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Carbonated Drinks Impact Follicle Development, Expression of Ovarian FSHR and Serum Caspase-3 in Mice

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Summary

Objectives: The present study aimed to assess the effects of Coca-Cola and Pepsi-Cola on the development of ovaries and follicles, and on the reproduction of animals.

Methods: 150 female non-cycling mice weighing 20.36±2.28g were divided into five groups (n=30). COC-1 and COC-2 mice drank freely 50% and 100% Coca-Cola for up to 25 days. PEP-1 and PEP-2 mice received orally 50% and 100% Pepsi-Cola. Mice in the control group (CG) drank tap water. Another five mice were used as a normal control per group on day 0. Ovarian and follicle indices were measured under the microscope. Western blotting was utilized to ascertain the expression levels of FSHR protein in ovaries. Serum concentrations of caspase-3, epidermal growth factor (EGF) and vascular endothelial growth factor receptor (VEGFR) were measured by ELISA.

Results: The ovarian weight of PEP-2 was significantly lower than that of CG ($P<0.05$) on day 25. Ovarian cortex thickness (OCT) of PEP-2 was significantly reduced in comparison with CG ($P<0.01$). In the 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 those of CG on day 25. FWT and OLD of PEP-1 were reduced as compared to CG. In COC and PEP groups, numbers of POF and MF were reduced slightly. Follicles developed poorly. FSHR protein was decreased slightly and EGF levels increased in Coca-Cola treated or Pepsi-Cola treated mice. Serum caspase-3 levels of COC-1 and PEP-1 were significantly lower than those of CG.

Conclusion: Coca-Cola and Pepsi-Cola treatment could reduce ovarian weights, inhibit the ovarian cortex thickness and impair the development of follicles.

Introduction

Carbonated beverages are the most popular types of beverage worldwide. Many people drink these almost every day (*Hatch et al., 2012*). However, the harmful effects of carbonated beverages have been associated with their increased consumption. The rising prevalence of overweight people, obesity and type-II diabetes or the metabolic syndrome is related to car-bonated beverages (*Cuomo et al., 2014; Guan et al., 2013*). Recent studies have shown that consuming ≥ 1.5 servings of sugar-sweetened beverage daily could result in girls between 9 and 18.5 years old menstruating on average of 2.7 months earlier than those consuming carbonated beverages ≤ 2 times per week (*Carwile et al., 2015*). Consumption of carbonated

drinks during menstruation could alter menstrual characteristics in nulliparous females (*Adienbo et al., 2016*).

It has already been demonstrated that carbonated beverages affect adversely reproductive behaviours and functions. Ovary weights and numbers of primary and secondary follicles were significantly reduced in rats (*Dorostghoal et al., 2011*). Diameters of secondary and antral follicles were also decreased significantly. Body weights of Sprague–Dawley rats were increased after they were exposed to Coca-Cola from 30, 39, or 55 weeks of age (*Belpoggi et al., 2006*).

Thus, carbonated beverages had noticeable effects on reproductive endocrinology, reproductive function and fertility in women and animals. However, most of these results were acquired based on questionnaire investigations, or the prospective analyses of clinical cases or epidemiological analyses, which means that the study results are susceptible to bias and are difficult to compare (*Chavarro et al., 2009*). Currently, little information exists concerning the quantitative effects of carbonated drinks on fertility (*Hatch et al., 2012*). Comparative experimental studies are few (*Chavarro et al., 2009; Cuomo et al., 2014*).

In order to examine thoroughly the influence of carbonated beverages on the reproductive endocrinology and functions in humans, the current investigation assessed the effects of different doses of Coca-Cola and Pepsi-Cola on the development of ovaries and follicles of mice. The impact of both drinks on expression levels of ovarian FSHR, and on serum concentrations of caspase-3, epidermal growth factor (EGF) and vascular endothelial growth factor receptor (VEGFR) was investigated. The aim was to provide the experimental basis for further mechanistic studies of the effects of carbonated beverages on reproductive functions in humans.

METHODS

Animals and Ethics Statement

In order to carry out a sufficiently large experiment and acquire meaningful results, 150 non-cycling Kunming mice *(Mus musculus)*, 28 days old and body weight of 20.4 4±2.45g, were purchased from the Experiment Animal Center, Lanzhou University [License No. SCXK (Gansu) 2005-0007]. All mice were randomly assigned into five groups (n=30): 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). All mice were accurately weighed each day using an electronic balance, and group housed in mouse cages equipped with automatic water dispensers in a room maintained at 22-24° 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 started following a 7-day adjustment period. All animal-treatment procedures were approved by the Experiment Animal Care and Use Committee of Gansu province, the People's Republic of China. All mice were treated according to humanitarian 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 outlined in Table 1. Coca-Cola and Pepsi-Cola in 5 litre bottles were bought from a supermarket in Lanzhou city. They were stored at a room temperature of 22± 3°C. 5 mice were randomly chosen from each group on days 5, 10, 15, 20 and 25. Each mouse was anesthetized by injecting 0.1mg/kg xylazine intramuscularly. Subsequently every animal was sacrificed by cervical dislocation. 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 2 h at room temperature, and then were centrifuged (3000×g, 20 min). The serum was stored at -20° until analysis.

The remaining five mice of each group and 2 healthy male mice (45 days old) were raised together on day 25 for a week so as to mate randomly.

Additionally, another five mice per group were used as normal controls on day 0. Bilateral ovaries and blood samples were collected on day 0 as described above.

Table 1. Administration doses of carbonated beverages and sampling. 50% Coca-Cola (Pepsi-Cola) represents one litre (1L) of pure Coca-Cola (Pepsi-Cola) diluted with the same volume of tap water (1L); 100% Coca-Cola (Pepsi-Cola) represents pure Coca-Cola (Pepsi-Cola).

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

Measurements of ovarian weight and ovarian cortex thickness (OCT)

Each ovary was weighed using an electronic balance. Under an optical microscope, ovarian cortex thickness (OCT) was determined immediately with a vernier calliper. The average ovarian weight of each mouse was determined on the basis of the right and left values.

Histological observations and image measurement of ovaries

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

Western blotting analysis of FSHR protein in ovaries

To evaluate the FSHR (follicle-stimulating hormone receptor) protein expression of ovaries following Coca-Cola and Pepsi-Cola treatment, western blotting was conducted. Briefly, ovary samples were lysed in lysis buffer, and the resulting proteins were subjected to 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis, before being transferred to polyvinylidene fluoride membranes (PVDF). These membranes were then blocked for 2 h in a 5% non-fat milk solution containing 10 mM Tris (pH 7.5), 100 mM NaCl, and 0.1% Tween 20 (w/v). Membranes were incubated with rabbit anti-sheep, FSHR (Sigma, diluted 1:200), and rabbit anti-sheep β-actin (1:1000) polyclonal antibodies at 4°C overnight, before being exposed to the appropriate secondary antibody (1:2000) for 1 h. Mouse anti-β-actin monoclonal antibody (1:10 000) was used as a sample loading control. Blots were developed using a chemiluminescent reagent (SuperSignal West Pico; Thermo Scientific, Rockford, IL, USA). The integrated optical densities of bands in the scanned images were measured with Quantity One software (Bio-Rad, Hercules, CA, USA). The protein expression of FSHR was determined as the ratio of the grey value of the target band to that of the β-actin band. Samples were run in triplicate. The negative control was not incubated with the primary antibodies.

Detection of serum caspase-3, epidermal growth factor (EGF) and vascular endothelial growth factor receptor (VEGFR)

Serum levels of caspase-3, epidermal growth factor (EGF) and vascular endothelial growth factor receptor (VEGFR) were measured using ELISA according to the manufacturer's instructions (Shanghai Bangyi, Biological Technology Co. Ltd, Shanghai, China). Samples were analysed in triplicate. Analytical sensitivities were 0.10ng/mL (caspase-3) and 0.40pg/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 of offspring

On day 25, female and male mice were raised together. The pregnancy rate and duration were calculated. Numbers of offspring and their gender ratio were also determined.

Statistical analyses

Data are reported as means ± standard errors of means. Statistical analysis was performed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). After square root transformation of the data, all variables complied with the assumptions of one-way analysis of variance (ANOVA).

Post-ANOVA comparisons between groups were carried out using the contrast option under the general linear model procedure (Scheffé test). When significant differences were identified, supplementary Tukey's *post-hoc* tests were conducted to investigate pairwise differences. P-values <0.05 were considered significant.

RESULTS

Ovarian weights of mice

As shown in Table 2, ovarian weights of all experimental group mice were less than that of control group (CG) during most of the experiment. On day 25, ovarian weight of PEP-2 was significantly lower than that of CG ($P<0.05$). There were no significant differences between Coca-Cola groups and Pepsi-Cola groups. The findings indicate that oral inges-

Table 2. Ovarian weights of mice (mean ± SEM, mg). Data on day 0 were measured from five normal control mice per group. *There was a significant difference when compared to control group (CG).

Group	0d	5d	10d	15d	20d	25d
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*
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

tion of Coca-Cola and Pepsi-Cola for a prolonged duration could reduce ovarian weights. Pepsi-Cola had a stronger effect.

Ovarian cortex thicknesses (OCT)

Data in Table 3 show that OCT values of experimental group mice were lower than that of CG during the experiment. On day 25, OCT of PEP-2 was significantly reduced in comparison with CG $(P<0.01)$. On day 25, OCT of COC-2 and PEP-1 groups was also significantly smaller than CG $(P<0.05)$. The results demonstrate that administration of Coca-Cola and Pepsi-Cola could reduce ovarian cortex thickness. The effects increased with the duration of exposure. The effect of Pepsi-Cola was slightly greater than that of Coca-Cola.

Values of FLD, FTD, FWT, OLD and OTD

Results in Table 4 indicate that FLD, FWT, OLD and OTD of COC-2 were less than that of CG *(mostly $P<0.05$)* on day 25. All five indices of PEP-2 were significantly lower than CG $(P<0.05$ or $P<0.01)$. FWT and OLD of PEP-1 were decreased compared to CG $(P<0.05)$.

Histology of ovaries

The primordial follicles (POF) and primary follicles (PF) were small. A few mature follicles (MF) existed. The structures of the ovaries and follicles were normal. Ovarian cortex and zona pellucida (ZP) were clear.

In COC-1 group, numbers of POF and MF were reduced slightly in comparison with CG. A thin gran-

Table 3. Ovarian cortex thickness of mice (mean ± SEM, μm). Data on day 0 were measured from five normal control mice per group. * There was a significant difference (P<0.05) when compared to control group (CG). **There was a highly significant difference (P<0.01) when compared to control group (CG).

Group	0d	5d	10d	15d	20d	25d
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±0.88*
PEP-1	8.06±0.78	8.12±0.79	8.27±0.75	8.96±0.81	9.01±1.04	9.31±1.09*
PEP-2	8.11±0.81	8.25±0.82	8.29±1.86	8.21±0.79*	8.12±0.76	8.31±0.91**
CG	8.12±0.79	8.89±0.92	9.28±0.91	10.32±0.98	11.38±1.12	12.60±1.23

Table 4. FLD, FTD, FWT, OLD and OTD of mice on day 25 (mean ± SEM, μm). Data on day 0 were measured from five normal control mice per group. FLD-Follicle longitudinal diameter; FTD-Follicle transverse diameter; FWT-Follicle wall thickness; OLD-Oocyte longitudinal diameter; OTD-Oocyte transverse diameter. *There was a significant difference (P<0.05) when compared to control group (CG). **There was a highly significant difference (P<0.01) when compared to control group (CG).

Group	FLD	FTD	FWT	OLD	OTD
COC-1	407.3±39.5	273.3±20.9	73.2±9.5	138.2±15.6*	127.1±12.3
COC-2	378.5±40.7*	251.2±21.2	65.4±7.6*	130.5±14.8*	117.3±3.5*
PEP-1	391.1±40.5	269.6±18.2	70.2±9.3*	137.7±16.6*	124.9±7.7
PEP-2	373.2±31.4*	242.0±19.1*	61.8±8.7**	130.7±15.6*	119.9±1.6*
CG	425.5±69.4	292.6±27.5	86.3±9.5	161.6±17.3	146.8±5.2

ular layer was distributed over the secondary follicles (SF). In COC-2 group, numbers of POF and PF were decreased in comparison with CG. Small SF and MF were present. Follicles did not develop fully.

In PEP-1, histological changes were very similar to COC groups. POF were scarcer than in COC-2. Few SF and mature MF were observed in comparison with CG and they were larger than in the CG. The granular layer in SF was distributed evenly. Zona pellucida (ZP) was small. For PEP-2 group, few SF and MF existed. PF and SF numbers were reduced as compared to COC-2. Follicles developed poorly. Apoptosis of granular cells was observed.

The results demonstrated that drinking Coca-Cola and Pepsi-Cola for a prolonged period could down-regulate ovary and follicle development and maturation. Pepsi-Cola had more noticeable effects than Coca-Cola.

Expression levels of FSHR protein in ovaries

To assess the impact of both Coca-Cola and Pepsi-Cola treatment on expression and mRNA level of FSHR, qRT-PCR was employed in each group. In comparison with CG, expression level of FSHR protein was decreased slightly in all experimental groups from day 10 (Figure 1). However, there were no significant differences between groups. These findings demonstrate that Coca-Cola and Pepsi-Cola had no marked effects on the expression level of ovarian FSHR protein in mice.

Detection of serum caspase-3

As shown in Figure 2, after day 10 serum caspase-3 levels of COC-1 and PEP-1 decreased in comparison to CG. The maximum reduction of caspase-3 levels was found in PEP-1 group *(P<0.05)*. The results indicated that Coca-Cola and Pepsi-Cola inhibited caspase-3 synthesis.

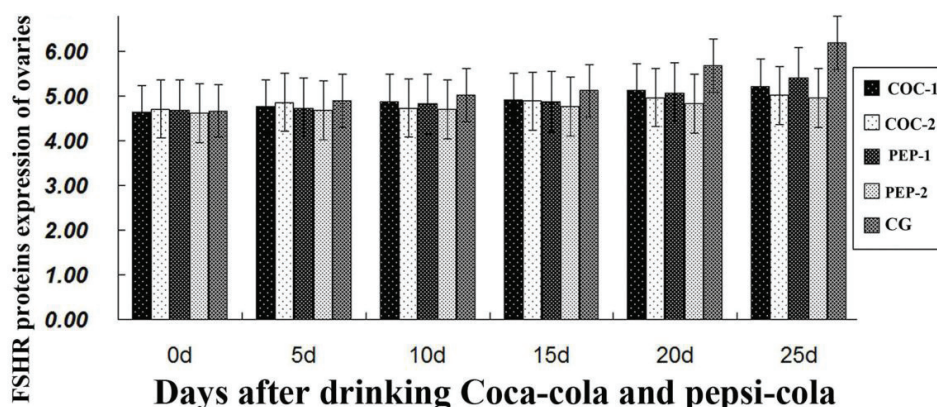**Figure 1.** Expression levels of ovarian FSHR protein

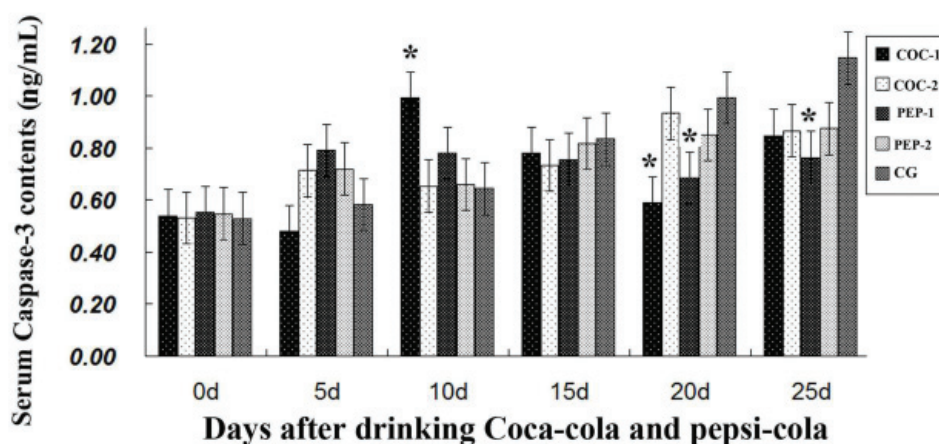**Figure 2.** Changes of serum caspase-3. *There was a significant difference (P<0.05) when compared to control group (CG).

Detection of serum EGF and VEGFR

As shown in Figure 3, the epidermal growth factor (EGF) levels of the four experimental groups were higher than that of CG after day 15. On day 20, the level for PEP-2 was increased significantly compared to CG *(P<0.05)*. On day 25, the level for PEP-1 was increased significantly as compared to CG *(P<0.05)*. Thus, Coca-Cola and Pepsi-Cola could enhance EGF activity.

Data in Figure 4 show that serum levels of vascular endothelial growth factor receptor (VEGFR) were increased in the four experimental groups. From Day 15, VEGFR levels of all experimental groups were higher than that of CG. The most significant increase was detected in PEP-2. The results indicate that Coca-Cola and Pepsi-Cola could increase serum VEGFR levels.

Pregnancy duration and gender ratio of offspring

Table 5 shows that there were no significant differences in pregnancy duration between the groups. Pregnancy rate of PEP-2 mice was clearly lower than that of CG. Mean offspring numbers were highest in COC-1 and lowest in COC-2 mice. The gender ratio (Male:Female) of offspring decreased in experimental mice. Gender ratios of COC-1, COC-2 and PEP-2 groups were significantly lower than that of CG *(P<0.05)*. The survival rate and body weights of 1 week old offspring were reduced in COC-2 when compared to CG *(P<0.05)*. The findings demonstrate that Coca-Cola and Pepsi-Cola clearly affected reproduction of female mice.
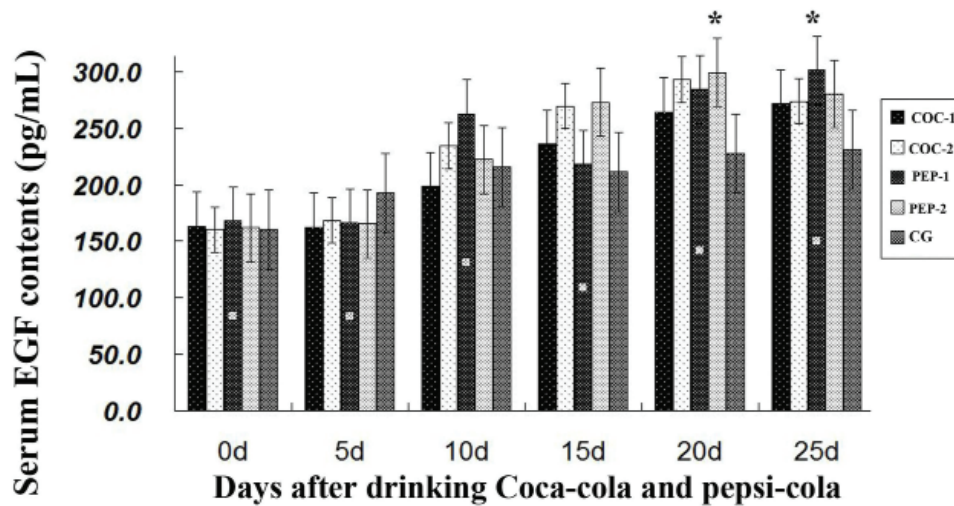

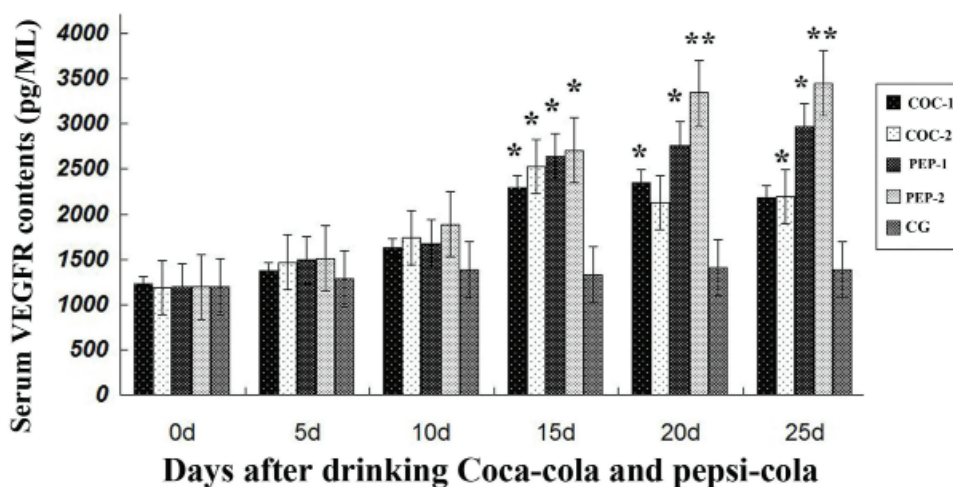**Figure 3.** Changes of serum EGF. *There was a significant difference (P<0.05) when compared to control group (CG).

Figure 4. Changes of serum VEFGR. *There was a significant difference (P<0.05) when compared to control group (CG). **There was a highly significant difference (P<0.01) when compared with control group (CG).

Table 5. Pregnancy and offspring results (mean ± SEM). Survival rate and body weights of each group were determined on day 7 after offspring birth (or 1 week old). The different capital letter superscripts represent that there was significant difference between experimental groups (P<0.05). *There was a significant difference (P<0.05) when compared to control group (CG).

Group	Pregnancy rate (%)	Pregnancy period (d)	Offspring numbers	Mean birth numbers	Gender ratio (M:F)	Body weights	Survival rate (%)
COC-1	4 (80.0)	20.0±0.8	48	12.0±1.1[A]	40.0:60.0[*]	4.05±0.41[A]	46 (95.8)
COC-2	4 (80.0)	21.0±1.2	37[*A]	9.0±1.6[B]	47.4:52.6[*]	5.17±1.33[*B]	33 (89.2)[*]
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)[*B]	19.67±1.1	28[*C]	9.3±1.0[B]	44.4:55.6[*]	4.84±0.45	26 (92.8)
CG	4 (100.0)	20.00±1.0	52	10.5±1.1	70.0:30.0	4.25±0.49	51 (98.1)

Discussion

Health concerns over carbonated soft drinks consumption have been widely reported. Many epidemiological studies have evaluated the association between caffeine and fertility, with inconsistent results. Some studies suggest that various caffeine-containing beverages may affect fertility differently (*Hatch et al., 2012*). Recent studies reported that caffeine (including coffee, cocoa, colas) has been associated with alterations in the levels of estradiol and other hormones in women (*Kotsopoulos et al., 2009*). However, the mechanisms by which caffeinated beverages affect fertility remain undetermined. Ovary weights of rats were decreased significantly in a caffeine-treated group at all stages of postnatal development (*Dorostghoal et al., 2011*). The number of primary and secondary follicles was decreased on days 7, 14 and 28 after birth in caffeine-treated Sprague–Dawley rats. In the present mouse study, ovarian weights of Coco-Cola and Pepsi-Cola groups were decreased compared to the control group (CG). Ovarian cortex thickness (OCT) values of the four experimental groups were decreased during the experiment in comparison with CG; the effect was most pronounced in PEP-2. FLD, FWT, OLD and OTD of COC-2 and PEP-2 were lower than that of CG on day 25. Additionally, numbers of POF and MF were slightly reduced. A thin granular layer was distributed over the secondary follicles (SF). Follicle development was inadequate. Our findings demonstrated that Coca-Cola and Pepsi-Cola affected the development of ovaries and follicles in mice. These findings were in agreement with early reports (*Chavarro et al., 2009; Dorostghoal et al., 2011*). But, they were in disagreement with the results of other studies (*Chavarro et al., 2009; Hatch et al., 2012*). Therefore, the effects and mechanism of Coca-Cola and Pepsi-Cola on ovarian and follicle development need to be thoroughly explored.

Follicle stimulating hormone (FSH) can promote the proliferation and differentiation of preantral follicles, and thus induce follicular growth and maturation of ovarian follicles (*Miró & Hillier, 1996*). The ovarian response to FSH stimulation depends on the FSHR genotype (*Perez et al., 2000; Ali et al., 2012*). It has previously been demonstrated that FSHR is expressed in multiple ovarian cell types, including pre-ovulation granule cells and luteinized cells. However, it was still unknown whether long-term drinking of Coca-Cola or Pepsi-Cola influences FSHR expression in ovaries and follicles (*Gormack et al., 2015*). Our study indicates that the expression level of ovarian FSHR protein was decreased slightly in the Coca-Cola treated and Pepsi-Cola treated mice. To date, little information on these effects has been reported (*Imai et al., 2010; Zeng et al., 2014*). Our results remain to be confirmed by future studies.

Apoptosis is characterized by specific structural changes. Although multiple genes are involved in apoptosis (*Li et al., 2013*), key mediators are aspartate specific cysteine proteases (Caspase). Caspase families play important roles in the process of apoptosis. Caspase-3 is the most critical apoptosis protease in the downstream of the caspase cascade (*Cao et al., 2014*). FSH down-regulates caspase-3 mRNA level in the granule cells of dominant follicles. As a result, FSH prevented atresia of dominant follicles (*Li et al., 2013*). In our present investigation, serum caspase-3 levels of COC-1 and PEP-1 decreased with the largest effect in the PEP-1 group. The mechanism needs to be further studied.

The epidermal growth factor (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 (*Zhang et al., 2014*). Vascular endothelial growth factor (VEGF) and its receptor 2 (VEGFR-2) are the main promoters of angiogenesis

and cellular protection during follicular and corpus luteum (CL) development (*Shibuya, 2013*). VEFG directly suppressed T-cell activation via VEGFR-2 (*Färkkilä et al., 2011*). The main functions of VEGF and its receptors are to control the formation of new blood vessels, and also to protect the endothelial and granulose cells (*Gavalas et al., 2012*). Vascular changes are important to regulate the follicular and CL development and ovulation (*Torres & Sanchez, 2012*). It is currently known that VEGFR can be expressed by a variety of cells (*Johnson & Wilgus, 2013; Friedrich et al., 2015*). Our findings indicate that serum VEGF levels of Coca-Cola treated and Pepsi-Cola treated groups were higher than that of CG; the highest increase was in PEP-2 mice from day 15 onwards. On day 25, EGF level of PEP-1 was significantly increased.

Administration of Coca-Cola or Pepsi-Cola could reduce foetus numbers and increase the proportion of female foetuses. This may be associated with changes in the uterine micro- environment after prolonged consumption of Coco-Cola or Pepsi-Cola. Our results need to be confirmed in other animals and humans.

Conclusion

Oral ingestion of Coca-Cola or Pepsi-Cola over a prolonged period reduced ovarian weights, inhibited ovarian cortex thickness, and affected the development of follicles and oocytes. Coca-Cola and Pepsi-Cola reduced serum levels of caspase-3, and increased EGF and VEGFR. They clearly affected reproduction of female mice, promoted growth of offspring, and reduced pregnancy rate and affected foetus numbers. Our study has laid a solid foundation and provided the experimental data for further investigating the effects and mechanism of Coca-Cola and Pepsi-Cola on development and reproduction in humans.

Competing Interest

None of the authors has any potential financial conflict of interest related to this manuscript.

Authors Contribution

Professor Wei Suocheng was responsible for the experimental designs and writing the manuscript. Professor Gong Zhuandi raised the experimental animals and took the samples. Dr Lu Huining detected the receptor gene expressions. Miss Liang Haoqin observed the histology structures and examined the ovarian parameters. Miss Lai Luju did the statistical analyses. All authors interpreted the measures serum indices and data, critically revised the manuscript and approved the final version.

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