Coca-cola and Pepsi-cola affect ovaries and follicles development.

Wei Suocheng[1], Lu Huining[1], Liang Haoqin[1], Lai Luju[2], Gong Zhuandi[2*]**

[1]Life Science and Engineering College, Northwest University for Nationalities, Lanzhou, PR China

[2]Affiliated Hospital of Medicine College, Northwest University for Nationalities, Lanzhou, PR China

Abstract

Objective: The present study investigated the effects of carbonated drinks on ovaries and follicles of animals. Method 150 female non-cycle mice (20.36 ± 2.28 g) were divided into five groups. COC-1 and COC-2 mice drank freely 50% and 100% Coca-cola. PEP-1 and PEP-2 mice received orally 50% and 100% Pepsi-cola. Mice in Control Group (CG) drank tap water. Ovarian and follicle indexes were measured under the light microscope. FSHR levels in ovaries, serum caspase-3, Epidermal Growth Factor (EGF) and Vascular Endothelial Growth Factor Receptor (VEGFR) were measured with ELISA kit. Results On day 25, ovarian weight of PEP-2 was significantly lower than that of CG (P<0.05). Ovarian Cortex Thickness (OCT) of PEP-2 was significantly reduced in comparison with CG (P<0.05). In COC-2 group, values of Follicle Longitudinal Diameter (FLD), Follicle Transverse Diameter (FTD), Follicle Wall Thickness (FWT), Oocyte Longitudinal Diameter (OLD) and Oocyte Transverse Diameter (OTD) were less than CG on day 25. FWT and OLD of PEP-1 were decreased compared to CG. Primordial Follicles (POF) and Primary Follicles (PF) were small. Numbers of POF and MF reduced slightly. Small Secondary Follicle (SF) and Mature Follicle (MF) were distributed. Follicles developed poorly. Serum levels of FSHR proteins and caspase-3 slightly decreased in experimental mice. VEGFR and EGF levels of COC and PEP groups increased during the experiment. Conclusion Administration of coca-cola and pepsi-cola for a longer duration could reduce ovarian weights, inhibited the ovarian cortex thickness, affect the development of follicles and oocytes.

Introduction

Currently Carbonated Soft Drinks (CSDs) are the most popular types of beverage worldwide and many people drink them daily [1,2]. However, CSDs have become one of the crucial harmful factors along with their consumption increase. The rising of prevalence rate of overweight, obesity and type-II diabetes or the metabolic syndrome is related to CSDs [3,4]. It has been found that CSDs adversely affect reproduction [5]. Ovary weights of decreased significantly [6]. The number of primary and secondary follicles decreased significantly on days 7, 14 and 28 as did the number of antral follicles on days 14 and 28 after birth in the high dose caffeine-treated group. The diameter of secondary and antral follicles decreased significantly in high dose caffeine-treated group on the early days of postnatal development. The male and female Sprague-Dawley rats were exposed to coca-cola from 30, 39, or 55 weeks of age. The results indicated that body weight increased

in the treated animals [7]. However, the findings of a prospective investigation did not support the hypothesis that caffeine affected the ovulation function and increased the frequency of infertility due to ovulation disorders [8].

Many studies have investigated whether caffeine and alcohol intake affect fertility in women. However, most of these studies have retrospectively collected information, the results were acquired based on the questionnaire investigation, or the prospective analyses of clinical cases or epidemiological observations, making the results susceptible to biases and inconsistent mutually [8]. So far, there has been little quantitative evaluation of the fertility and carbonated drink intake [1]. Comparative experimental studies are scanty [2,9].

The present study aimed to investigate comparatively the effects of coca-cola and pepsi-cola on development of ovaries and follicles female mice at the different doses, also to explore

thoroughly the long-term impacts of CSDs on microstructure and serum levels of FSHR, caspase-3, EGF and VEGFR so as to provide the experimental basis for further studying effects and mechanisms of CSDs on reproduction in humans.

Material and Methods

Animals and ethics statement

One hundred and fifty Kunming female non-cycle mice (Mus musculus), 28 days old and body weight of 20.4 4 ± 2.45 g, were purchased from Experiment Animal Center, Lanzhou University [License No. SCXK (Gansu) 2005-0007]. All mice were randomly assigned into five groups: Coca-Cola group 1 (COC-1), Coca-Cola group 2 (COC-2), Pepsi-cola group 1 (PEP-1), Pepsi-cola group 2 (PEP-2) and control groups (CG, n=30). All mice were accurately weighed each day using an electronic balance, and raised in the group and kept in mice cages equipped with automatic water dispensers under the same conditions in the room maintained at 22-24°C and 30% to 50% relative humidity. The light cycle in the room provided 12h light/day. Mice freely received a commercial diet (Lanzhou Taihua Feed Co. Ltd, Lanzhou, China). Water was provided ad libitum. The experiment was launched following 7 days adjustment period. All procedures referring to animal treatment were approved by the Experiment Animal Care and Use Committee of Gansu province, the People's Republic of China. All mice were treated in the humanitarianism and ethical rules.

Animal treatments and sample collection

Mice in COC-1, COC-2, PEP-1, PEP-2 and CG drank coca-cola, pepsi-cola or tap water as summarized in table 1. Coca-cola and pepsi-cola in 5 Liter bottles were bought from a supermarket in Lanzhou city. They were stored at a room temperature of 22°C ± 3°C. After 5 female mice from each group were anesthetized by injecting 0.1 mg/kg xylazine intramuscularly, they were sacrificed by cervical dislocation on days 5, 10, 15, 20 and 25, respectively.

Bilateral ovaries were harvested aseptically and accurately weighed using an electronic balance. Blood samples were taken aseptically using vacutainers (Zhejiang Gongdong Medical Technology Co Ltd, Zhejiang, China). Blood samples were allowed to coagulate during 2h at room temperature, and then were centrifuged (3000 Xg, 20 min). The serum was stored at -20°C until analysis. The rest five mice of each group and 2 male mice (45 days old) were raised together from day 25 on and mated randomly.

Table 1. Adeministration doses of carbonated drinks and sampling.

Group	Numbers	Treatment	Sampling
COC-1	30	50% Coca-Cola	
COC-2	30	100% Coca-Cola	
PEP-1	30	50% Pepsi Cola	The ovaies, uteri and blood were collected at days 0, 5, 10, 15, 20 and 25, respectively.
PEP-2	30	100% Pepsi Cola	
CG	30	tap water	

Notes: 50% Coca-Cola (Pepsi Cola) represent one liter (1 L) of pure Coca-Cola (Pepsi Cola) was released by adding the same volume of tap water (1 L); 100% Coca-Cola (Pepsi Cola) represent one liter (1 L) of pure Coca-Cola (Pepsi Cola) was released by adding the same volume of tap water (1 L).

Measurements of ovarian weight and Ovarian Cortex Thickness (OCT)

The ovarian weight was weighed for each ovary using an electronic balance. Under an optical microscope, OCT was determined immediately with a vernier caliper. The average ovarian weight of each mouse was calculated on the basis of the right and left values.

Histological observation and image measurement of ovaries

Ovary tissues fixed in 10% formaldehyde were sliced (5μm), and stained with hematoxylin and eosin (H&E). The sections were observed under the light microscope (Leica, Japan). Microscopic images of the ovaries were photographed. Six sites in each section (5 sections in every group, totaling 150 sites for each group) were evaluated. Data of ovaries and follicles were measured using Images Advanced 3.2 and Image Pro-Plus 2.0 (MOTIC Company, Hong Kong, China). The indexes included the follicle (secondary follicles and mature follicles) longitudinal diameter (FLD), FTD, FWT, oocyte (including the secondary oocytes and mature oocytes) longitudinal diameter (OLD) and OTD.

Western blotting analysis of FSHR protein in ovaries

To evaluate the FSHR protein expression of ovaries following oral ingestion of Coca-cola and pepsi cola, the Western blotting was performed. Briefly, the ovary samples were lysed in lysis buffers (0.5% Nonidet P [NP] 40, 10 mmol/L Tris, pH 7.4, 150 mmol/L NaCl, 1 mmol/L ethylene diamine tetra-acetic acid [EDTA], 1 mmol/L Na_3VO_4) containing protease inhibitor (1 mmol/L Phenylmethylsulfonyl Fluoride [PMSF]).

Proteins were loaded onto 10% Sodium Dodecyl Sulfate Polyacrylamide gel Electrophoresis (SDS-PAGE), then transferred to Polyvinylidene Fluoride (PVDF) membranes and

blocked in 5% non-fat milk in 10 mmol/L Tris, pH 7.5, 100 mmol/L NaCl, 0.1% (w/v) Tween 20 for 2 hrs. Mouse anti-rabbit FSHR polyclonal antibodies (Sigma, 1:200) and β-actin polyclonal antibodies (1:1000) were diluted and incubated at 4°C overnight, followed by 1 h incubation with the appropriate secondary antibody (1:2000). Anti-β-actin mouse monoclonal antibodies were diluted in 1:10000 for sample loading control. The blots were performed using a chemiluminiscence reagent (SuperSignal West Pico, Rockford, IL, USA).

The Integral Optical Density (IOD) of the scanned band images was done by using Quantity One software (Bio-Rad Company, Hercules, CA, USA). The relative contents of FSHR proteins were presented as the ratio of FSHR gray values divided by that of β-actin. A negative control was performed without primary antibodies. The experiments were executed in triplicate.

Detections of serum caspase-3, EGF and VEGFR

Serum caspase-3 levels were detected using caspase-3 detection kit. Serum levels of the EGF and VEGFR were also assayed using EGF detection kit and VEGFR detection kit for rabbits (ELISA) respectively according to the manufacturer's instructions (Shanghai Bangyi, Biological Technology Co. Ltd, Shanghai, China). The samples were executed in triplicate. Analytical sensitivities were 0.10 ng/mL (caspase-3) and 0.40 pg/mL (EGF and VEGFR). The inter-assay CV was lower than 6%. The correlation coefficient of the standard curve was 0.9986.

Pregnancy duration of maternal mice and gender ratio offspring rats

The rest five mice of each group and 2 health male mice (45 days old) were raised together and mated randomly from day 25 on. Pregnancy rates and duration of every mouse were calculated. Numbers of offspring rats and their gender ratio were also determined.

Statistical analyses

Statistical analysis was done using SPSS v. 18.0 (SPSS Inc. Chicago, IL, USA). Data is presented as means ± SEM. All variables of three groups complied with the assumptions for a one-way ANOVA. Pearson's correlation analysis was utilized to analyze the correlations between the ovarian parameters. When significant differences were identified, supplementary Tukey's post-hoc tests were conducted to investigate pair wise differences. P value lower than 0.05 was considered to be a significant difference ($^{*}P<0.05$ and $^{**}P<0.01$).

Results

Ovarian weights of mice

As shown in table 2, ovarian weights of all experimental group mice were less than that of CG during the whole experiment. On day 25, ovarian weight of PEP-2 was significantly lower than that of CG (P<0.05). There was no significant difference between coca-cola group and pepsi-cola group. The findings indicated that oral ingestion of coca-cola and pepsi-cola for a longer duration could reduce ovarian weights. Pepsi-cola had a strong efficacy.

Ovarian Cortex Thickness (OCT)

Data in table 3 showed that OCT values of experimental group mice were lower than CG within the experiment. But there were no significant difference on the first 10 days among all groups. On days 15, 20 and 25, OCT of PEP-2 was significantly reduced in comparison with CG (P<0.05, or P<0.01).

On day 25, OCT of COC-2 and PEP-1 were also significantly reduced in comparison with CG (P<0.05). The results demonstrated that administration of coca-cola and pepsi-cola could inhibit the ovarian cortex thickness. The effects increased along with drinking time. Efficacy of pepsi-cola was slightly greater than coca-cola.

Table 2. Ovarian weights of mice (mg).

Group	0 d	5 d	10 d	15 d	20 d	25 d
COC-1	5.67 ± 0.29	5.67 ± 0.47	7.67 ± 0.31	8.33 ± 0.50	9.67 ± 0.80	10.17 ± 0.85
COC-2	5.33 ± 0.49	5.98 ± 0.60	7.02 ± 0.65	8.18 ± 0.70	9.33 ± 0.89	9.80 ± 0.96
PEP-1	5.87 ± 0.51	6.13 ± 0.73	7.33 ± 0.74	8.07 ± 0.72	9.27 ± 0.58	9.73 ± 0.85
PEP-2	5.83 ± 0.51	6.07 ± 0.63	7.01 ± 0.72	8.07 ± 0.74	9.17 ± 0.82	9.37 ± 0.93[b]
CG	5.67 ± 0.52	6.20 ± 0.71	7.33 ± 0.71	8.67 ± 0.60	9.67 ± 0.88	10.83 ± 1.03[a]

Note: Compared to control group, the different superscripts mean that there was significant difference between groups (P<0.05).

Values of FLD, FTD, FWT, OLD and OTD

Results in table 4 indicated that FLD, FWT, OLD and OTD of COC-2 were less than that of CG (P<0.05) on day 25. All five indexes of PEP-2 were significantly lower than CG (P<0.05 or P<0.01). Meanwhile, FWT and OLD of PEP-1 were decreased compared to CG (P<0.05).

Table 3. *Ovarian cortex thickness of mice (μm).*

Group	0 d	5 d	10 d	15 d	20 d	25 d
COC-1	8.08 ± 0.65	8.54 ± 0.78	9.03 ± 0.81	9.69 ± 0.86	10.20 ± 1.06	10.46 ± 1.13
COC-2	7.98 ± 0.62	8.31 ± 0.91	8.37 ± 1.01	8.87 ± 0.81	9.12 ± 0.85	9.55 ± 0.88b
PEP-1	8.06 ± 0.78	8.12 ± 0.79	8.27 ± 0.75	8.96 ± 0.81	9.01 ± 1.04	9.31 ± 1.09b
PEP-2	8.11 ± 0.81	8.25 ± 0.82	8.29 ± 1.86	8.21 ± 0.79b	8.12 ± 0.76b	8.31 ± 0.91c
CG	8.12 ± 0.79	8.89 ± 0.92	9.28 ± 0.91	10.32 ± 0.98a	11.38 ± 1.12a	12.60 ± 1.23a

Notes: Compared to control group, the different superscripts mean that there was significant difference between groups, of which adjacent superscript (such as ab, bc) indicate the difference was significant (P<0.05), while interval superscript (such as ac) the difference was highly significant (P<0.01).

Table 4. *FLD, FTD, FWT, OLD and OTD of mice on day 25 (μm).*

Group	FLD	FTD	FWT	OLD	OTD
COC-1	407.3 ± 39.5	273.3 ± 20. 9	73.2 ± 9.5	138.2 ± 15.6b	127.1 ± 12.3
COC-2	378.5 ± 40.7b	251.2 ± 21. 2	65.4 ± 7.6b	130.5 ± 14.8b	117.3 ± 3.5b
PEP-1	391.1 ± 40.5	269.6 ± 18.2	70.2 ± 9.3b	137.7 ± 16.6b	124.9 ± 7.7
PEP-2	373.2 ± 31.4b	242.0 ± 19.1b	61.8 ± 8.7c	130.7 ± 15.6b	119.9 ± 1.6b
CG	425.5 ± 69.4a	292.6 ± 27.5a	86.3 ± 9.5a	161.6 ± 17.3a	146.8 ± 5.2a

Notes: 1. Compared to control group, the different superscripts mean that there was significant difference between groups, of which adjacent superscript (such as ab, bc) indicate the difference was significant (P<0.05), while the interval superscript (such as ac) show the difference was highly significant (P<0.01).
2. FLD: Follicle Longitudinal Diameter; FTD: Follicle Transverse Diameter; FWT: Follicle Wall Thickness; OLD: Oocyte Longitudinal Diameter; OTD: Oocyte Transverse Diameter.

Histology structures in ovaries of mice

CG: The Primordial Follicles (POF) and Primary Follicles (PF) were small. A few of the Mature Follicles (MF) existed. The structures of the ovaries and follicles were complete (Figure 1A). Ovarian cortex and Zona Pellucida (ZP) were clear.

COC: In COC-1 group, numbers of POF and MF reduced slightly in comparison with CG. Loose granular layer distributed over the Secondary Follicles (SF). In COC-2 group, numbers of POF and PF decreased in comparison with CG (Figures 1A and 1B). Small SF and MF were distributed. Follicles did not develop fully. A few apoptotic follicles were observed.

PEP: In PEP-1, histological changes in PEP were highly similar to COC groups. POF was scarcer than in COC-2. Few SF and mature MF were observed in comparison with CG. They became larger in comparison with that of CG. The granular layer in SF distributed tightly. Zona pellucida (ZP) became small. For PEP-2 group, few SF and MF existed. PF and SF numbers reduced as compared to COC-2. Follicles

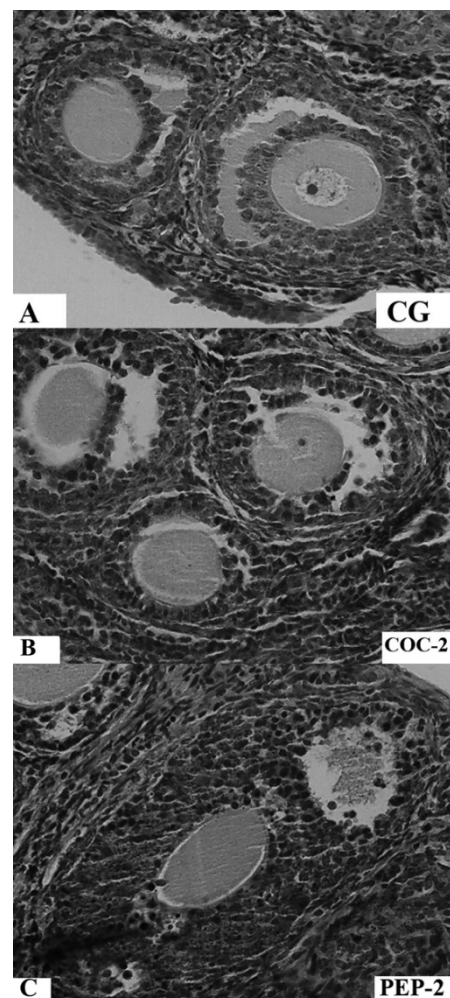developed poorly. The apoptosis of granular cells was found (Figure 1C).

Figure 1. *Microstructure changes of ovaries under optic microscope in mice (x400). Drinking beverages of coca-cola and pepsi-cola for a longer time can block the ovary and follicle development and maturation. A, B and C represent CG, COC-2 and PEP-2 groups, respectively.*

The results demonstrated that drinking coca-cola and pepsi-cola for a longer time could down-regulate the ovary and

follicle development and maturation. Pepsi-cola had more noticeable effects than coca-cola.

Expression levels of FSHR protein in ovaries

Western blotting of ovarian FSHR proteins was performed for each group. In comparison with CG, FSHR protein levels decreased slightly in all experimental mice during the whole experiment (Figure 2). However, there was no significant difference between groups. These findings demonstrated coca-cola and pepsi-cola had no obvious effects on expressions of FSHR protein in ovaries of mice.
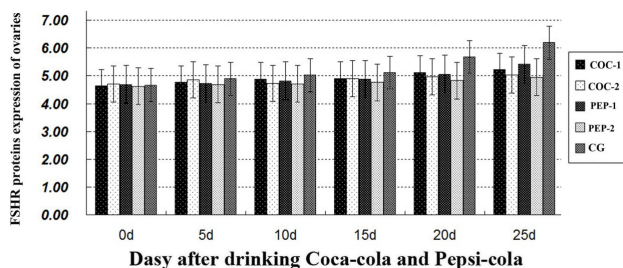
Figure 2. Expression levels of ovarian FSHR proteins, FSHR protein levels decreased slightly in all experimental mice during the whole experiment when compared to CG.

Detections of serum caspase-3

As shown in figure 3, on day 10 serum caspase-3 levels of COC-1 and PEP-1 were increased in comparison with CG. From day 15, serum caspase-3 levels in COC and PEP groups are all less than in CG. The maximum reduction of caspase-3 levels was found in PEP-1 group (P<0.05). The results indicated high doses of coca-cola and pepsi-cola reduced caspase-3 synthesis.
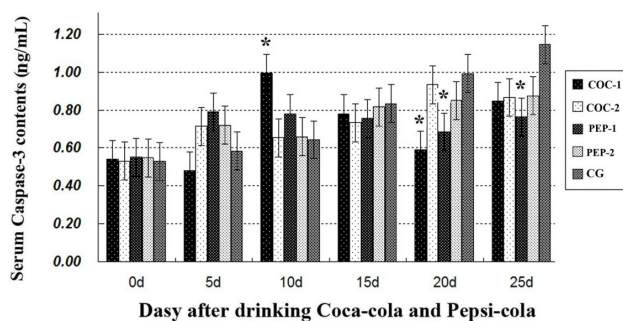
Figure 3. Changes of serum caspase-3. Serum caspase-3 levels of COC-2, PEP-1 and PEP-2 increased in comparison with CG. The maximum increment of caspase-3 levels was found in PEP-2 group.

Detections of serum EGF and VEGFR

As shown in figure 4, the EGF levels of four experimental groups were slightly higher than that of CG on day 15. On day 20, EGF level of PEP-2 was increased significantly compared to CG (P<0.05). EGF level of PEP-1 was higher than that of CG on day 25 (P<0.05). That demonstrated coca-cola and pepsi-cola could enhance EGF activity.
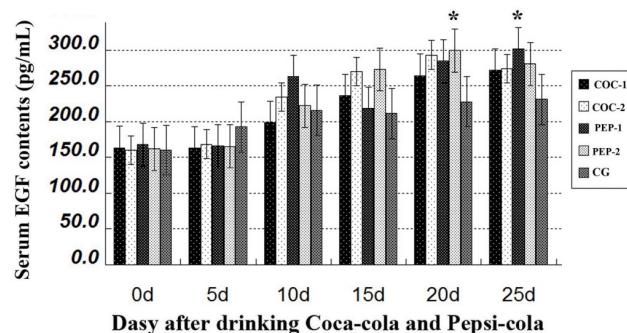
Figure 4. Changes of serum EGF, On day 20, EGF levels of COC-2 and PEP-2 were increased significantly compared to CG (P<0.05). On day 25, level of PEP-1 were increased significantly compared to CG.

Data in figure 5 showed that serum levels of VEGFR varied similar to EGF levels in four experimental groups. VEGFR levels of both COC and PEP groups were increased during the experiment. Day 15 afterwards, VEGFR levels of all experimental mice were higher than that of CG (P<0.05 or P<0.01). The most significant increase was detected in PEP-2. The results indicated that coca-cola and pepsi-cola could promote serum VEGFR levels and up-regulated VEGFR activity.
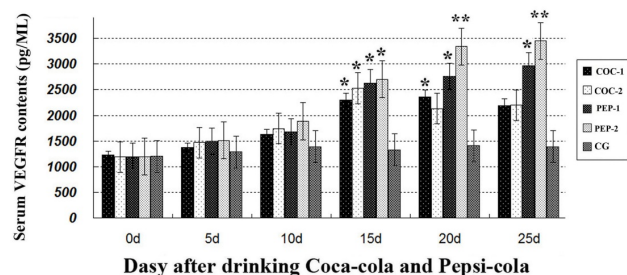
Figure 5. Changes of serum VEFGR, VEGFR levels of all experimental mice were higher than that of CG on day 15, the maximum increase was detected in PEP-2.

Pregnancy duration of maternal mice and gender ratio offspring rats

Data in table 5 showed that the pregnancy rate of PEP-2 mice was obviously lower than that of CG and PEP-1 group (P<0.05). Pregnancy duration of mice had no significant differences between all groups. Total offspring numbers of pregnancy female mice were maximum COC-1 and minimum in and COC-2 mice. Mean birth numbers of COC-1 was greater than that of COC-2, PEP-2 (P<0.05) and control groups. The gender ratio (Male : Female) of offspring mice decreased in experimental mice as compared with CG. The gender ratio of COC-1, COC-2 and PEP-2 groups were significantly lower than that of CG (P<0.05). The body weights of COC-2, PEP-1 and PEP-2 offspring mice in 1 week old were increased in COC-2 and PEP-1 in comparison with CG (P<0.05). On day 7 (1 week old) after birth, the survival rates of offspring mice in COC and PEP groups were reduced with

the maximum reduction in COC-2 and PEP-1 (P<0.05). The findings demonstrated coca-cola and pepsi-cola obviously affected reproduction behaviors of female mice, additionally impacted the growth and development offspring mice.

Table 5. Mice pregnancy duration and offsprings gender ratio.

Group	Pregnancy rate (%)	Pregnancy period (d)	Offspring numbers	Mean numbers birth	Gender ratio (M:F)	Body weights (g)	Survival rate (%)
COC-1	4 (80.0)	20.0 ± 0.8	48	12.0 ± 1.1[A]	40.0: 60.0[b]	4.05 ± 0.41[A]	46 (95.8)
COC-2	4 (80.0)	21.0 ± 1.2	37b[A]	9.3 ± 1.6[B]	47.4: 52.6[b]	5.17 ± 1.33b[B]	33 (89.2)[b]
PEP-1	5 (100.0)[A]	20.6 ± 1.5	55[B]	11.0 ± 1.2	60.0: 40.0	4.64 ± 0.17	53 (96.4)
PEP-2	3 (60.0)[bB]	19.7 ± 1.1	28b[C]	9.3 ± 1.0B	44.4: 55.6[b]	4.84 ± 0.45	26 (92.8)
CG	4 (100.0)[a]	20.0 ± 1.0	52[a]	10.5 ± 1.1	70.0: 30.0[a]	4.25 ± 0.49[a]	51 (98.1)[a]

Note: Compared to control group, the different lowercase letter superscripts mean that there was significant difference between groups (P<0.05). The different capital letter superscripts represent that there was significant difference between experimental groups (P<0.05). Survival rate and body weights of each group were determined on day 7 after offspring birth (or 1 week old).

Discussion

Caffeine (including coffee, cocoa, chocolate, colas) has been associated with alterations in estradiol and other hormones [10] which may affect ovulation, the length of the follicular or luteal phase. The mechanisms that caffeinated beverages could affect fertility are undetermined. Ovary weights decreased significantly in caffeine-treated group at all stages of postnatal development [6]. The number of primary and secondary follicles decreased significantly on days 7, 14 and 28 after birth in caffeine-treated group. The diameter of secondary and antral follicles decreased significantly at the early days of postnatal development. In the present study, the results demonstrated that ovarian weights of experimental groups decreased compared to normal CG, especially in PEP-2 group. OCT values of experimental groups decreased during the experiment. OCT of PEP-2 was significantly reduced in comparison with CG. Meanwhile, FLD, FWT, OLD and OTD of COC-2 and PEP-2 were lower than CG on day 25. Numbers of POF and MF reduced slightly. The granular layer in the SF distributed loosely. SF and MF were decreased. Follicle development was inadequate. A few follicles displayed apoptosis. ZP was thinned. Our findings indicated that oral ingestion of high dose of coca-cola and pepsi-cola could decrease numbers of SF and MF, also reduced the sizes of FLD, FWT, OLD and OTD when compared to normal control mice. The changes of micro-histology and ovarian indexes were consistent. Such coca-cola and pepsi-cola affected the development of ovaries and follicles. The actual mechanism need to be further studied. These findings are in agreement to early reports [6,11]. But, they are inconsistent with other results [1,8]. Therefore the accurate effects of carbonated drinks (including coca-cola and pepsi-cola) still need to be thoroughly explored.

Follicle Stimulating Hormone (FSH) can promote the proliferation and differentiation of preantral follicles, thus induce follicular growth and maturation of ovarian follicles [12]. Ovarian response to FSH stimulation depends on the FSHR genotype [13,14]. FSHR has been found to be expressed in multiple ovarian cell types, including pre-ovulation granule cells and luteinized cells. FSHR is expressed in all sizes of preantral follicles. However, it is unknown whether high doses of coca-cola or pepsi-cola influence FSHR expression in ovaries [15]. Our study indicated FSHR protein levels were decreased in experimental mice with a maximum reduction of PEP-2. Up to date, little information on this aspect has been reported [16,17]. Such our results remain to be testified by the future studies.

Apoptosis, a programmed cell death, is characterized by specific structural changes. Although multiple genes are involved in apoptosis, the key mediators are aspartate specific cysteine proteases (Caspases). The caspases play important roles in the process of apoptosis. Caspase-3 is the most critical apoptosis protease in the downstream of caspase cascade [18]. FSH down-regulates caspase-3 mRNA levels in the granule cells of dominant follicles. As a result, FSH prevented atresia in dominant follicles [19]. However, there is scarce information about CSDs administration affecting the activity of caspase family [20]. In this comparative investigation, serum caspase-3 levels of COC-2, PEP-1 and PEP-2 increased with a maximum increment in PEP-2 group. This may be a reason for poor follicle development and apoptosis as well as reduction of ovarian FSHR expression levels. Its mechanism needs to be further studied.

The EGF enhances epidermal regeneration, cell motility and proliferation, and stimulates cellular migration, proliferation and angiogenesis. The protective effect of EGF against apoptosis is known to occur through the activation of PI3K/AKT [21]. VEGF is one of the most potent proangiogenic growth factors [22]. VEGF and its receptor 2 are the main promoters of angiogenesis and cellular protection during follicular and CL development. Angiogenic factors probably play a role in the pathology of ovarian Granulosa Cell Tumors (GCTs) [23]. VEFG directly suppressed T-cell activation via VEGFR-2. The main function of VEGF and receptors is to control the new blood vessels formation and the protection of endothelial and granulosa cells [24]. The vascular changes are important to regulate the follicular and Corpus Luteum development (CL), as well that the ovulation [25]. It is currently known that VEGFR can be expressed by a variety of

other cell types [26,27]. Our findings indicated that serum VEGF levels of experimental groups were higher than that of CG. On day 25 EGF level of PEP-1 increased significantly. Day 15 afterwards, serum VEGFR levels of experimental mice were higher than CG with a maximum increase in PEP-2 mice. However, similar reports are not documented. The accurate effects have to be further explained.

High doses of coca-cola and pepsi-cola reduced fetus numbers, also increased proportion of female fetus. This may be associated to the changes of micro environment within uterus after a longer drinking coco-cola and pepsi-cola. Our results have to be testified in other animals and humans [28].

Conclusion

Oral ingestion of coca-cola and pepsi-cola for a longer duration could reduce ovarian weights at the different degrees, inhibited the ovarian cortex thickness, affect the development of follicles and oocytes. Coca-cola and pepsi-cola increased serum levels of EGF and VEGFR. They decreased serum Caspase-3 contents, obviously affected reproduction behaviors of female mice, promoted growth of offspring mice, additionally reduced pregnancy rate and fetus numbers of female mice, additionally increased proportion of female fetuses. Efficacy of pepsi-cola was slightly greater than coca-cola. Our studies laid a solid foundation and provided the scientific bases to further investigating effects and mechanism of Coca-cola and Pepsi-cola on development and reproduction in humans.

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***Correspondence to:**

Gong Zhuandi

Affiliated Hospital of Medicine College

Northwest University for Nationalities

PR China