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 blood vessels were significantly lower in 80E compared to 20E. However, the numbers of blood 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 reported 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).

Several non-pharmacological therapies such as increased caloric intake reduced exercise energy expenditure, and pharmacological treatment has been described to prevent and/ or treatment of

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

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, Universiti Sains Malaysia [16 August 2011,
100   Reference: USM/Animal Ethics Approval/ 2011/ (71) (325)], and has been described previously
101   (6,14).
102
103   The estrous cycle is the main reproductive cycle of rodents that lasts four days and is categorized
104   as: proestrus, estrus, metestrus (diestrus I) and diestrus (diestrus II), which can be determined
105   according to the cell types observed in the vaginal smear (15). Diestrus phase, which lasts 55–57
106   h and more than half of the cycle (16), suits to observe the population of different types of cells.
107   To aim of standardization in hormonal phase, the rats were scrutinized to detect diestrus phase two
108   times; beginning and end of the experiment. Vaginal secretion of each rat was collected by flushing
109   the vagina with 1 μL of normal saline (0.9%) using a clean pipette. The unstained wet smear was
110   observed under a light microscope at 100x magnifications, similar pattern was used as standard
111   for determining diestrus phase of the rats. The rats were assigned into 6 experimental groups (12
112   rats in each group) by block-randomization; control group with free cage activity and no
113   intervention (C), low intensity; 20 jumps per day at 5 days per week for 8 weeks (20E), high
114   intensity; 80 jumps per day for 5 days per week for 8 weeks (80E), honey supplementation for 7
115   days per week for 8 weeks (H), 20 jumps per day for 5 days per week combined with honey
116   supplementation for 8 weeks (20EH), and 80 jumps per day for 5 days per week combined with
117   honey supplementation for 8 weeks (80EH). Each rat in the training groups was positioned at the

118 bottom of a specially designed wooden box and jumping was commenced by applying an electrical
119 grid and after a few days of training, the rats jumped without electrical stimulation. Each jump
120 took 4 seconds. The rats in the control group (C) were also handled during the duration of the study
121 to imitate the stress induced by handling. At the end of the experiment, the rats were anaesthetized
122 using chloroform by lying for 2-3 minutes in a dried jar containing chloroform-soaked gauze pad
123 and then they were decapitated in diestrus phase (Scientific Research Instrument, U. K)(6,14).
124

125 After decapitation, the uterus horns and ovaries were carefully removed and cleaned. The required
126 organs were fixed in 10% neutral buffered formalin. These specimens were stored in a screw-
127 capped specimen container prior to the next step procedures within a reasonable time. Each tissue
128 was subjected to tissue fixation, processing, paraffin wax embedding, section cutting, and staining
129 according to the previous study (17). Two histological slices were made from each organ for each
130 rat. The standard hematoxylin and eosin (H& E) stained tissues of ovaries and uterine horns were
131 observed for any changes under a light microscope attached with an image analysis system.
132 Primary follicle consists of a central oocyte surrounded by a single layer of cuboidal granulose
133 cells. Graafian follicles is a large and mature follicle that has an antrum containing follicular fluid.
134 Corpus hemorrhagicum is a temporary structure formed immediately after ovulation from
135 the ovarian follicle as it collapses and is filled with blood clot. Corpus luteum is formed after
136 ovulation which consists of luteinized granulosa and theca cells. Ovarian luteal cyst is corpus
137 luteum that has large cystic centre. Endometrium is the tissue lining the inner cavity of the
138 uterus and is bordered with simple epithelium line and comprises plentiful tubular glands. The
139 myometrium is the middle layer of the uterine wall and comprised of a three smooth muscle layers,
140 which are microscopically difficult to distinct. Uterine blood vessels is the myometrium that has
141 strong and well-perfused vascular layer that is quite running around the uterus. The uterine gland
142 or endometrial gland is a simple tubular gland shaped by invagination of the uterine endometrium.
143 Qualitative and quantitative parameters of ovaries and uterine horns including the thickness of
144 endometrium and myometrium, as well as the number of uterine glands and blood vessels were
145 observed and measured. The mean of uterus thickness, including endometrium and myometrium
146 of each rat was measured based on the maximum and minimum sagittal section thickness in μm.
147 The number of uterine glands and blood vessels was counted once per slide for each rat and then
148 the mean was calculated (Figure 1). Regarding ovaries, the number of Graafian follicles, primary
149 follicle, cyst, haemorregia, corpora lutea, and blood vessels were counted twice for each rat and
150 then the mean was calculated (Figure 2).

151 **Honey supplementation**
152 The rats were fed with Malaysian Tualang honey at a dosage of 1 g/ kg body weight/ day by oral
153 gavage for 7 days per week for 8 weeks. The rats in combined honey with jumping exercise groups
154 (20EH & 80EH) were fed with honey, 30 minutes prior to the exercise session (6). The honey
155 dosage was prescribed based on the rat biweekly body weight.
156

157 **Statistical analysis**
158 The numerical data were studied using SPSS version 18.0. All variables were assessed for
159 normality using the Normality Test as well as the Levene's Test to check for homogeneity of
160 variance at 5% level of significance. One-way analysis of variance (ANOVA) with post-hoc test
161 were performed to determine the significance of the differences between groups. The results are
162 presented as mean ± standard error (SE). The *p*-value of < 0.05 was defined as statistically
163 significant and used for all the comparisons.
164
165

Results
167 The data of body weights and reproductive organ weights presented in Table 1. The rats were
168 weight matched at the beginning of the study, and there was no significant difference in weight
169 gain percentage among all the experimental groups. There were also no significant differences in
170 weight of ovarian among all the groups. Mean uterine weight in 20EH group was significantly
171 greater compared to control, and there were no significant differences in uterine weight among all
172 the experimental groups compared to control.
173
174 The quantitative histopathological findings and cross sections of uterine glands and blood vessels,
175 the thickness of myometrium and endometrium are presented in Table 2 and Figure 1, respectively.
176 Mean number of primary and Graafian follicles, corpora lutea, cysts, haemorregia, blood vessels
177 and cross sections of the ovaries are presented in Table 3 and Figure 2, respectively.
178
179 The present data show that the 80 jumps/per day were associated with lower endometrium
180 thickness in comparison with the control group, however, results were not significant at 0.05 level.
181 The endometrium was significantly thicker in the rats in H, 20EH and 80EH groups compared to
182 the rats in C, 20E and 80E. The myometrium thicknesses of the rats in 80E were significantly
183 lower compared to controls. However, the myometrium thickness was significantly higher in 80EH
184 compared to C. There was significantly higher myometrium thickness in 20EH and 80EH
185 compared to 20E and 80E. Additionally, the myometrium thickness was significantly higher in
186 20EH and 80EH compared to H.
187
188 Regarding numbers of glands and blood vessels of the uterus, it was found that the numbers of
189 glands of the uterus in 20E and 80E were significantly lower compared to C. However, there was
190 a significantly high level of glands number in H, 20EH, and 80EH compared to 20E and 80E. The
191 numbers of uterus blood vessels were significantly lower in 80E compared to 20E. However, the
192 numbers of blood vessels were significantly higher in H, 20EH, and 80EH compared to 80E.
193
194 The histopathological finding of ovaries was revealed that the number of Graafian follicles did not
195 differ between all experimental groups with exception of 20EH with significantly (P<0.05) greater
196 count of follicles compared to C, J20 and J80. The number of primary follicles (P<0.05) were

197 higher in 80E, 20EH and 80EH compared to C. There were significantly (P<0.05) higher of
198 primary follicles in 20EH and 80EH compared to H. There was no significant difference in the
199 number of ovarian cysts among the experimental groups. There was no count of haemorregia in
200 20EH group. The number of haemorregia were significantly (p<0.05) lower in 20E, 80E, H and
201 80EH compared to C. The number of haemorregia were significantly (p<0.05) lower in 20E, H
202 and 80EH compared to J80. The number of corpora lutea were significantly (p<0.05) lower in
203 80EH compared to H. However, the number of corpora lutea were significantly (p<0.05) higher in
204 20EH compared to J20 and H. The number of ovary blood vessels was significantly (p<0.05)
205 higher in H compared to 80E. However, the levels of ovary blood vessels in HJ20 were
206 significantly higher compared to C, J20, J80, and 80EH.
207
208

Discussion

210 This study firstly evaluated the probable histomorphometric changes of female reproductive
211 organs influenced by different jumping exercise intensities. We observed that jumping exercise
212 mainly 80 jumps per day (high-intensity exercise) induced negative effects on the measured
213 histopathological parameters with significantly decreased in the number of uterus glands and
214 myometrium thickness. Consequently, we observed that honey supplementation has played an
215 effective role in diminishing these adverse effects induced by jumping exercise on uterus as well
216 as improvement in ovary parameters characteristics.
217
218 It has been shown that intense exercise is commonly correlated with the boosted generation of
219 ROS which may damage tissue and organ redox homeostasis (18). Furthermore, it has also been
220 shown that intense exercise-induced ischemic/reperfusion actions can be related to increased ROS
221 production due to the deviation of the cardiac output to muscle mass inactivity and skin,
222 consequently causing ischemia in the pelvis section (19). Our finding showed that honey
223 supplementation may have potential in increasing the endometrium thickness in the rats feeding
224 with honey alone or combined with 20 and 80 jumps/ day compared to the rats in control and
225 exercise alone groups. Similar effects of honey supplementation on reproductive organs have been
226 reported in the study done by Zaid et al. (15) on ovariectomized rats, in which administration of
227 honey supplementation for 2 weeks significantly increased the weight of the uterus and the
228 thickness of vaginal epithelium. This previous study showed that improvement of uterus and
229 vagina atrophy which might be attributed to the biologically active estrogen-like molecules or
230 phytoestrogens exists in honey supplementation. In the present study, the myometrium thickness
231 was significantly lower in 80 jumps/ day group compared to the control group suggesting that there
232 were negative effects of high exercise intensities on the female reproductive system. Previously,
233 we have shown that high intensity of jumping exercises elicited negative effects on follicle
234 stimulating hormone (FSH), luteinizing hormone (LH) and progesterone concentrations in female
235 rats which can possibly explain the changes in the thickness of endometrium and myometrium
236 (6,14). It has been suggested by Warren and Perlroth (1) that stress induced by exercise can arrest

237 the gonadal function, through the increased glucocorticoids and catecholamines levels with
238 activation of the corticotropin-releasing hormone neurons. Meanwhile, low caloric intake and high
239 caloric expenditure, which can occur among athletes, suppress reproductive function (20), and this
240 could be a suppressor factor for the gonadotropin-releasing hormone. In the present study, we
241 observed that the myometrium was thicker in 20 and 80 jumps/ day combined with honey
242 supplementation groups compared to the control group and the rats with jumping alone, i.e.20 and
243 80 jumps/ day groups. Additionally, the myometrium thickness was significantly higher in 20EH
244 and 80EH compared to H. Regarding the number of glands and blood vessels of the uterus, it was
245 found that the numbers of glands of the uterus in 20E and 80E were significantly lower than C,
246 and the numbers of uterus blood vessels were significantly lower in 80E compared to 20E. These
247 results implied that jumping exercise mainly high intensity with 80E /day may elicit negative
248 effects on these measured parameters. We also found that there were increases in these measured
249 parameters in jumping groups with honey supplementation compared to exercise groups without
250 honey supplementation. Flavonoids contained in the honey with their antioxidant compounds may
251 able to retard biologically damaging chemical reactions in living organisms through their ability
252 to scavenge oxidants and free radicals (21). This positive effects of honey on endometrium may
253 due to its high nutritional contents, particularly flavonoids which have antioxidant property (22)
254 in scavenging ROS that may occur during exercise (16, 17). Furthermore, based on Oh et al (12)
255 and Jaganathan (11) findings, flavonoids specially kaempferol and quercetin are natural
256 phytoestrogens as they show the estrogenic property. It also was reported that phytoestrogens were
257 associated with an increased incidence of endometrial proliferation (23). Furthermore, these
258 positive effects of honey particularly in combination with jumping exercise on myometrium
259 thickness, number of gland and blood vessels may also be due to increase in supply of honey's
260 components, i.e. sugars, phenolic acid and flavonoids through the increased of blood flow to the
261 muscle (24) induced by jumping exercise and these vital elements contained in honey may have
262 beneficial effects on uterus.
263
264 Our finding showed that high and low intensity jumping combined with honey supplementation
265 were most effective in primary follicle generation in female rats. This observation is similar to the
266 findings of the study conducted by Zaid et al. (23) in which the researcher showed that animals
267 treated with Bisphenol A together with Tualang honey exhibited an improvement in the percentage
268 of normal oestrous cycle compared to those treated with Bisphenol A. This finding may be
269 attributed to the effect of honey on improving the normal oestrous cycle. The noteworthy
270 histological findings of ovaries showed that low-intensity jumping exercise containing 20 jumps
271 per day combined with 1 g per kg body weight of daily honey supplementation caused more
272 obvious beneficial effects on the number of Graafian follicles, corpora lutea, and ovarian blood
273 vessels formation compared to other groups in female rats.
274
275 The appearance of corpora lutea is considered the occurrence of ovulation. These results could be
276 explained with this fact that exercise might promote follicular maturation and ovulation by

277 decreasing sympathetic activity. The possible justification for the improvement of cyclicity is that
278 reduced sympathetic activity caused by exercise may have a direct impact on the ovaries and sex
279 steroid synthesis pathways (26). It has been shown that physical exercise decreased sympathetic
280 nerve activity, improve menstrual frequency, and improved hyperandrogenism in women with the
281 polycystic ovarian syndrome (27).
282
283 The findings of this study showed that honey supplementation induced by different jumping
284 exercise intensities caused no significant difference in the number of ovarian cysts in rats. The
285 cystic follicle is formed from anovulatory follicle encircled by thin layers of granulosa cells with
286 non-detectable theca cell layers (25). These findings suggest that exercise promotes follicular
287 maturation and ovulation. The possible clarification for the improvement of cyclicity is that
288 reduced sympathetic action may have a direct effect on the ovaries and female steroid production
289 pathways. It has been reported that physical activity improved menstrual cycle through a reduction
290 in sympathetic nerve activity and the levels of several sex steroids in women diagnosed with the
291 polycystic ovarian syndrome (27).
292

Conclusions

294 In summary, this study demonstrated that honey has a protective effect against the histological and
295 structural changes induced by jumping exercise on uterus and ovary in rats.
296

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377

Body weights and reproductive organ weights (mean ± SE)

*significant from C (p<0.05)

1 **Title**
2 **Table 1.** Body weights and reproductive organ weights **(mean ± SE)**

Group	Initial body weight (g)	Weight gain% (g)	Ovarian weight (mg)	Uterine weight (mg)
C	186.7(16.5)	76.2 (2.0)	0.10(0.02)	0.41(0.1)
H	192.5(16.3)	76.6 (1.5)	0.10(0.03)	0.46(0.1)
20E	188.9 (10.5)	75.4 (1.1)	0.10(0.02)	0.48(0.1)
80E	189.1(9.3)	75.8(1.9)	0.11(0.02)	0.48(0.1)
20EH	191.6 (13.5)	78.1(2.3)	0.10(0.02)	0.53(0.3)*
80EH	193.3(8.6)	76.3(1.8)	0.10(0.01)	0.45(0.1)

4 *significant from C (p<0.05)
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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 **Table 2.** 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)[b,c]	18.5(3.9)[c]	7.5(0.9)[a,b,c]	96.3(6.6)
20EH	21.6(2.1)[b,c]	19.3(3.8)[c]	6.3(0.9)[a,b,c]	121.9(9.2)[b,c,d]
80EH	21.6(3.6)[b,c]	21.5(2.8)[c]	6.1(0.5)[a,b,c]	140.6(8.2)[a,b,c,d]

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 **Table 3.** Ovary quantitative histopathological findings and presented as mean ± SE

Groups	Primary follicle	Graafian follicle	Corpora lutea	Ovarian luteal cysts	Corpus hemorrhagicum	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)^{a,b}	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)^{a,c}	11.9(2.8)^c
20EH	6.4(1.1)^{b,d}	12.4(1.6)^{a,b,c}	12.5(2.3)^{b,d}	6.0(1.9)	0.0	15.1(2.4)^{a,b,c}
80EH	7.3(1.6)^{b,d}	8.7(1.7)	7.0(1.2)^e	7.6(2.1)	0.2(0.2)^{a,c}	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)

Figure 1

Cross sections of the uterus (Magnification 4X with scale bar: 500μm and 10X with scale bar: 200μm) 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 vessels.
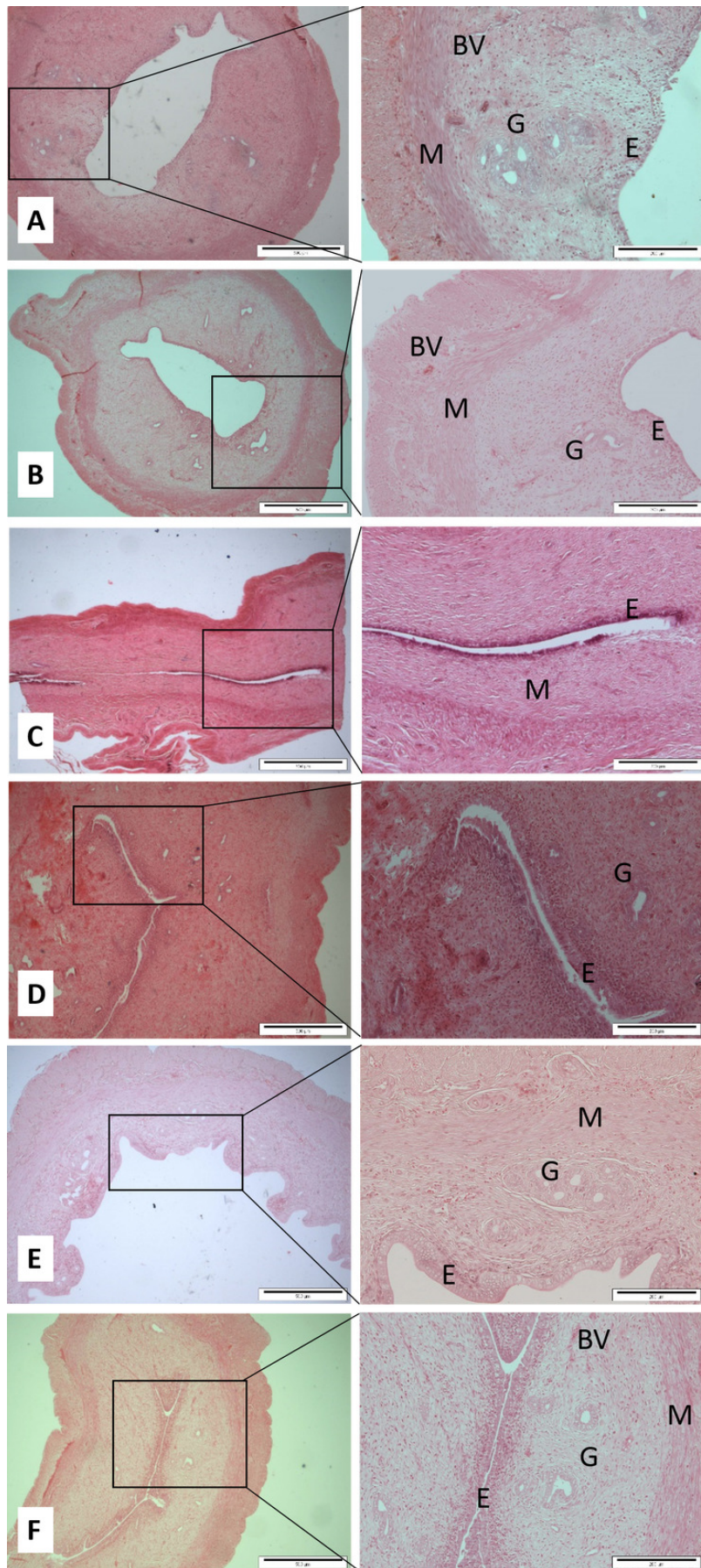
Figure 2

Cross sections of the ovaries (Magnification 4X with scale bar: 500µm and 10X with scale bar: 200µm). 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). PF: Primary follicle; CL: Corpus Lutea; GF: Graafian follicle; BV: Blood vessels; C: Ovarian luteal cyst; H: Corpus hemorrhagicum.