculated (Figure 2).
135
136   **Honey supplementation**
137   The rats were fed with Malaysian Tualang honey at a dosage of 1 g/ kg body weight/ day by oral
138   gavage for 7 days per week for 8 weeks. The rats in combined honey with jumping exercise
139   groups (20EH & 80EH) were fed with honey, 30 minutes prior to the exercise session (6). The
140   honey dosage was prescribed based on the rat biweekly body weight.
141
142   **Statistical analysis**
143   The numerical data were studied using SPSS version 18.0. All variables were assessed for
144   normality using the Normality Test as well as the Levene's Test to check for homogeneity of
145   variance and also to determine if the groups had unfit variance at 5% level of significance. Then
146   One-way analysis of variance (ANOVA) was performed. The parametric data were assessed and
147   presented as mean ± standard error (SE). The p-value of < 0.05 was defined as statistically
148   significant and used for all the comparisons.
149
150

151   # Results
152   The quantitative histopathological findings of uterus glands and vessels, the thickness of
153   myometrium and endometrium are presented in Table 1. Mean number of primary and Graafian
154   follicles, corpora lutea, cysts, haemorregia, and blood vessels are presented in Table 2.
155

156 The present data show that the 80 jumps/per day were associated with lower endometrium
157 thickness in comparison with the control group, however, results were not significant at 0.05
158 level. The endometrium was significantly thicker in the rats in H, 20EH and 80EH groups
159 compared to the rats in C, 20E and 80E. The myometrium thicknesses of the rats in 80E were
160 significantly lower compared to controls. However, the myometrium thickness was significantly
161 higher in 80EH compared to C. There was significantly higher myometrium thickness in 20EH
162 and 80EH compared to 20E and 80E. Additionally, the myometrium thickness was significantly
163 higher in 20EH and 80EH compared to H.
164
165 Regarding numbers of glands and vessels of the uterus, it was found that the numbers of glands
166 of the uterus in 20E and 80E were significantly lower compared to C. However, there was a
167 significantly high level of glands number in H, 20EH, and 80EH compared to 20E and 80E. The
168 numbers of uterus vessels were significantly lower in 80E compared to 20E. However, the
169 numbers of vessels were significantly higher in H, 20EH, and 80EH compared to 80E.
170
171 The histopathological finding of ovaries was revealed that the number of Graafian follicles did
172 not differ between all experimental groups with exception of 20EH with significantly (P<0.05)
173 greater count of follicles compared to C, J20 and J80. The number of primary follicles (P<0.05)
174 were higher in 80E, 20EH and 80EH compared to C. There were significantly (P<0.05) higher of
175 primary follicles in 20EH and 80EH compared to H. There was no significant difference in the
176 number of ovarian cysts among the experimental groups. There was no count of haemorregia in
177 20EH group. The number of haemorregia were significantly (p<0.05) lower in 20E, 80E, H and
178 80EH compared to C. The number of haemorregia were significantly (p<0.05) lower in 20E, H
179 and 80EH compared to J80. The number of corpora lutea were significantly (p<0.05) lower in
180 80EH compared to H. However, the number of corpora lutea were significantly (p<0.05) higher
181 in 20EH compared to J20 and H. The number of ovary vessels was significantly (p<0.05) higher
182 in H compared to 80E. However, the levels of ovary vessels in HJ20 were significantly higher
183 compared to C, J20, J80, and 80EH.
184
185

186 **Discussion**
187 This study firstly evaluated the probable histomorphometric changes of female reproductive
188 organs influenced by different jumping exercise intensities. We observed that jumping exercise
189 mainly 80 jumps per day (high-intensity exercise) induced negative effects on the measured
190 histopathological parameters with significantly decreased in the number of uterus glands and
191 myometrium thickness. Consequently, we observed that honey supplementation has played an
192 effective role in diminishing these adverse effects induced by jumping exercise on uterus as well
193 as improvement in ovary parameters characteristics.
194

195 It has been shown that intense exercise is commonly correlated with the boosted generation of
196 ROS which may damage tissue and organ redox homeostasis (16). Furthermore, it has also been
197 shown that intense exercise-induced ischemic/reperfusion actions can be related to increased
198 ROS production due to the deviation of the cardiac output to muscle mass inactivity and skin,
199 consequently causing ischemia in the pelvis section (17). Our finding showed that honey
200 supplementation may have potential in increasing the endometrium thickness in the rats feeding
201 with honey alone or combined with 20 and 80 jumps/ day compared to the rats in control and
202 exercise alone groups. Similar effects of honey supplementation on reproductive organs have
203 been reported in the study done by Zaid et al. (15) on ovariectomized rats, in which
204 administration of honey supplementation for 2 weeks significantly increased the weight of the
205 uterus and the thickness of vaginal epithelium. This previous study showed that improvement of
206 uterus and vagina atrophy which might be attributed to the biologically active estrogen-like
207 molecules or phytoestrogens exists in honey supplementation. In the present study, the
208 myometrium thickness was significantly lower in 80 jumps/ day group compared to the control
209 group suggesting that there were negative effects of high exercise intensities on the female
210 reproductive system. Previously we have shown that high intensity of jumping exercises elicited
211 negative effects on follicle stimulating hormone (FSH), luteinizing hormone (LH) and
212 progesterone concentrations in female rats which can possibly explain the changes in the
213 thickness of endometrium and myometrium (6,14). It has been suggested by Warren and Perlroth
214 (1) that stress induced by exercise can arrest the gonadal function, through the increased
215 glucocorticoids and catecholamines levels with activation of the corticotropin-releasing hormone
216 neurons. Meanwhile, low caloric intake and high caloric expenditure, which can occur among
217 athletes, suppress reproductive function (18), and this could be a suppressor factor for the
218 gonadotropin-releasing hormone. In the present study, we observed that the myometrium was
219 thicker in 20 and 80 jumps/ day combined with honey supplementation groups compared to the
220 control group and the rats with jumping alone, i.e.20 and 80 jumps/ day groups. Additionally, the
221 myometrium thickness was significantly higher in 20EH and 80EH compared to H. Regarding
222 the number of glands and vessels of the uterus, it was found that the numbers of glands of the
223 uterus in 20E and 80E were significantly lower than C, and the numbers of uterus vessels were
224 significantly lower in 80E compared to 20E. These results implied that jumping exercise mainly
225 high intensity with 80E /day may elicit negative effects on these measured parameters. We also
226 found that there were increases in these measured parameters in jumping groups with honey
227 supplementation compared to exercise groups without honey supplementation. Flavonoids
228 contained in the honey with their antioxidant compounds may able to retard biologically
229 damaging chemical reactions in living organisms through their ability to scavenge oxidants and
230 free radicals (19). This positive effects of honey on endometrium may due to its high nutritional
231 contents, particularly flavonoids which have antioxidant property (20) in scavenging ROS that
232 may occur during exercise (16, 17). Furthermore, based on Oh et al (12) and Jaganathan (11)
233 findings, flavonoids specially kaempferol and quercetin are natural phytoestrogens as they show
234 the estrogenic property. It also was reported that phytoestrogens were associated with an

235 increased incidence of endometrial proliferation (21). Furthermore, these positive effects of
236 honey particularly in combination with jumping exercise on myometrium thickness, number of
237 gland and vessels may also be due to increase in supply of honey's components, i.e. sugars,
238 phenolic acid and flavonoids through the increased of blood flow to the muscle (22) induced by
239 jumping exercise and these vital elements contained in honey may have beneficial effects on
240 uterus.
241
242 Our finding showed that high and low intensity jumping combined with honey supplementation
243 were most effective in primary follicle generation in female rats. This observation is similar to
244 the findings of the study conducted by Zaid et al. (23) in which the researcher showed that
245 animals treated with BPA full spelling for BPA together with Tualang honey exhibited an
246 improvement in the percentage of normal oestrous cycle compared to those treated with BPA.
247 This finding may be attributed to the effect of honey on improving the normal oestrous cycle.
248 The noteworthy histological findings of ovaries showed that low-intensity jumping exercise
249 containing 20 jumps per day combined with 1 g per kg body weight of daily honey
250 supplementation caused more obvious beneficial effects on the number of Graafian follicles,
251 corpora lutea, and ovarian vessels formation compared to other groups in female rats.
252
253 The appearance of corpora lutea is considered the occurrence of ovulation. These results could
254 be explained with this fact that exercise might promote follicular maturation and ovulation by
255 decreasing sympathetic activity. The possible justification for the improvement of cyclicity is
256 that reduced sympathetic activity caused by exercise may have a direct impact on the ovaries and
257 sex steroid synthesis pathways (24). It has been shown that physical exercise decreased
258 sympathetic nerve activity, improve menstrual frequency, and improved hyperandrogenism in
259 women with the polycystic ovarian syndrome (25).
260
261 The findings of this study showed that honey supplementation induced by different jumping
262 exercise intensities caused no significant difference in the number of ovarian cysts in rats. The
263 cystic follicle is formed from anovulatory follicle encircled by thin layers of granulosa cells with
264 non-detectable theca cell layers (23). These findings suggest that exercise promotes follicular
265 maturation and ovulation. The possible clarification for the improvement of cyclicity is that
266 reduced sympathetic action may have a direct effect on the ovaries and female steroid production
267 pathways. It has been reported that physical activity improved menstrual cycle through a
268 reduction in sympathetic nerve activity and the levels of several sex steroids in women diagnosed
269 with the polycystic ovarian syndrome (25).
270

Conclusions

272 In summary, this study demonstrated that honey has a protective effect against the histological
273 and structural changes induced by jumping exercise on uterus and ovary in rats.
274

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Cross sections of the uterus (Magnification 4X and 10 X)

(Magnification 4X and 10 X) of A: Control (C), B: H (honey), C: 20E (20 jumps/day), D: 80E (80 jumps/ day), E: 20EH (20 jumps and honey supplementation), F: 80EH (80 jumps and honey supplementation). G: Gland, M: Myometrium, E: Endometrium, BV: Blood vessel

A: Magnification 4X

A: Magnification 10 X

B: Magnification 4X

B: Magnification 10 X

C: Magnification 4X

C: Magnification 10 X

D: Magnification 4X

D: Magnification 10 X

E: Magnification 4X

E: Magnification 10 X

F: Magnification 4X

F: Magnification 10 X
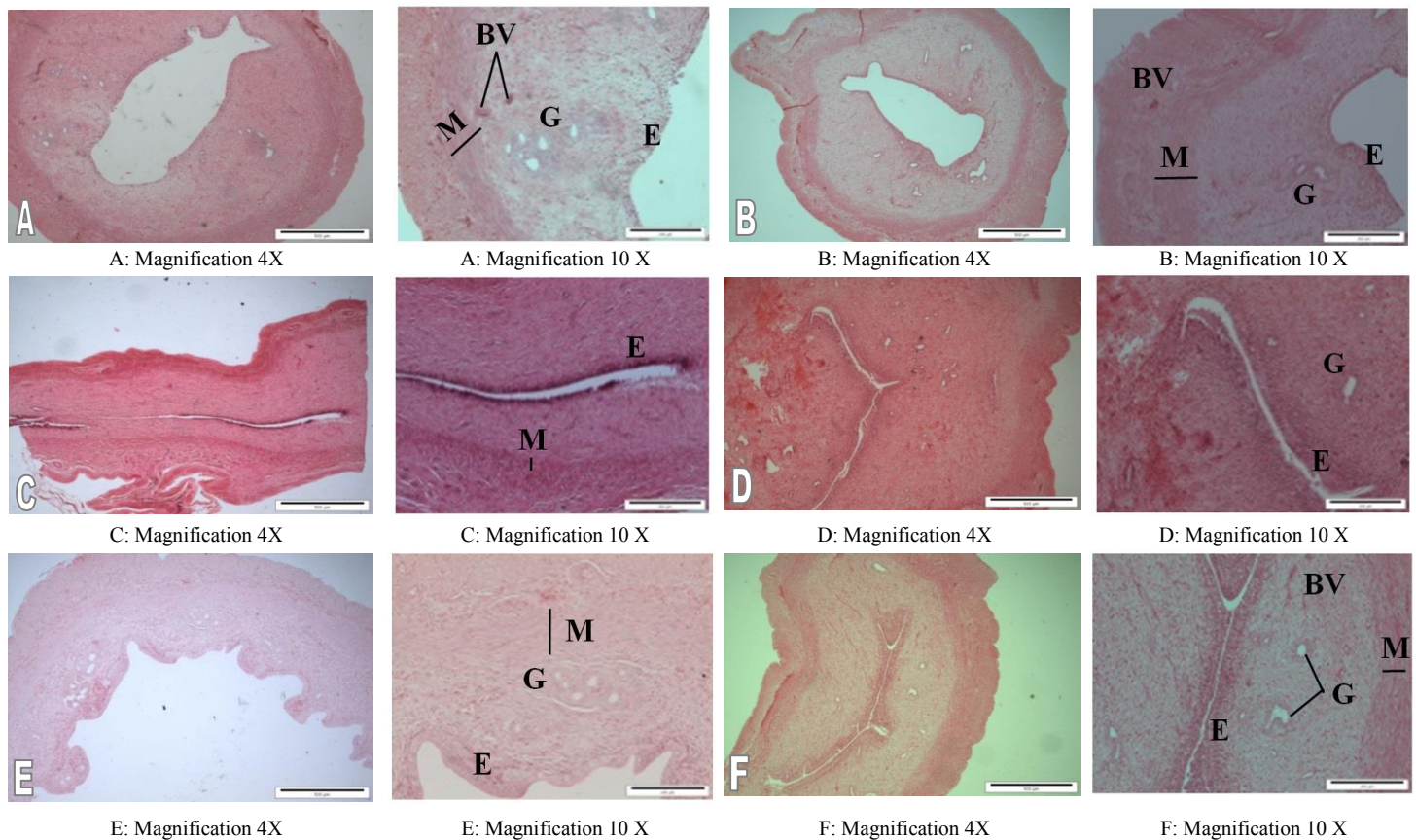
Figure 1. Cross sections of the uterus (Magnification 4X and 10 X) of A: Control (C), B: H (honey), C: 20E (20 jumps/day), D: 80E (80 jumps/ day), E: 20EH (20 jumps and honey supplementation), F: 80EH (80 jumps and honey supplementation). G: Gland, M: Myometrium, E: Endometrium, BV: Blood vessel

Cross sections of the ovaries of (Magnification 4X and 10 X)

(Magnification 4X and 10 X) A: Control (C), B: H (honey), C: 20E (20 Jumps/day), D: 80E (80 jumps/ day), E: 20EH (20jumps and honey supplementation), F: 80EH (80jumps and honey supplementation). PF: Primary follicle; CL: Corpus lutea; GF: Graafian follicle.

A: Magnification 4X   A: Magnification 10 X   B: Magnification 4X   B: Magnification 10 X

C: Magnification 4X   C: Magnification 10 X   D: Magnification 4X   D: Magnification 10 X

E: Magnification 4X   E: Magnification 10 X   F: Magnification 4X   F: Magnification 10 X
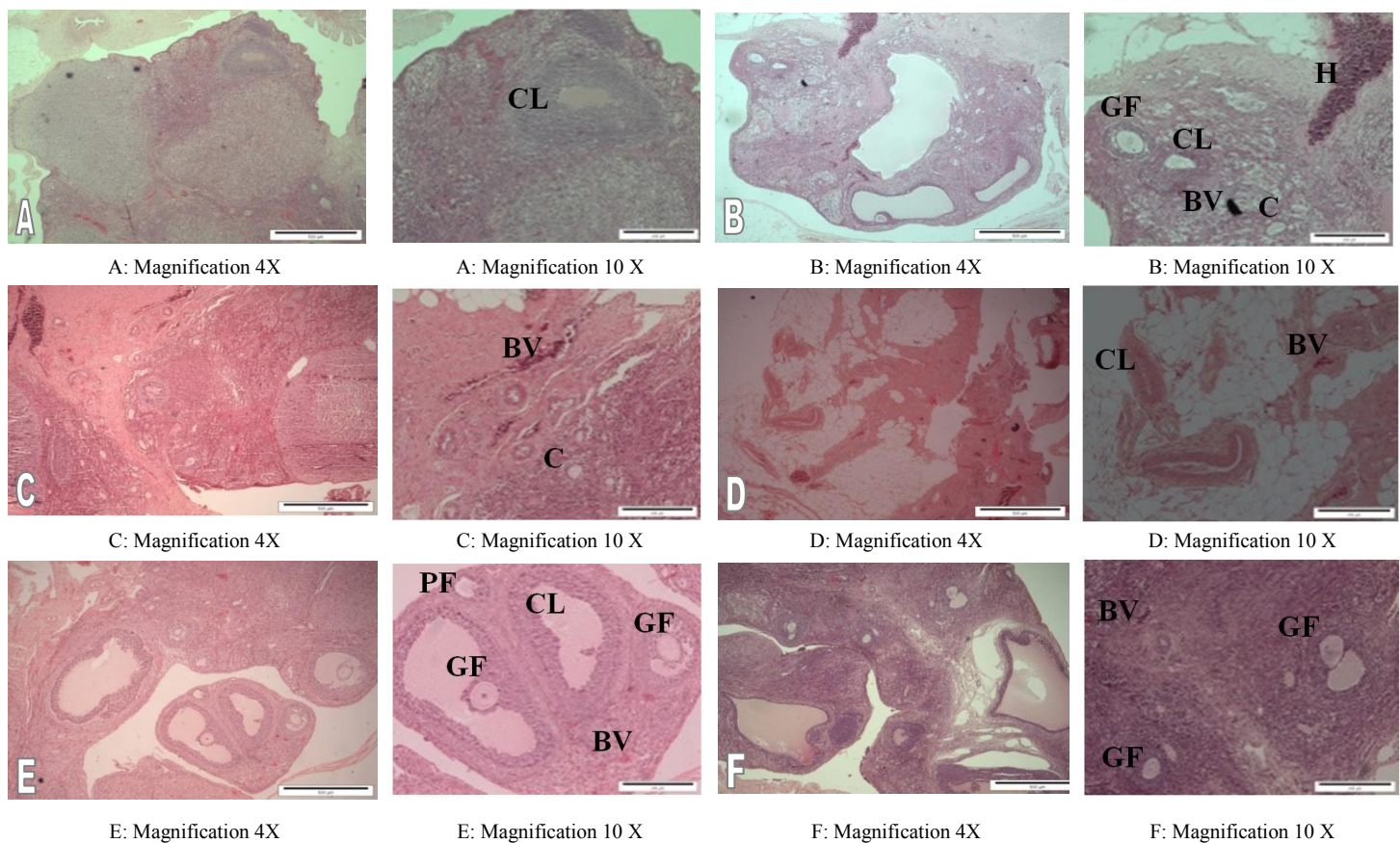
Figure 2. Cross sections of the ovaries of (Magnification 4X and 10 X) A: Control (C), B: H (honey), C: 20E (20 Jumps/day), D: 80E (80 jumps/ day), E: 20EH (20jumps and honey supplementation), F: 80EH (80jumps and honey supplementation). PF: Primary follicle; CL: Corpus lutea; GF: Graafian follicle.

Uterus quantitative histopathological findings and presented as mean ± SE

[a] significant from C (P<0.05), [b] significant form 20E (P<0.05), [c] significant from 80E(P<0.05), [d] significant from H (P<0.05)

1 **Title**

2 **Table1. Uterus quantitative histopathological findings and presented as mean ± SE**

Groups	No. uterus glands	No. uterus vessels	Endometrium thickness (μm)	Myometrium thickness (μm)
C	22.2(1.9)	8.4(2.4)	3.6(0.5)	107.1(7.9)
20E	13.9(1.2)[a]	13.2(3.9)	3.6(0.6)	89.2 (6.6)[a]
80E	12.6(2.1)[a]	7.3(1.7)[b]	2.2(0.4)	79.9(7.7)[a]
H	21.9(2.3)[bc]	18.5(3.9)[c]	7.5(0.9)[abc]	96.3(6.6)
20EH	21.6(2.1)[bc]	19.3(3.8)[c]	6.3(0.9)[abc]	121.9(9.2)[bcd]
80EH	21.6(3.6)[bc]	21.5(2.8)[c]	6.1(0.5)[abc]	140.6(8.2)[abcd]

5 [a] significant from C (P<0.05), [b]significant form 20E (P<0.05), [c] significant from 80E(P<0.05),

6 [d] significant from H (P<0.05)

Ovary quantitative histopathological findings and presented as mean ± SE

[a] significant from C (P<0.05),[b] significant form 20E (P<0.05), [c] significant from 80E(P<0.05), [d] significant from H (P<0.05), [e] significant from 20EH (P<0.05)

1   **Title**

2   **Table2. Ovary quantitative histopathological findings and presented as mean ± SE**

Groups	Primary follicle	Graafian follicle	Corpora lutea	Cyst	Haemorregia	Blood vessels
C	4.1(1.4)	7.7(1.4)	8.4(1.0)	7.3(1.3)	2.2(0.9)	7.0(1.3)
20E	2.2(0.3)	6.0(0.6)	6.7(0.9)	7.9(2.1)	0.3(0.2)^{a}	7.2(1.8)
80E	5.9(1.5)^{b}	7.4(1.4)	8.8(1.7)	6.1(2.0)	1.8(0.5)^{ab}	6.0(1.3)
H	2.3(0.4)^{c}	9.7(1.0)	8.1(1.6)	6.5(1.5)	0.2(0.2)^{ac}	11.9(2.8)^{c}
20EH	6.4(1.1)^{bd}	12.4(1.6)^{abc}	12.5(2.3)^{bd}	6.0(1.9)	0.0	15.1(2.4)^{abc}
80EH	7.3(1.6)^{bd}	8.7(1.7)	7.0(1.2)^{e}	7.6(2.1)	0.2(0.2)^{ac}	7.8(2.5)^{e}

4   ^{a} significant from C (P<0.05), ^{b} significant form 20E (P<0.05),

5   ^{c} significant from 80E(P<0.05), ^{d} significant from H (P<0.05), ^{e} significant from 20EH (P<0.05)