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Original Research Article

Effects of quinestrol and levonorgestrel on prolactin serum concentration in lactating Mongolian gerbils (*Meriones unguiculatus*) and reproductive parameters of their offspring

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ABSTRACT

The effects of the two sterilants, quinestrol (QE) and levonorgestrel (LNG) on serum prolactin (PRL) level in lactating Mongolian gerbils and reproductive parameters of their offspring were examined in the study. Both sterilants increased the serum PRL level in lactating gerbils. The body weight as well as weights of the ovary, testis, epididymides, and seminal vesicles were lower, whereas that of the uterus was higher in the pups originating from QE-treated mothers in comparison to controls. Histological ovarian sections of the offspring from QE-treated mothers contained only growing follicles, whereas their uterine sections showed a thinner endometrium, thicker myometrium, and greater epithelial-cell height than in controls. The histometrical testis characteristics as well as sperm concentration and motility of male pups from QE-treated mothers were lower compared to those of the control group. The serum gonadotropin levels of female pups from mothers treated with QE were lower, whereas the serum estradiol (E_2) and progesterone (P_4) levels were higher than in control gerbils. In contrast, serum gonadotropin and testosterone (T) levels of male pups from QE-treated mothers were lower compared to controls. LNG did not affect the examined parameters of the offspring. The offspring from QE-treated mothers was infertile, whereas the offspring from LNG-treated mothers was fertile. In summary, QE and LNG have a stimulatory effect on PRL level in lactating gerbils. It also appears that QE administered *via* milk to mothers affects reproductive processes of their offspring.

1. Introduction

The Mongolian gerbil (*Meriones unguiculatus*, Milne Edwards, 1867) belongs to the subfamily *Gerbillinae* and mainly inhabits an area encompassing the arid steppes, semideserts, and adjacent pastoral areas of northern China, Mongolia, and Russia's Baikal Lake region [1,2]. Mongolian gerbils seriously damage crops in agricultural areas where their populations are overabundant and are also the main host of *Yersinia pestis*

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which causes the plague [1]. In addition, the Mongolian gerbil has been used as an experimental animal model in many areas of research [2].

Contraception is effective in the control of overabundant wildlife populations [3]. Chemosterilants were shown to be more advantageous over conventional poisoning for curbing rat populations [4]. Quinestrol (QE) is a synthetic estrogen that has been used as a potential contraceptive agent in humans and rodents [5–8]. Levonorgestrel (LNG) is a synthetic progestin widely used for hormonal contraception, alone or in combination with estrogen, and is effective in women. Additionally, it is extensively used in the fertility control of several wildlife species [9–12].

QE and LNG have been used as sterilants in studies on reproductive endocrinology of Mongolian gerbils [7,13]. There is no data, however, concerning their effects on the reproductive parameters of gerbils' offspring. In addition, there is only a few reports [14] on the effects of the sterilants on the maternal PRL, a hormone controlling milk production [15]. Therefore, the aim of the study was to examine the effects of QE and LNG on the maternal PRL level in lactating Mongolian gerbils and reproductive parameters of their offspring.

2. Materials and methods

2.1. Animals

The Mongolian gerbils used in the study were from a domesticated colony bred from animals captured in the Xilinguole League of Inner Mongolia. The gerbils were maintained at 23 ± 1 °C, with automatically controlled lighting from 07:00 to 21:00 h (14 h light:10 h dark). The Mongolian gerbils were provided with a food mixture containing equal parts of corn and sunflower seeds and water *ad libitum*. The estrous cycle of the Mongolian gerbil is four to seven days, pregnancy is 24–26 days, lactation is 21–22 days, and the litter size at birth ranges from one to nine offspring [16]. Mongolian gerbils reach sexual maturity when they are 70–90 days old [17]. Virgin, 4-month-old female gerbils were paired with experienced males in the afternoon and were examined by vaginal smear the next morning. After sperm were found in the vaginal smear, each female gerbil was placed in an individual cage until delivery. Forty-five pregnant gerbils were used in the study. The study was conducted according to the Guidelines for Animal Experiments and approved by the Animal Care and Use Committee at China Agricultural University.

2.2. Experimental design

QE and LNG (Zizhu Medicine Co. Ltd., Beijing, China) were dissolved in peanut oil. The delivery gerbils ($n = 45$) were randomly divided into nine groups, and QE or LNG was given intragastrically within 4 h after delivery at single doses of 0.6–16.2 μg/g body weight (BW; [7,18]). The gerbil groups were as follows: group 1 (control gerbils) – peanut oil; group 2 – 0.6 μg/g QE; group 3 – 1.8 μg/g QE; group 4 – 5.4 μg/g QE; group 5 – 16.2 μg/g QE; group 6 – 0.6 μg/g LNG; group 7 – 1.8 μg/g LNG; group 8 – 5.4 μg/g LNG; and group 9 – 16.2 μg/g LNG. The parturition day was considered day 0 of lactation, while the day when the pups could obtain food by themselves was considered the last day of lactation. Blood samples (0.2–0.3 ml) were collected every three days during lactation *via* orbital venous puncture following light ether anesthesia. Three male and three female pups from each group were sacrificed on days 70 and 90, respectively [7]. Blood samples were collected for hormonal assay, and the body and reproductive organs were weighed. The reproductive organ weight was expressed as mg per 100 g body weight [18].

2.3. Histological and quantitative analysis of reproductive organs

The ovaries, uteri, and testes were fixed in 4% paraformaldehyde, gradually dehydrated in ethanol, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for histological and histometrical examination. The sections were subjected to light microscopy. The mean diameter of the uterine lumen, height of the uterine epithelium, and thickness of the endometrium and myometrium as well as mean diameter of seminiferous tubule and the Leydig cell nuclei were measured [800× magnification; [19]]. In addition, sperm density and motility from the cauda epididymides of the male pups were determined [20].

2.4. Hormone assays

The serum was separated by centrifugation at 1000 × *g* for 20 min at 4 °C and stored at −80 °C until analysis. The concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) were measured using rat ELISA kits (EIAab Science Co. Ltd., Wuhan, China) as described and validated previously [21]. The concentration of growth hormone (GH) was measured by rat ELISA kits (EIAab Science Co. Ltd., Wuhan, China). The assay sensitivity for FSH, LH, PRL and GH was 0.078 mIU/ml, 0.195 mIU/ml, 0.039 ng/ml and 0.078 ng/ml, respectively. The intra-assay variability for FSH, LH, and PRL was 4.8% and the inter-assay variability for the three hormones was 7.4%. The intra- and inter-assay variability for GH were 4.5% and 7.2%, respectively. The serum estradiol (E_2), progesterone (P_4), and testosterone (T) levels were determined by a chemiluminescence immunoassay (CLIA) using CLIA kits (Furui Biotechnology Co. Ltd., Beijing, China). The assay sensitivity for E_2, P_4 and T was 1.50 pg/ml, 0.05 ng/ml and 0.1 ng/ml, respectively. The intra- and inter-assay variability for the three hormones were less than 10% and 15%, respectively.

2.5. Fertility test

Three subsequent female (90-day-old) and three male (70-day-old) pups from each group were paired with experienced male gerbils and fertile female gerbils, respectively. The female gerbils were examined by vaginal smear the next morning. The first day sperm was found in a vaginal smear was designated as day 0 of gestation. The parturition day was regarded as the last day of gestation. The gestational length and litter size were recorded.

2.6. Statistical analysis

The data were analyzed by one-way ANOVA followed by Tukey's test. The values were considered statistically significant at $p < 0.05$ and highly significant at $p < 0.01$. The analysis was performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The data are presented as mean ± SEM or mean ± SD.

3. Results

3.1. Effects of QE and LNG on maternal serum PRL level

The highest QE dose increased ($p < 0.05$) maternal PRL serum on days 9, 12, and 24 of lactation (Fig. 1A). QE did not affect PRL level on the remaining days of lactation. The highest LNG dose increased ($p < 0.05$) maternal PRL serum on days 0, 9, 12, and 15 of lactation (Fig. 1B). No other LNG dose affected maternal PRL level. Similarly, LNG did not affect PRL level on the remaining days of lactation.

3.2. Effects of QE and LNG on the weight of body and reproductive organs in offspring

Along with the increase in QE dose, the body weight gradually decreased, the uterus weight increased and the ovary weight remained unchanged in female pups from QE-treated mothers. In contrast, no alterations in these parameters were found in female pups from LNG-treated mothers (Table 1). The uteri of the female pups from mothers treated with QE at doses of 5.4 and 16.2 μg/g appeared abscessed, and the percentage of the uterine abscesses was 66.7 and 100%, respectively.

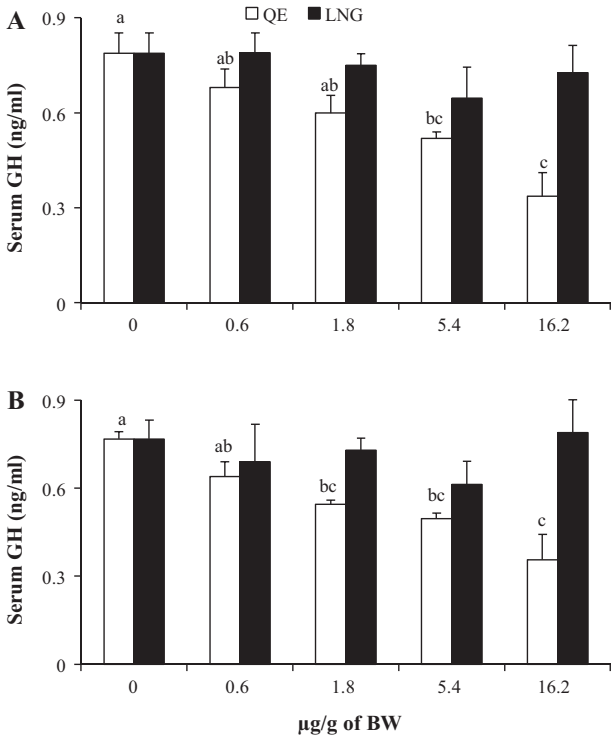

Fig. 1 – Effects of (A) quinestrol (QE) and (B) levonorgestrel (LNG) on the maternal serum prolactin (PRL) level (mean ± SEM) in lactating gerbils ($n = 5$). Bars with different superscripts are significantly different ($p < 0.05$).

Table 1 – Body and organ weights (mean ± SD) of the female pups from the gerbils treated with quinestrol (QE) and levonorgestrel (LNG).

Treatment and dose (μg/g)	Body weight (g)	Weight (mg/100 g BW)	
		Ovaries	Uteri
Control gerbils	53.8 ± 2.1[a]	27.1 ± 5.0	55.2 ± 32.2[a]
QE-treated gerbils			
0.6	41.5 ± 2.4[b]	28.9 ± 13.4	56.6 ± 31.3[a]
1.8	33.0 ± 5.0[b]	24.9 ± 1.2	129.7 ± 81.5[a]
5.4	32.8 ± 5.0[b]	24.9 ± 7.3	1431.4 ± 2214.1[a]
16.2	32. 7 ± 5.0[b]	22.1 ± 2.6	15,647.6 ± 6247.3[b]
LNG-treated gerbils			
0.6	53.0 ± 1.0	24.5 ± 1.7	78.4 ± 15.7
1.8	50.5 ± 7.6	23.8 ± 3.3	58.1 ± 5.0
5.4	42.0 ± 5.3	21.8 ± 4.6	52.7 ± 4.8
16.2	41.7 ± 7.6	27.1 ± 3.7	74.0 ± 25.6

$n = 3$ per group; different superscripts depict significant differences ($p < 0.05$); no differences were found for LNG.

The ovaries of pups from QE-treated mothers contained no mature follicles, only growing ones (Fig. 2B). The uteri of this group displayed a thinning endometrium and thickening myometrium (Fig. 2E). Additionally, the endometrium showed epithelial hyperplasia, apoptosis, and keratinization; there was an abundance of inflammatory cells in the endometria lamina propria. The uterine histometrical parameters of the female pups are shown in Table 2. A histological examination showed that the ovaries and uteri of the female pups from LNG-treated mothers were not different from those of control gerbils (Fig. 2C and F).

The weight of body and reproductive organs in male pups from QE-treated mothers were significantly ($p < 0.05$) lower than those of controls, whereas the weight in male pups from LNG-treated mothers did not differ from those of control gerbils (Table 3). A histological examination of the testes of pups from QE-treated mothers showed a thinning of the seminiferous tubules and a widening of gaps among the seminiferous tubules (Fig. 2H). A histological examination of the testes of the pups from LNG-treated mothers revealed normal morphology. The histometrical parameters of testes as well as sperm concentration and motility of the male pups are shown in Table 4.

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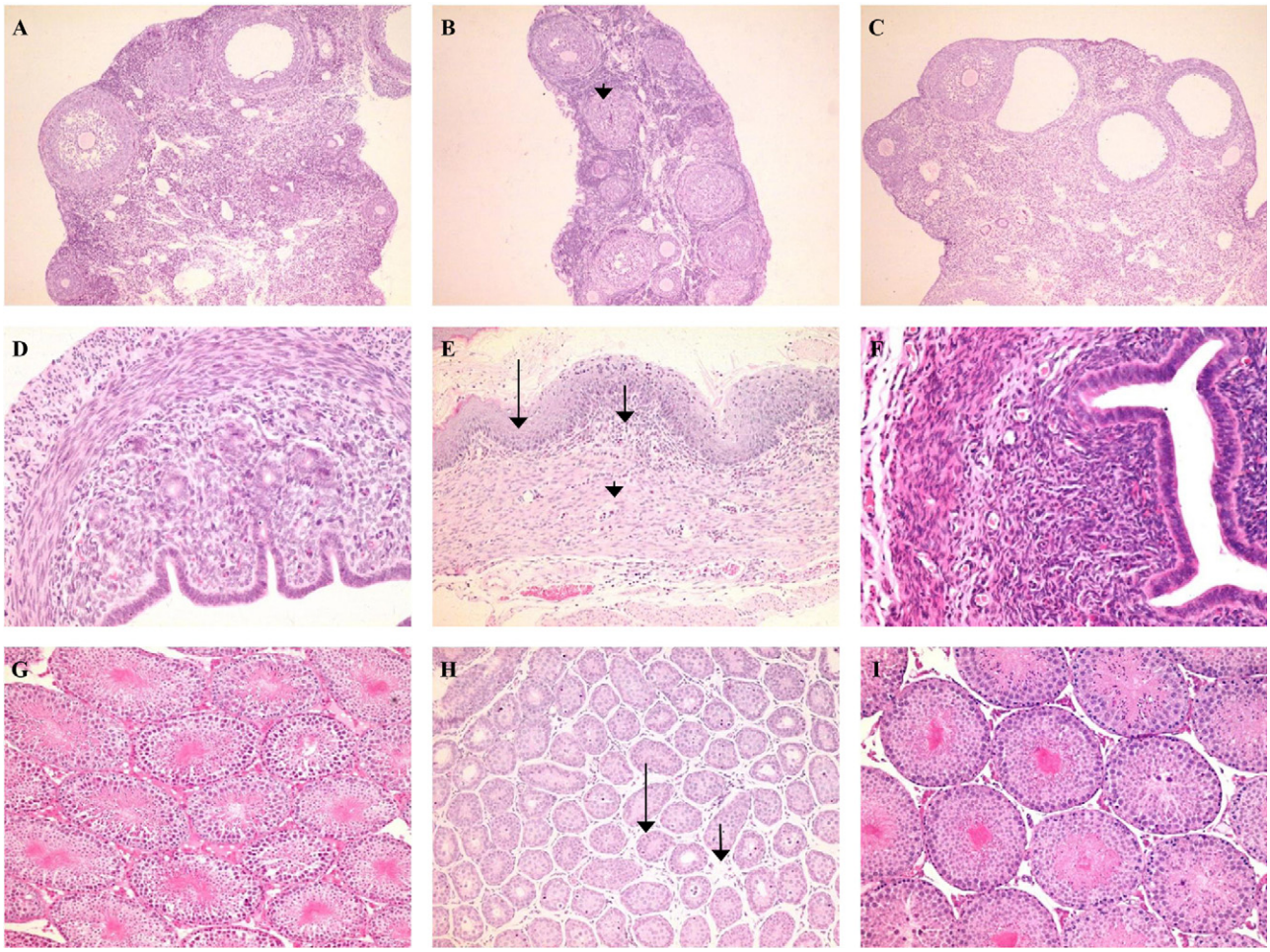

Fig. 2 – Histological images of the ovaries (A–C), uteri (D–F), and testes (G–I) in the pups of gerbils treated with quinestrol (QE) and levonorgestrel (LNG). Control gerbils: (A), (D), and (G); QE (16.2 μg/g)-treated gerbils: (B) only growing follicles (arrowhead), without mature follicles are visible; (E) a thickening myometrium (arrowhead) and thinning endometrium with epithelial hyperplasia (long arrow) and inflammatory cells (short arrow) are visible, and (H) thinning seminiferous tubules (long arrow) and the widening gap among the seminiferous tubules (short arrow) are visible; LNG (16.2 μg/g)-treated gerbils: (C), (F), and (I), no obvious pathological changes were observed. The sections were stained with hematoxylin and eosin. Magnification: (A–C): 100×; (D and F): 400×; (E and G–I): 200×.

Table 2 – Uterine histometrical parameters (mean ± SEM) of female pups from the gerbils treated with quinestrol (QE) and levonorgestrel (LNG).

Treatment and dose (μg/g)	Uterine diameter (mm)	Height/thickness (μm)		
		Epithelial cells	Endometrium	Myometrium
Control gerbils	1.0 ± 0.0^{a}	19.3 ± 0.4^{a}	257.7 ± 17.4^{a}	128.5 ± 3.2^{a}
QE-treated gerbils				
0.6	1.1 ± 0.0^{a}	22.1 ± 0.9^{a}	202.4 ± 8.7^{b}	132.2 ± 1.6^{a}
1.8	1.1 ± 0.0^{a}	24.3 ± 1.4^{a}	189.4 ± 4.7^{b}	140.7 ± 5.0^{a}
5.4	2.9 ± 0.8^{b}	57.5 ± 15.3^{b}	154.1 ± 21.0^{bc}	246.7 ± 39.8^{b}
16.2	4.9 ± 0.3^{c}	89.0 ± 0.5^{c}	108.3 ± 1.6^{c}	315.8 ± 3.9^{b}
LNG-treated gerbils				
0.6	1.0 ± 0.0	20.8 ± 0.8	232.6 ± 27.3	126.5 ± 2.5
1.8	1.1 ± 0.0	21.1 ± 0.4	216.7 ± 19.1	132.6 ± 4.5
5.4	1.0 ± 0.0	20.3 ± 0.6	230.8 ± 19.0	132.0 ± 2.5
16.2	1.1 ± 0.0	20.8 ± 0.7	220.7 ± 10.2	131.5 ± 6.2

$n = 3$ per group; different superscripts depict significant differences ($p < 0.05$); no differences were found for LNG.

Table 3 – Body and organ weights (mean ± SD) of male pups from the gerbils treated with quinestrol (QE) and levonorgestrel (LNG).

Treatment and dose (μg/g)	Body weight (BW; g)	Weight (mg/100 g BW)		
		Testis	Epididymides	Seminal vesicles
Control gerbils	53.1 ± 6.8^{a}	1379.3 ± 376.9^{a}	364.4 ± 130.8^{a}	193.8 ± 17.9^{a}
QE-treated gerbils				
0.6	40.2 ± 6.7^{ab}	256.9 ± 264.1^{b}	62.5 ± 8.7^{b}	43.8 ± 6.3^{b}
1.8	39.0 ± 3.0^{b}	226.4 ± 268.4^{b}	48.9 ± 5.6^{b}	33.9 ± 5.7^{b}
5.4	32.0 ± 4.5^{b}	210.4 ± 63.9^{b}	46.8 ± 13.2^{b}	37.8 ± 12.1^{b}
16.2	26.8 ± 3.8^{b}	138.4 ± 63.9^{b}	43.0 ± 10.1^{b}	29.8 ± 10.2^{b}
LNG-treated gerbils				
0.6	49.3 ± 0.6	831.4 ± 167.0	197.5 ± 27.0	125.7 ± 101.6
1.8	45.7 ± 4.6	1097.4 ± 315.0	246.9 ± 192.3	84.5 ± 110.2
5.4	47.3 ± 10.0	910.8 ± 463.5	184.6 ± 155.8	125.9 ± 96.7
16.2	49.0 ± 12.8	1099.4 ± 442.7	299.9 ± 293.2	129.6 ± 88.2

$n = 3$ per group; different superscripts depict significant differences ($p < 0.05$); no differences were found for LNG.

Table 4 – Histometrical parameters of testis as well as sperm concentration and motility (mean ± SEM) in male pups from the gerbils treated with quinestrol (QE) and levonorgestrel (LNG).

Treatment and dose (μg/g)	Seminiferous tubule diameter (μm)	Leydig cell nuclear diameter (μm)	Sperm concentration (million/ml)	Sperm motility (%)
Control gerbils	176.2 ± 6.8^{a}	4.7 ± 0.2^{a}	54.3 ± 4.8^{a}	64.7 ± 3.3^{a}
QE-treated gerbils				
0.6	98.0 ± 3.0^{b}	3.5 ± 0.1^{b}	16.7 ± 0.9^{b}	23.0 ± 3.2^{b}
1.8	88.6 ± 3.3^{bc}	3.5 ± 0.1^{b}	14.3 ± 2.6^{b}	18.3 ± 1.8^{bc}
5.4	78.6 ± 3.2^{cd}	3.4 ± 0.1^{b}	13.0 ± 1.2^{b}	16.0 ± 1.2^{bc}
16.2	61.8 ± 4.2^{d}	3.3 ± 0.1^{b}	9.7 ± 1.2^{b}	11.0 ± 2.1^{c}
LNG-treated gerbils				
0.6	194.0 ± 2.7	4.2 ± 0.1	55.3 ± 2.7	59.0 ± 2.1
1.8	177.2 ± 3.9	4.3 ± 0.2	51.0 ± 1.5	56.0 ± 3.6
5.4	186.8 ± 3.6	4.2 ± 0.1	54.7 ± 5.2	60.0 ± 4.2
16.2	182.8 ± 5.0	4.1 ± 0.1	55.0 ± 3.5	59.3 ± 3.8

$n = 3$ per group; different superscripts depict significant differences ($p < 0.05$); no differences were found for LNG.

3.3. *Effects of QE and LNG on serum hormone levels in offspring*

Mean serum FSH, LH, GH, E_2 and P_4 levels in control female pups were 15.04 ± 0.84 mIU/ml, 14.39 ± 0.72 mIU/ml, 0.77 ± 0.02 ng/ml, 31.39 ± 2.63 pg/ml and 5.34 ± 0.23 ng/ml, respectively. Along with the increased QE dose, serum FSH, LH and GH levels gradually decreased and serum E_2 and P_4 levels gradually increased in female pups from QE-treated mothers (Figs. 3 and 5).

The serum FSH, LH, GH and T levels were 13.37 ± 0.42mIU/ml, 14.10 ± 0.52 mIU/ml, 0.79 ± 0.06 ng/ml and 0.88 ± 0.08 ng/ml in control male pups, respectively. Along with the increased QE dose, serum concentrations of all examined hormones gradually decreased in the male pups from QE-treated gerbils (Figs. 4 and 5). In contrast, none of the serum hormone levels in female and male pups from LNG-treated gerbils were affected by the dose of LNG.

3.4. *Effects of QE and LNG on fertility of offspring*

All female and male pups from QE-treated mothers were infertile, whereas all female and male pups from LNG-treated gerbils were fertile. The gestation length and litter size of the female pups from mothers treated with LNG are shown in Table 5.

4. Discussion

This study is the first to report the stimulatory effects of QE and LNG on maternal PRL level in Mongolian gerbils and to demonstrate the infertility of female and male pups born from QE-treated lactating gerbils. QE and LNG were used as sterilants in studies on fertility control in several rodent species [7,8,18]. Estrous cycle-long administration of QE at doses from 0.1 to 2.7 μg/g inhibited the fertility of Mongolian gerbils [7]. In contrast to multiple doses, a single dose of QE effectively inhibited lactation [22]. Hence, the lactating gerbils in the present study were treated with a single-dose of QE or LNG. Although hormonal contraception is an effective method of fertility control, there are potential problems with its application in fields [23]. Several doses of QE and LNG were examined in our laboratory, but the establishment of the proper dose and delivery time of the sterilants for fertility control in Mongolian gerbils in the wild requires additional studies.

The decreased weights of body (females and males) and reproductive organs (males) in pups from QE-treated mothers

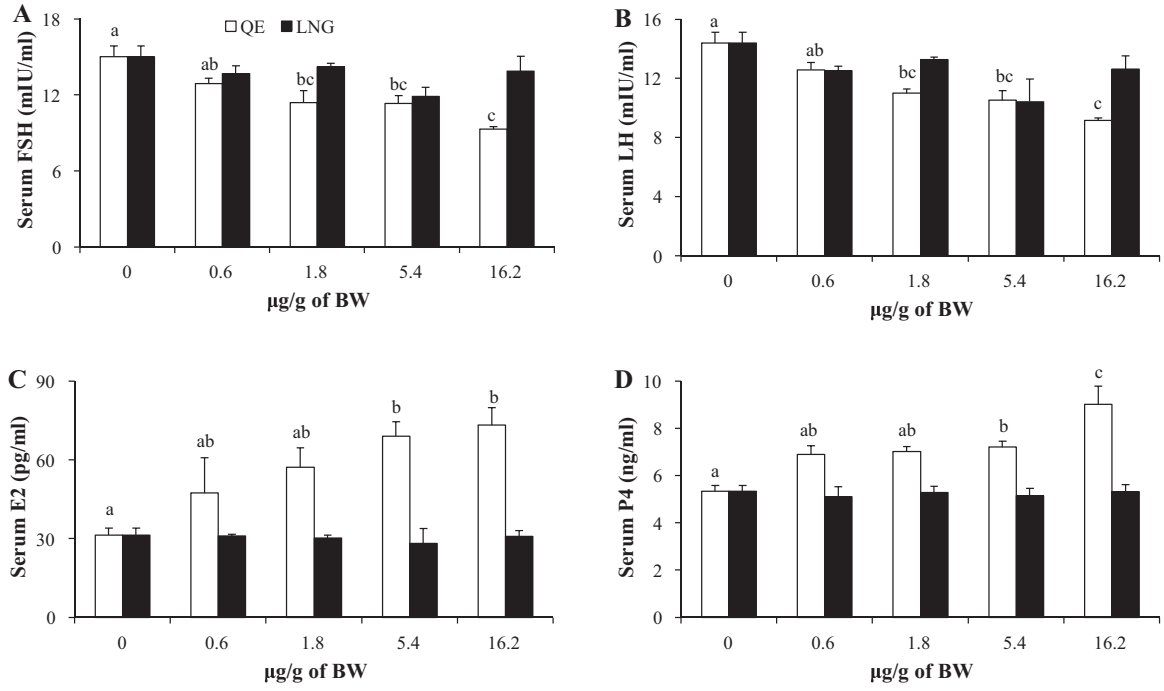

Fig. 3 – Serum concentrations (mean ± SEM) of (A) follicle-stimulating hormone (FSH), (B) luteinizing hormone (LH), (C) estradiol (E_2), and (D) progesterone (P_4) in female pups from gerbils (n = 3/per group) treated with quinestrol (QE; □) or levonorgestrel (LNG; ■). Bars with different superscripts are significantly different ($p < 0.05$). No differences were found for LNG; BW: body weight.

suggest that QE may affect growth of the offspring. This may be associated with the decreased GH level in these pups. In addition, a reduction in milk secretion has also been observed [24] in women receiving QE. The effects of the contraceptives on offspring growth due to a decrease in milk output or quality were demonstrated previously [25,26]. On the other hand, the contraceptives in Mongolian gerbils increased serum level of maternal PRL, a hormone important for milk production [15]. This result was in agreement with previous reports on humans [14]. Thus, it is not very likely that QE affected lactation *via* changes in PRL level. The mechanism of QE action on milk production in Mongolian gerbils requires further studies.

It is of interest that serum PRL level was higher in LNG-treated lactating gerbils, but the body weight and the gonadosomatic indices of their offspring were not affected by the treatment. It has been shown that contraception with synthetic progestin did not affect milk production or the growth and development of the offspring [27–30]. LNG, in contrast to QE, had no obvious effect on lactation in gerbils.

We found that pups from the QE-treated lactating gerbils were infertile. QE may be transferred to the pups *via* milk and affect reproduction of the offspring. Ovaries of the female pups lacked mature follicles and had only growing follicles. These results are in agreement with those obtained in gerbils treated directly with QE [7]. Moreover, female pups from the QE-treated lactating gerbils exhibited lower FSH and LH levels and higher E_2 and P_4 levels compared to pups from control animals. Since the levels of gonadotropins and steroid hormones are associated with the reproductive status of Mongolian gerbils [16,31], the abnormal reproductive hormone levels could cause the observed ovarian abnormalities.

Reproductive success in females requires the coordination of the processes taking place in the ovary and uterus. In this study, we report the presence of uterine abscesses in female pups of the QE-treated mothers. Previous reports concerning this issue were inconsistent [7,18], probably due to the differences in QE doses. These results indicate that protocols with lower dosage of QE or combinations of QE and LNG are needed.

Abnormal ovarian and uterine parameters may be responsible for infertility of female pups of the QE-treated mothers. The infertility of the male pups from the QE-treated mothers was also related to the abnormal serum levels of reproductive hormones. In addition, the histological and histometrical data obtained during the examination of the reproductive organs of the male pups from QE-treated mothers were consistent with hormonal data. Moreover, the histological and histometrical data of the male pups support the results obtained in other rodents treated directly with QE [8,32].

The reproduction of female pups from LNG-treated gerbils was not significantly affected by the treatment. It was reported previously that transfer ratio of LNG from the maternal milk to the offspring was very low [33]. No obvious effects on reproductive hormones or organs were observed in female pups from LNG-treated gerbils, which suggests that the amount of LNG received through the maternal milk was not sufficient to impact offspring reproduction [34]. A similar conclusion can be drawn with regards to the male pups from the LNG-treated mothers.

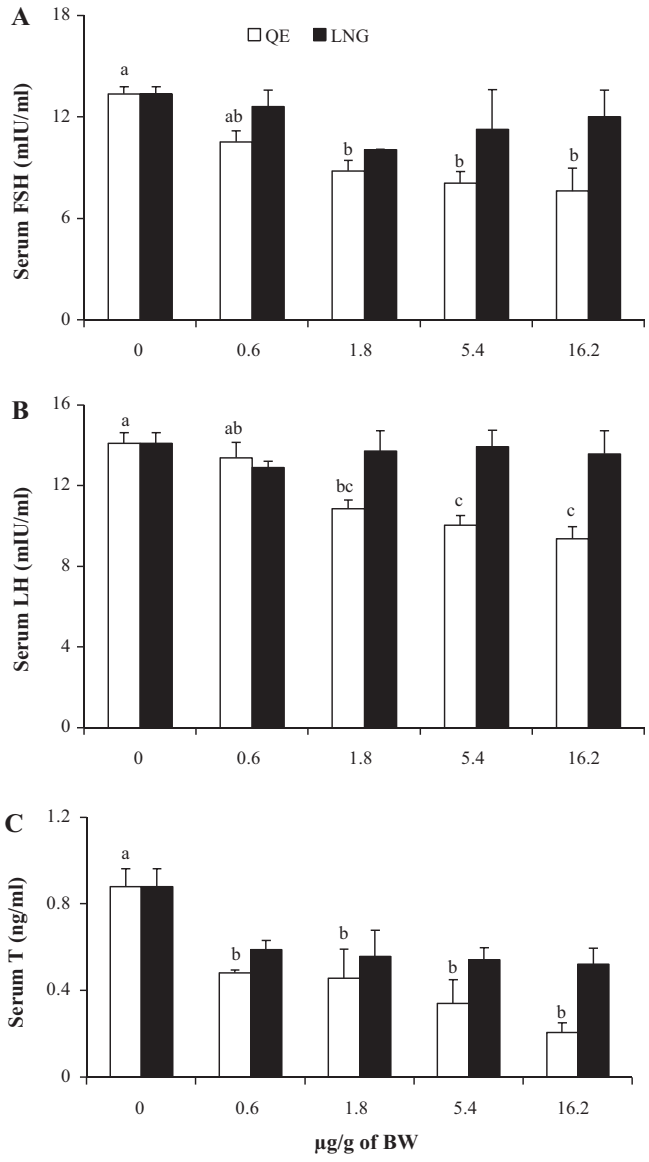

Fig. 4 – Serum concentrations (mean ± SEM) of (A) follicle-stimulating hormone (FSH), (B) luteinizing hormone (LH), and (C) testosterone (T) in male pups from gerbils (n = 3/per group) treated with quinestrol (QE; □) or levonorgestrel (LNG; ■). Bars with different superscripts are significantly different ($p < 0.05$). No differences were found for LNG; BW: body weight.

Table 5 – The fertility (mean ± SEM) of the female pups from the gerbils treated with levonorgestrel (LNG).

Dose (μg/g)	Gestation length (days)	Litter size	Number of females	Number of males
Control gerbils	24.0 ± 0.0	5.7 ± 0.3	2.3 ± 0.3	3.3 ± 0.7
LNG-treated gerbils				
0.6	24.3 ± 0.3	5.3 ± 0.3	2.7 ± 0.3	2.7 ± 0.7
1.8	24.0 ± 0.0	4.7 ± 0.9	1.7 ± 0.7	3.0 ± 0.6
5.4	24.0 ± 0.0	6.0 ± 0.6	2.3 ± 0.7	3.7 ± 0.3
16.2	24.3 ± 0.3	4.7 ± 0.3	2.0 ± 0.6	2.7 ± 0.3

n = 3 per group; no differences were found.

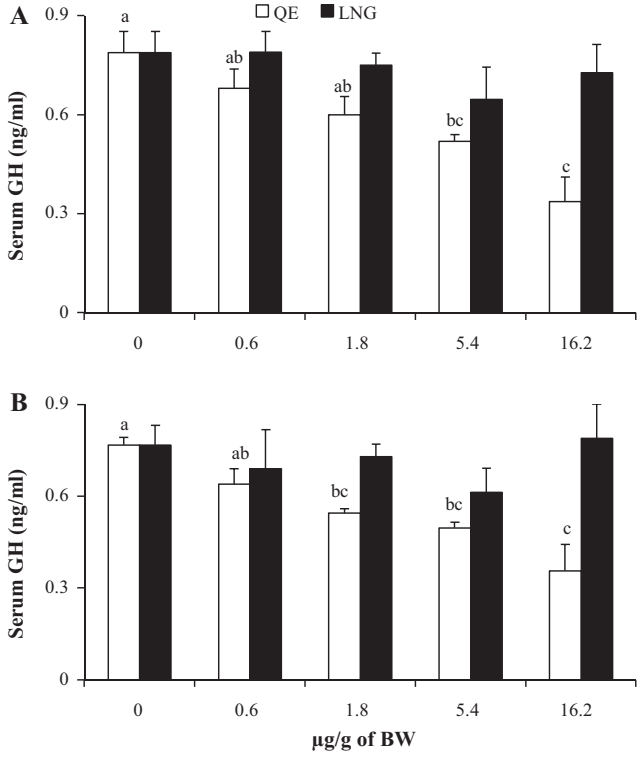

Fig. 5 – Serum growth hormone (GH) concentrations (mean ± SEM) in (A) female and (B) male pups from gerbils (n = 3/per group) treated with quinestrol (QE; □) or levonorgestrel (LNG; ■). Bars with different superscripts are significantly different ($p < 0.05$). No differences were found for LNG; BW: body weight.

In summary, we demonstrated that that QE and LNG increased serum PRL levels in lactating Mongolian gerbils. In contrast to LNG-treated gerbils, the reproduction of the pups from the QE-treated gerbils was entirely inhibited. It is probable that QE is transferred *via* maternal milk to the pups and affects the reproductive process of the offspring.

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