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Effects of coenzyme q10 supplementation on metabolic and reproductive outcomes in obese rats

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Abstract

Obesity, a global epidemic, is linked to adverse reproductive outcomes, including infertility and ovulation dysfunction. The cafeteria diet (CAF) serves as an animal model mirroring Western diet habit. Coenzyme Q10 (CoQ10), known for enhancing reproductive outcomes in various pathologies, is not fully understood for its effects on obesity treatment. Here, obesity was modeled using CAF-fed rats to assess CoQ10's impact on metabolic and ovarian disruptions caused by obesity. Wistar rats were divided into control (standard diet) and obese (CAF diet) groups. After 75 days, half of each group received oral CoQ10 (5 mg/kg) for 13 days, while the rest received a vehicle. Animals were euthanized during the estrus phase, and blood and ovaries were collected for analysis. CAF caused increased body weight gain ($p < 0.01$) associated with hyperglycemia, hypertriglyceridemia, and hypercholesterolemia ($p < 0.05$). Moreover, it caused a reduction in the number of AMH + follicles ($p < 0.001$), increasing follicular atresia ($p < 0.05$) and serum estradiol levels ($p < 0.05$). Obesity also altered the estrous cycle and reduced the ovulation rate ($p < 0.05$). CoQ10 administration showed beneficial effects on all ovarian disruptions but had no effect on the metabolic alterations induced by obesity. In summary, CoQ10 could be an additional treatment for obesity-related infertility in patients with normal metabolic profiles. While CoQ10 does not affect metabolic parameters influenced by obesity, crucial for reproductive issues and offspring health, it is recommended as part of a treatment plan that includes a balanced diet and increased physical activity for obese individuals with metabolic alterations seeking pregnancy.

Keywords Obesity, Coenzyme Q10, Metabolism, Ovulation

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Introduction

The World Health Organization (WHO) defines obesity as a condition of abnormal or excessive fat accumulation in adipose tissue such that health may be impaired. The prevalence of obesity is dramatically increasing, affecting more than 650 million adults worldwide [1]. The incidence of obesity worsened during the COVID-19 pandemic, with 48% of surveyed US adults gaining body weight in the first year of the pandemic [2], though it cannot be determined at this point if this weight gain resulted in obesity. Obesity has contributed to an increased prevalence of related metabolic dysfunction, cardiovascular diseases, diabetes, musculoskeletal disorders, and cancers [3]. Furthermore, obesity exerts a negative impact on female fertility and is associated with numerous adverse prenatal maternal and fetal effects [4].

The ovary is the female gonad whose main functions include the production, maturation, and release of oocytes, as well as the synthesis of sex steroid and peptide hormones that regulate reproductive functions [5]. Environmental factors can exhaust the oocyte pool, induce the depletion of follicular cells, premature ovarian failure, early menopause, and infertility. Obese women are more likely to suffer ovulatory dysfunction than women of a normal weight, due to dysregulation of the hypothalamic-hypophysis-ovarian axis [6, 7]. Despite the vast evidence describing the negative effects of obesity on ovarian physiology, the mechanisms underlying them remain unknown.

Although it is a multifactorial disease, the high consumption of Western diet is associated with this pandemic [8]. Westernized diet is characterized by a reduced consumption of fruits and vegetables that are replaced by a high content of saturated fats, sugar, alcohol, salt, proteins, refined grains, and corn-derived fructose syrup [9]. Different animal models have been used for the study of obesity, such as high-fat, high-sugar, and cafeteria diet CAF [10, 11]. CAF diet-induced obesity animal model has been shown to be able to induce obesity and reduce the reproductive capability in adult female rats when administered from weaning age, showing that this model can aid in gaining a deeper understanding of the mechanisms that contribute to reproductive dysfunction in individuals who are obese [12]. t has been shown that the duration of energy-dense diet consumption has differential regulatory mechanism in altering the ovarian steroid production [13]. We previously showed that CAF induces obesity and alters the glucose metabolism, without altering serum triglycerides or cholesterol concentrations in rats. The ovarian function was also altered, obese rats showed reduced ovarian reserve, the presence of follicular cysts and disturbance of the ovulatory process [14]. Ovaries from obese rats showed decreased glucose uptake and became insulin resistant, showing increased follicular nitric oxide synthase expression that may be responsible for the ovulatory disruptions and for inflammation, a common feature in obesity. Obese rats resulted less fertile than controls and their pups were macrosomic [15].

Obesity is related to an increase in inflammation which is associated with an increase in oxidative stress. Recently, antioxidants have been used extensively to overcome the effects of excess reactive oxygen species in several pathologies [16]. Among the antioxidants used, vitamins E and coenzyme Q (CoQ10) are included. Increased CoQ10 levels were described in several tissues, including the ovaries after CoQ10 supplementation [17]. CoQ10 administration prevents the development of insulin resistance that occurs due to suboptimal intrauterine nutrition [18]. At a reproductive level, there are multiple positive effects described due to the administration of CoQ10. For example, it improves the response in patients with low ovarian reserve [19, 20] and is associated with an increase in pregnancy rates in women undergoing in vitro fertilization procedures [21]. Elevated levels of CoQ10 in follicular fluid are associated with higher pregnancy rates [22, 23]. In addition, it increases ovulation rates in patients with polycystic ovary syndrome [24]. Despite the promising effects of CoQ10 on reproductive outcomes, more studies are needed to assess its clinical potential [25]. There is little literature where CoQ10 is administered as a therapeutic for obesity and even less where its effects on reproductive parameters are evaluated [26–28].

CoQ10 appears, depending on its redox state, in either oxidized form (ubiquinone) or reduced form (ubiquinol). The reduced form, rich in energy, exhibits better cellular penetration capacity, particularly across the mitochondrial membrane [29]. Supplementing CoQ10 with antioxidants like vitamin E can decrease its oxidation, thereby enhancing its bioavailability and function [30]. Therefore, the aim of this study was to evaluate the effects of exogenously administered CoQ10, supplemented with vitamin E, on the metabolic and ovarian status altered by obesity.

Materials and methods

Animals and experimental design

Twenty-two days old female Wistar rats (*Rattus norvergicus*) were, first, included in a diet-protocol, and then, in a treatment-protocol. Diet-protocol: twenty the animals were fed with standard rodent chow diet (Control group) and 20 were also offered with CAF, that consists in a varying menu of highly palatable human foods comprising sausages, cheese, snacks, peanuts, biscuits, and chocolate biscuits (Obese group). The nutritional composition of each food is described in Supplementary Fig. 1.

This animal model of obesity was adapted from previous studies [31–34] and has been previously implemented by us successfully [14, 15, 35, 36]. The diet-protocol was continued until each animal in that group showed significantly higher body weight than the average of the control group (here, on day 75th of the diet protocol). Afterwards, treatment-protocol was started. During this period rats continued receiving the same diet-protocol. For that purpose, 10 rats of each group were randomly selected and then daily administered, orally, with 5 mg/kg CoQ10 for 13 days [37], while the others received vehicle. Commercial tablets containing 30 mg of CoQ10, as ubiquinol, and 10 mg of Vitamin E (Nutrifarma, Buenos Aires, Argentina) were used. Each tablet was dissolved in 3 ml of 1% Tween 80 then suspended in water to reach the necessary concentration [38, 39]. The treatment duration aligned with previous literature [39], encompassing approximately 3 estrous cycles, meeting the minimum recommended duration for clinical practice. At the conclusion of this 88-day experimental design, four groups were established ($n=10$/group): Control, CoQ10, CAF and CAF + CoQ10.

All rats had ad libitum access to all diet components as well as to water and were kept on a 12:12 h light–dark cycle at 22 °C. Weight gain was monitored twice a week.

Estrous cycle staging

During the treatment-protocol, vaginal smears were collected daily by lavage with 0.9% saline solution between 09.00 and 11.00 AM from each animal. The fluid was spotted thinly on a microscope slide, and the dried slides were stained with 0.1% trypan blue in deionized water. The estrous cycle stage was determined by microscopic examination, as described by Westwood [40].

Glucose tolerance test

The glucose tolerance test (GTT) was conducted on day 88 of the experimental protocol. For that purpose, 6 h fasted control and obese rats were intraperitoneally injected with a bolus of glucose (2 g/kg) and blood glucose levels were determined at 0, 15, 30, 60, and 120 min after glucose challenge. Glycemia was measured in tail blood using glucose strips on an Accu-Chek Performa II instrument (Roche, Buenos Aires, Argentina). Data was collected for each individual animal and expressed as mean blood glucose concentration over time. The area under the curve (AUC) for glucose was calculated to evaluate glucose tolerance in control and obese animals [15].

Insulin tolerance test

The insulin tolerance test (ITT) was conducted on day 89 of the experimental protocol in rats fasted for 2 h, following the diet and treatment protocols. For that, a single intraperitoneal insulin injection (0.5 U/kg diluted in PBS) was administered, and blood glucose was sampled at times 0, 15, 30, 45, 60, 90, 120 and 150 min after insulin injection. Glycemia was measured in tail blood using glucose strips on an Accu-Chek Performa II instrument (Roche, Buenos Aires, Argentina) [15, 41].

Anesthesia and tissue collection

Euthanasia was performed by cardiac exsanguination in rats during the first estrous phase after treatment-protocol. For that purpose, animals were previously subjected to anesthesia with 50 mg/kg solution of ketamine (Brouwer, Argentina) associated with 10 mg/kg xylazine (Alfasan, Holland) that was injected intramuscularly into the inner side of one of the hind legs. Blood and ovaries were collected. Ovaries were weighed and fixed in 4% (w/v) formaldehyde for 24 h. Afterwards, they were dehydrated, embedded in paraffin, cut into seven-micron sections, and mounted on gelatin-coated glass slides for histology and immunohistochemical studies. Blood was immediately used for evaluating glucose levels and then, centrifuged at 2000 g to obtain serum that were frozen at −70 °C and thawed on the day of the metabolic/hormonal characterization.

Metabolic characterization

These determinations were performed according to the manufacturer's instructions. Briefly, fasting blood glucose was determined by using the glucose oxidase–peroxidase enzymatic colorimetric assay (GOD/PAP; GT Laboratories, Rosario, Argentina). Serum triglycerides were determined by using the glycerol-3-phosphate oxidase enzymatic colorimetric assay (GPO-PAP; GT Laboratories); and total and HDL cholesterol were measured, in sera, by using Colestat Kit (GT Laboratories). Animals were fasted for 8 h prior to sample collection.

Hormonal characterization

Serum estradiol, estrone and progesterone (P4) concentrations were determined. For estradiol (E2), an enzyme immunoassay was used (EIA 2693, DRG Instruments GMBH, Germany). The lower limits of detection for E2 and estrone were 9.714 pg/ml and 10.00 pg/mL, respectively. Cross-reactivity of the E2 antibodies is 0.2% for estrone and 0.05% for estriol.

Estrone and P4 concentrations were determined by radioinmunoassays (RIA) using a gamma counter (Multi Crystal LB 2111, Berthold Technologies, GmbH & Co., Bad Wildbad Germany). For estrone a coated-tubes commercial RIA kit was used (DSL 8700, IMMUNOTECH s.r.o., Praga, Czech Republic). All measurements

were completed in a single assay. The detection limit was 18.45 pg/ml. Mean intra-assay CV was 4.27%.

Progesterone concentrations were determined using a coated-tubes commercial RIA kit (RIA progesterone, Ref IM1188, Immunotech s.r.o. Hostivar, Czech Republic). All measurements were also completed in a single assay. The detection limit was 0.059 ng/ml. Mean intra-assay CV was 1.85%.

Ovarian histology and follicle counting

Tissue sections were stained with Masson trichrome stain, which was used to show the smooth muscle according to standard protocols, and then analyzed using an Olympus light microscope. Seven-micrometer step sections were mounted at 50-mm intervals onto microscope slides to prevent counting the same structure twice, according to the method described by Woodruff et al. [42]. To prevent multiple counts of the same follicle, only follicles with a visible oocyte nucleus were included [43]. Follicles were classified as either primary, secondary, antral or preovulatory. In addition, the number of corpora lutea (CL) was counted in each section analyzed. The abundance of each type of follicle or corpora lutea was normalized by the total ovarian area in the section, as reported previously [44]. The ovary area was measured with Image J (version 1.42q) and expressed per 10 mm^2.

Immunohistochemistry

The expression of anti-Müllerian hormone (AMH) was detected by immunohistochemistry as previously [15]. Briefly, ovarian sections were subjected to an antigen retrieval technique by heat. Tissue slides were placed in a solution containing 0.01 M citrate buffer, pH 6.2 for 5 min in a microwave oven at 100 °C at 600 W. Endogenous peroxidase activity was blocked by incubation in 3% (v/v) hydrogen peroxide at room temperature for 15 min. Background blocking was achieved by incubating with 5% (w/v) non-fat milk at room temperature for 30 min. The tissue sections were then incubated at 4 °C overnight with 1:50 mouse monoclonal anti-AMH (AbD Serotec, Oxford, UK) as primary antibody. The sections were incubated with 1:500 biotinylated goat anti-mouse antibody (Dako Cytomation, Carpinteria, CA, USA) at room temperature for 40 min. Then, 40-min incubation with 1:400 streptavidin–biotin horseradish peroxidase complex reagent (Dako) was done. Color development was performed with a solution containing 3,3′-diaminobenzidine (Dako), and then the sections were counterstained with hematoxylin. Finally, the sections were dehydrated, mounted with Entellan New (Merck, Darmstadt, Germany) and observed with an FV-300 Olympus light microscope. Control sections were performed by omitting the primary antibody.

Follicles were, first, classified according to the stage of development and then according to immunoreactivity for AMH (as positive or negative). The abundance of immunoreactive follicles was normalized by the total number of the same structure detected in the ovarian section.

Ovarian RNA extraction and retrotranscription

Total ovarian RNA was extracted from the ovary using Trizol reagent (Invitrogen, CA, USA) according to the manufacturer's protocol. cDNA was synthesized by incubating 2 µg of extracted RNA in a buffer containing 3U AMV Reverse transcriptase (Promega, Madison, WI, USA), 1uM oligo d(T)15 Primer (Dongsheng Biotech, Guangdong, China) and 1 mM Mix dNTPS (Dongsheng Biotech). The reaction mixture was incubated for 60 min at 42 °C followed by 15 min at 70 °C.

Polymerase chain reaction

The obtained cDNA (2 µl, selected to work within the linear range) was amplified by Polymerase Chain Reaction (PCR) in a buffer containing: 0.5 U Taq-DNA polymerase (Invitrogen), 0.2 mM of each primer (Invitrogen), 0.2 mM of each dNTP, 1.5 mM MgCl2 and each specific primer.

For amplification of Steroidogenic acute regulatory protein (StAR) cDNA, the primers were: sense 5′-GCC TGAGCAAAGCGGTGTC-3′, antisense 5′-CTGGCG AACTCTATCTGGGTCTGT-3′; for aromatase were: sense 5′-GAACGGTCCGCCCTTTCT-3′, antisense 5′-TGGATTCCACACAGACTTCT-3′, and for hydroxysteroid (17-beta) dehydrogenase 1 (Hsd17b1) were: sense 5′-TTCTGCAAGGCTTTACCAGG-3′, antisense 5′-ACAAACTCATCGGCGGTCTT-3′. The specific annealing temperature and the number of cycles used were primer dependent. The optimum cycle number was determined for each primer pair, so that signals were always in the exponential portion of the amplification curve. Each cycle consisted of denaturation at 95 °C for 15 s, primer annealing at the specific temperature for 30 s and extension at 72 °C for 15 s. Negative controls were performed without reverse transcriptase or RNA. PCR products were electrophoresed on 2% agarose (Biodynamics, Buenos Aires, Argentina) gels. Gel images were taken with the ImageQuant RT ECL (General Electric) software and quantified using Image J software (version 1.42q, National Institute of Health, USA). Density of the bands of interest was normalized to that of GADPH in each sample.

Statistical analysis

Statistical analyzes were performed with the GraphPad Prism 5.0 program (GraphPAD software, San Diego, CA, USA) considering a value of $p < 0.05$ to be significant. Homogeneity of variances was verified with the Levenne

test and normal distribution of the data was evaluated using the Shapiro-Willks test using the R-studio program (version 3.5.1). Results were expressed as the mean ± SD except where otherwise specified.

Body weights, glucose tolerance and insulin resistance curves were analyzed using repeated measures analysis of variance (ANOVA) followed by the Bonferroni test. Metabolic parameters and follicular counting were analyzed using a two-way ANOVA (Diets and Treatment) followed by the Bonferroni test.

The evaluation of the estrous cycle was carried out by comparing the percentage of days spent in each stage with respect to the total time evaluated by Chi^2 analysis.

Results

Cafeteria diet induces obesity in rats and CoQ10 treatment has no effect on body weight.

After 60 days of diet-protocol, rats fed with CAF diet showed a significant increase in body weight compared to controls. Despite this, diet-protocol was continued until each animal in that group showed significantly higher body weight than the average of the control group (day 75; $p < 0.05$; Fig. 1). Afterwards, during the 13 days of treatment protocol, CAF diet-fed rats continued showing higher body weights than controls despite whether or not they were treated with CoQ10 ($p < 0.05$; Fig. 1).

Metabolic profile of cafeteria diet-induced obese rats after CoQ10 treatment

Obese rats showed no alteration in HDL cholesterol levels. However, they showed higher levels of total cholesterol due to an increase in LDL + VLDL cholesterol when compared to controls ($p < 0.05$; Table 1). Moreover, higher levels of triglycerides and fasting blood glucose levels were detected in obese rats respect to controls ($p < 0.05$). Obese animals treated with CoQ10 showed the same metabolic alterations than those that were not treated, i.e., they showed higher total cholesterol, LDL + VLDL cholesterol, triglycerides, and fasting blood glucose levels than control rats ($p < 0.05$), without alterations in HDL cholesterol levels.

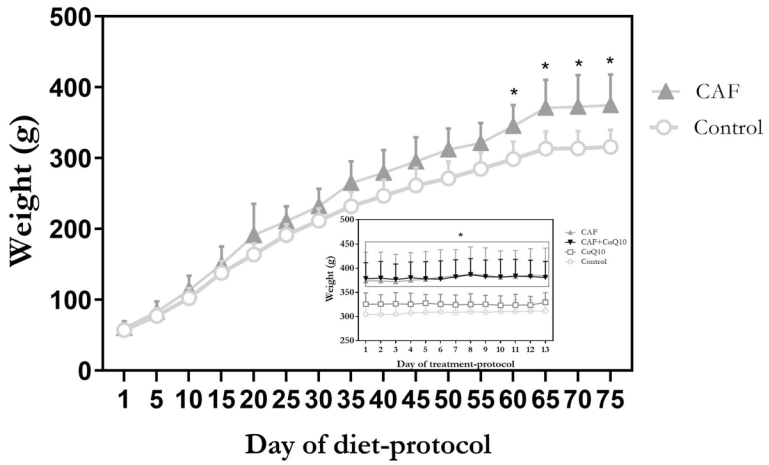

Fig. 1 Body weight gain of rats fed with standard chow (Control, open circles) and CAF diet (Obese, grey triangles) during diet-protocol. After that, half the animals of each group were administered orally with CoQ10 while the other half received vehicle, resulting in 4 groups: Control, ControlQ10, Obese and ObeseQ10 (*insert*). Each time point represents the mean ± S.D. ($n = 10$). Two-way ANOVA (repeated measurements) followed by Bonferroni comparisons test: * $p < 0.05$ between groups

Table 1 Metabolic profile of control and obese rats that were treated, or not, with Coenzyme Q10. Data is expressed as mean ± SEM ($n = 10$). Two-way ANOVA followed by Bonferroni comparisons test: different letters indicate significant differences ($p < 0.05$) among groups

	Control	CoQ10	CAF	CAF + CoQ10
Total Cholesterol (mg/dL)	51.40 ± 4.53, **a**	47.39 ± 2.89, **ab**	68.15 ± 2.05, **b**	72.72 ± 3.02, **ab**
HDL Cholesterol (mg/dL)	4.32 ± 0,44	4.03 ± 0.36	3.67 ± 0.47	4.39 ± 0.33
LDL + VLDL Cholesterol (mg/dL)	47.08 ± 4.60, **a**	43.66 ± 2.62, **a**	64.47 ± 1.74, **b**	68.16 ± 2.79, **b**
Triglycerides (mg/dL)	59.91 ± 10.97, **ab**	42.03 ± 9.83, **b**	92.15 ± 30.57, **ac**	121.77 ± 25.86, **c**
Glucose (mg/dL)	111 ± 5.55, **a**	112 ± 5.36, **a**	128.60 ± 3.64, **b**	135 ± 3.47, **b**

GTT (Fig. 2) showed that, after glucose injection, all groups showed the expected rapid increase in the glycemia (15 min, $p < 0.001$) followed by clearance of glucose. Thirty and sixty minutes after glucose challenge all groups showed higher glycemia than fasting levels ($p < 0.001$ and $P < 0.01$, respectively), reaching basal concentrations 2 h after glucose challenge in all groups.

In Fig. 3 the ITT curves show that all groups exhibit the expected decrease of glycemia 15, 30 and 60 min after insulin challenge ($p < 0.05$). Glycemia reached basal concentrations within 2 h of insulin challenge in all groups. Despite obese rats showing higher fasting blood glucose levels than controls, whether or not they received CoQ10 treatment, all rats showed similar blood

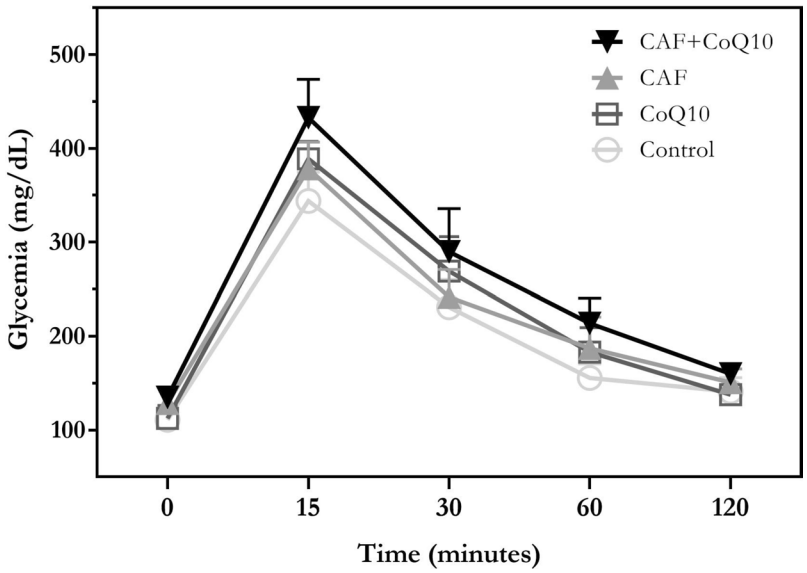

Fig. 2 Blood glucose levels during the glucose tolerance test in control and obese rats that were treated (controlQ10: open squares, and obeseQ10: filled triangles), or not (control: open circles, obese: grey triangles), with Coenzyme Q10. Each time point represents the mean ± S.D. ($n = 5$). Two-way ANOVA (repeated measurements) followed by Bonferroni comparisons test. No significant differences between groups were detected

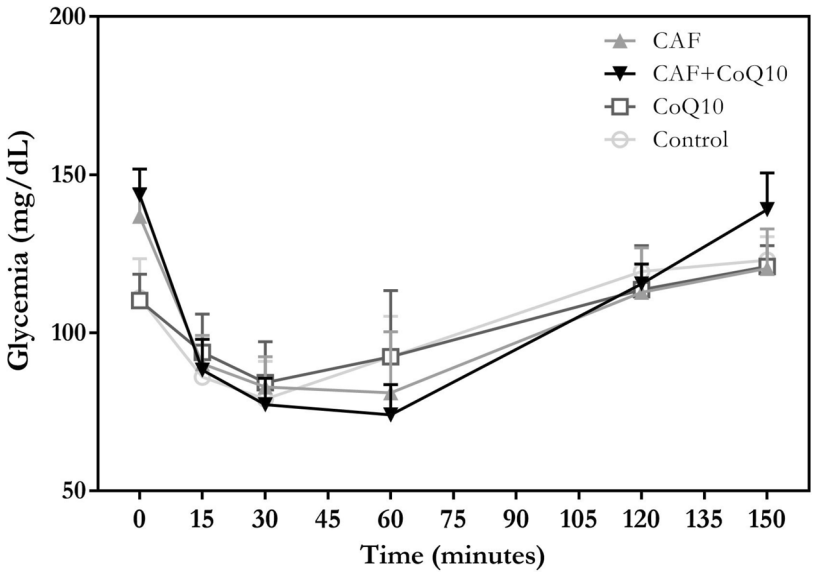

Fig. 3 Blood glucose levels during the insulin tolerance test in control and obese rats that were treated (controlQ10: open squares, and obeseQ10: filled triangles), or not (control: open circles, obese: grey triangles), with Coenzyme Q10. Each time point represents the mean ± S.D. ($n = 5$). Two-way ANOVA (repeated measurements) followed by Bonferroni comparisons test. No significant differences between groups were detected

glucose levels after 15, 30, 60, 120 and 150 min of insulin administration.

Cafeteria diet-induced obesity alters the estrous cycle and estradiol serum levels and CoQ10 normalizes them

As a prelude to analyze the effects of CoQ10 treatment on obese rat ovarian function, the duration of the estrous cycle was studied. Daily examination of vaginal cytology revealed that the duration of proestrus and metestrus phases were not modified by obesity (Fig. 4A and 4B). However, it altered the estrous cycle since obese animals had fewer days in the estrous phase than controls (Fig. 4C), remaining predominately in persistent diestrus phase (Fig. 4D). The duration of estrous and diestrus phases was restored by CoQ10 in obese rats, i.e., CoQ10-treated rats had no alteration in the duration of the phases respect to control.

As obesity and CoQ10 treatment affected the estrous cycle, we further analyzed the ovarian steroidogenesis. No alteration on serum progesterone nor estrone levels were detected among all groups (Fig. 5A and B). However, increased serum estradiol levels were observed in obese rats compared to controls ($p < 0.05$), and this effect was reverted by CoQ10 treatment in obese animals (Fig. 5C).

Ovulation rate is decreased, and follicular atresia is increased by obesity, and all are normalized by CoQ10

Estradiol is produced by granulose cells in the developing follicles. The number of granulosa cells increases during follicular development. So, one possible reason for the increased estradiol levels could be an alteration in the follicular dynamic in obese rats. Then, we analyzed the number of follicles in each stage of development. The number of viable primordial, primary, secondary, antral and preovulatory follicles were not altered by obesity (Fig. 6A-E); however, the number of corpora lutea was lower in obese rats compared to controls ($p < 0.05$, Fig. 6F). When obese rats were treated with CoQ10, the number of viable secondary and antral follicles was decreased compared to control rats ($p < 0.05$, Fig. C and

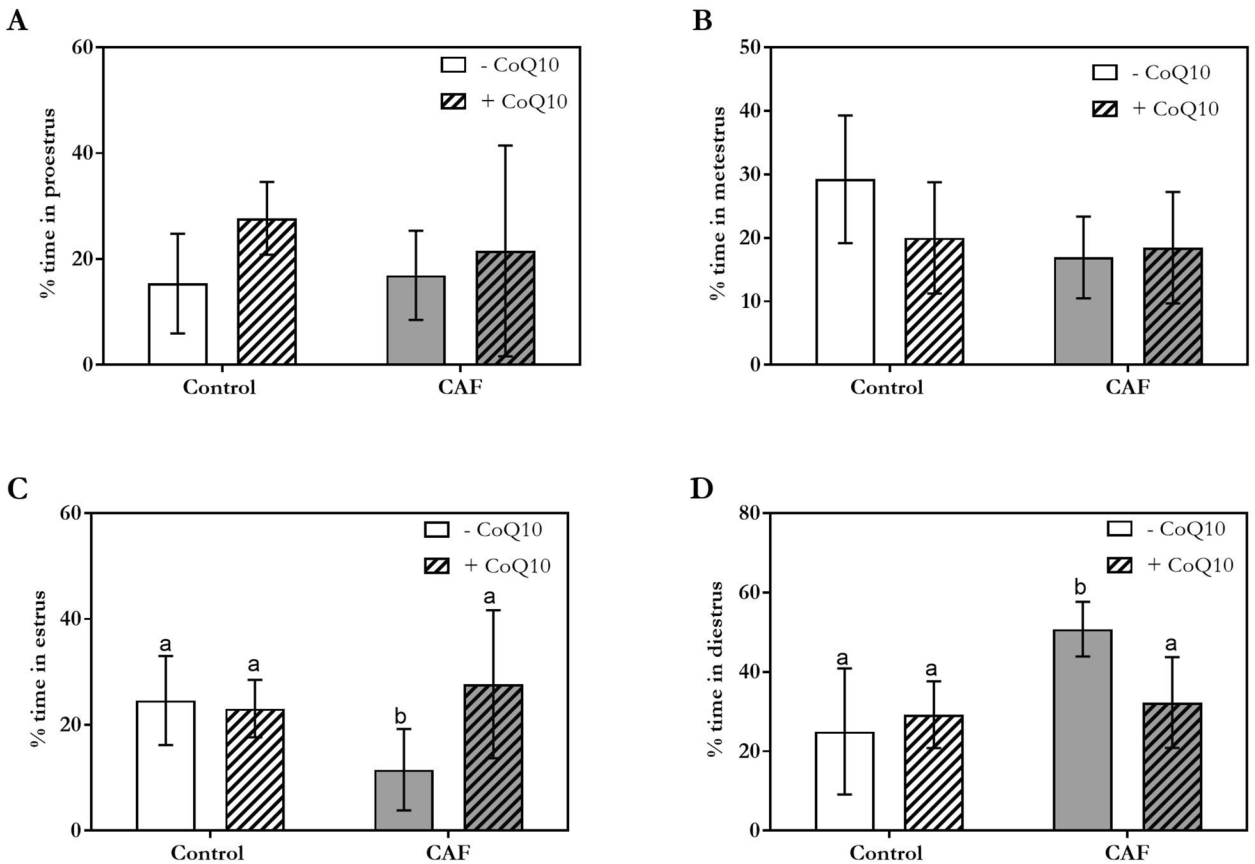

Fig. 4 Estrous cycle evaluation in control and obese rats that were treated, or not, with Coenzyme Q10. Percentage of time spent in each stage (**A**: proestrus, **B**: metestrus, **C**: estrus and **D**: diestrus). Data is expressed as mean ± S.D. ($n = 10$). Two-way ANOVA followed by Bonferroni comparisons test. Groups sharing the same letter (a, b, c) are not significantly different from each other. a vs. b $p < 0.05$ in C and $p < 0.005$ in D

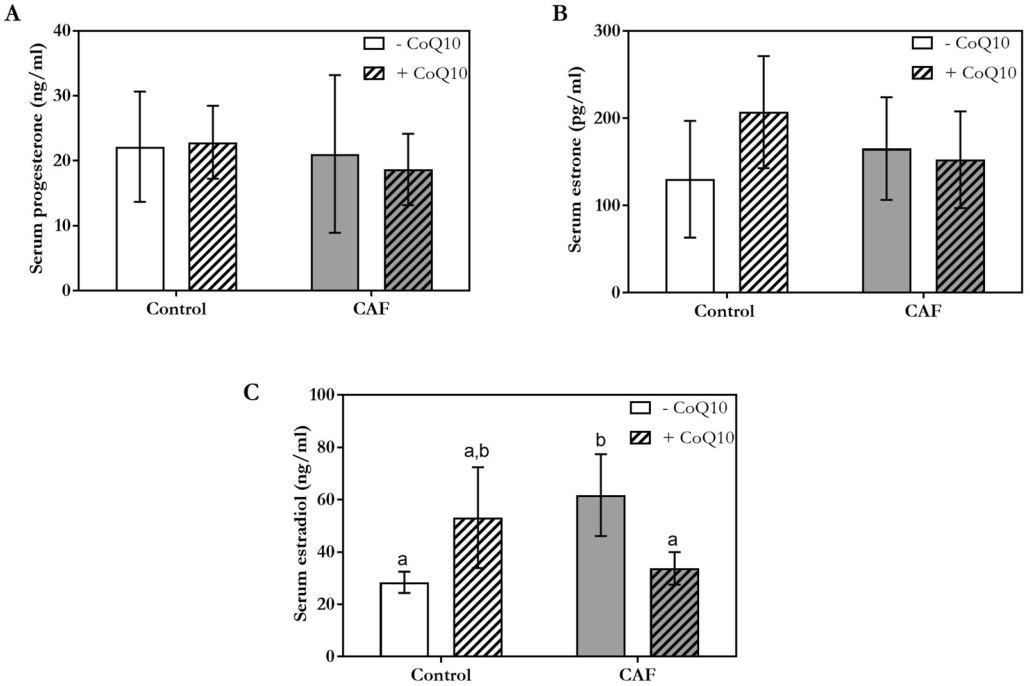

Fig. 5 Serum progesterone (**A**), estrone (**B**) and estradiol (**C**) levels in control and obese rats that were treated, or not, with Coenzyme Q10. Data is expressed as mean ± S.D. ($n = 5$). Two-way ANOVA followed by Bonferroni comparisons test. Groups sharing the same letter (a, b, c) are not significantly different from each other. a vs. b $p < 0.05$

D) and the number of corpora lutea was normalized to control levels (Fig. 6F).

A higher number of atretic antral follicles was detected in obese rats ($p < 0.05$) that was partially restored after CoQ10 administration (Fig. 7A). No alteration in the number of atretic preovulatory follicles were detected in any group (Fig. 7B).

The ovarian mRNA levels of StAR, Aromatase and Hsd17b1 are not altered neither by obesity nor CoQ10

To investigate potential reasons for the increased estradiol levels observed in obese rats, we analyzed the ovarian mRNA expression of the main steroidogenic enzymes involved in estradiol synthesis: StAR, Aromatase and Hsd17b1.

No alterations in the ovarian mRNA expression of StAR (Fig. 8A), Aromatase (Fig. 8B) and Hsd17b1 (Fig. 8C) were detected in any group, suggesting that the alteration in the transcription of these steroidogenic enzymes is not responsible either for the increase in serum estradiol levels produced by obesity or its normalization after CoQ10 treatment. Additionally, the protein expression of aromatase were assessed by immunohistochemistry in ovarian sections from the CTRL and CAF groups (Supplementary Fig. 1). No differences were found in the levels or localization of ovarian aromatase between the two groups.

The ovarian AMH expression is altered by obesity and CoQ10 normalizes it

The ovarian reserve is constituted by a resting pool of primordial follicles. AMH is synthesized by granulosa cells of growing follicles (from primary up to small antral stage) into circulation. Since there is no serum marker that directly can measure the number of primordial follicles, currently the best proxy to estimate the ovarian reserve is AMH that reflects the number of growing follicles.

To ask whether obesity and/or CoQ10 modify the ovarian reserve, the ovarian AMH expression was analyzed. Positive immunostaining for AMH (AMH+) was detected in granulosa cells of primary, secondary, and antral follicles in ovaries from all groups. No immunoreactivity was detected in pre-ovulatory follicles nor corpora lutea (Fig. 9, left panel).

When the relative number of AMH+follicles in each developmental stage was compared among groups (Fig. 9, right panel), it was found that obese rats have a reduction in the proportion of antral AMH+follicles when compared to controls ($p < 0.001$, Fig. 9C, right panel). No alteration in the proportion of AMH+primary nor secondary follicles was observed in those animals (Fig. 9A and B). Besides, the alteration in the proportion of antral AMH+follicles detected in obese rats was normalized after CoQ10 treatment.

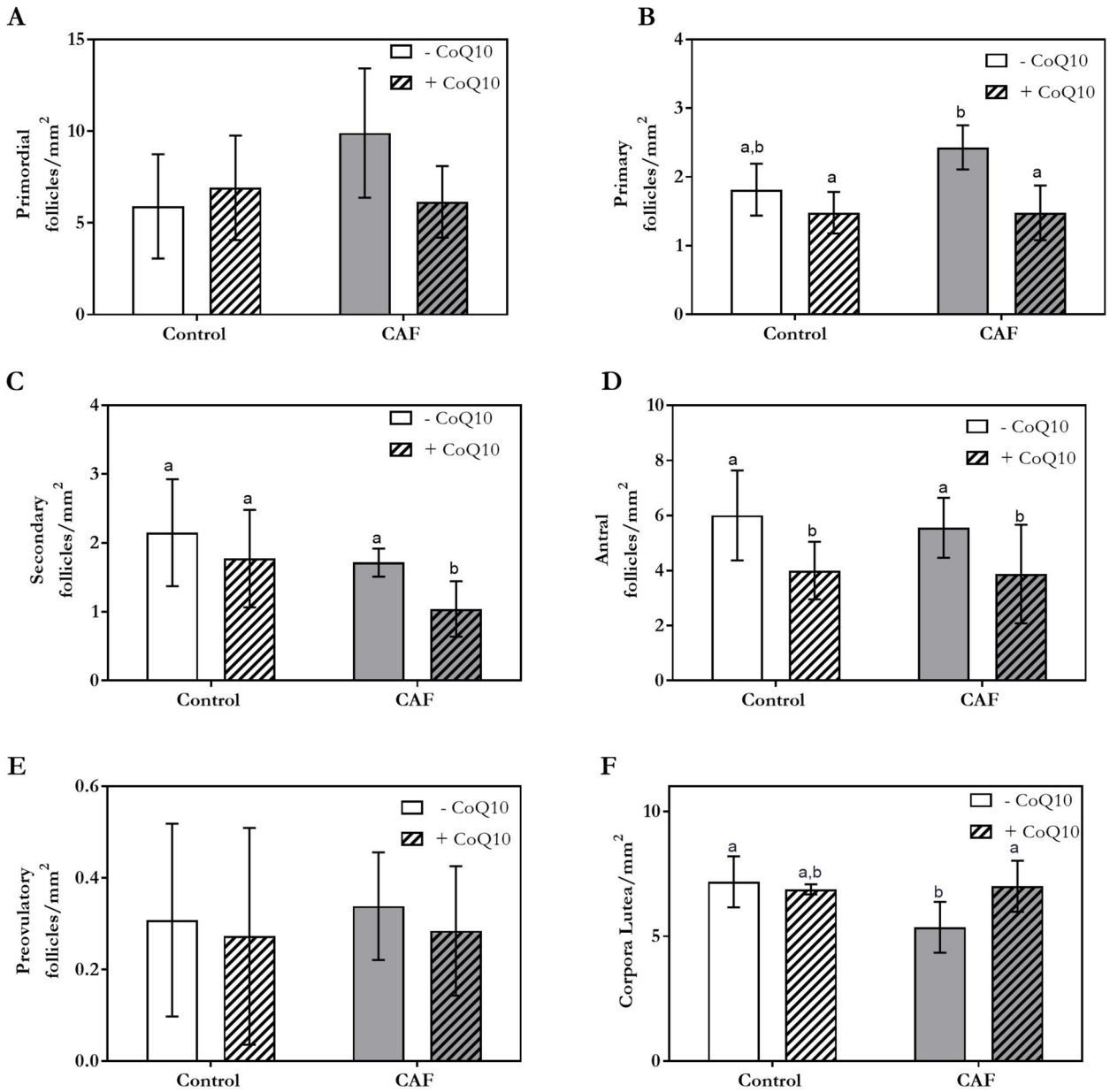

Fig. 6 Quantification of viable follicles in control and obese rats that were treated, or not, with Coenzyme Q10: primordial (**A**), primary (**B**), secondary (**C**), antral (**D**), preovulatory (**E**) follicles and corpora lutea (**F**). Data is expressed as mean ± S.D. ($n = 5$). Two-way ANOVA followed by Bonferroni comparisons test. Groups sharing the same letter (a, b, c) are not significantly different from each other. a vs. b $p < 0.01$ in B and $p < 0.05$ in C, D and F

Discussion

In recent decades, global obesity rates have surged, largely linked to the widespread adoption of the Western diet, despite its multifactorial nature. [8]. Among diet-induced obesity models, CAF's high palatability mirrors the effects of the Western diet, leading to hyperphagia and contributing to weight gain and metabolic changes [10]. In the present study, a higher body weight was detected after 75 days of CAF administration compared to controls, confirming that CAF effectively induces obesity in rats. Similar effects were previously described by us and other authors [14, 15, 35, 36, 45, 46]. Using this animal model, we previously showed that CAF induced obesity, worsened the reproductive performance, and decreased the ovarian reserve as well as altered the ovulatory and the ovarian endocrine function [14, 15, 36].

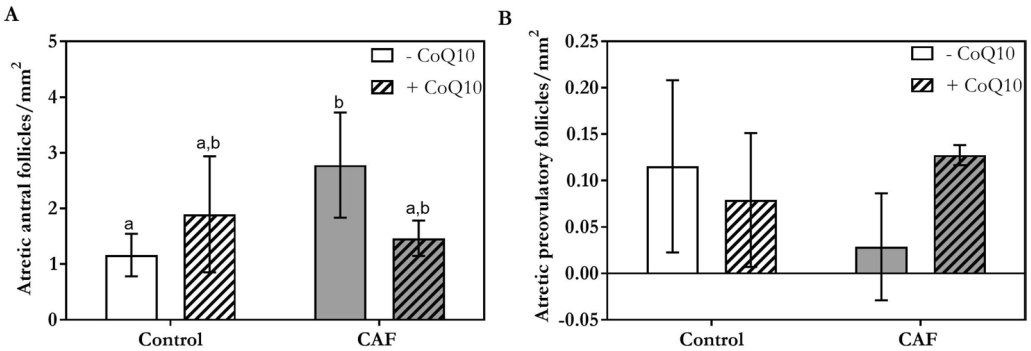

Fig. 7 Quantification of atretic follicles in control and obese rats that were treated, or not, with Coenzyme Q10: antral (**A**) and preovulatory (**B**) follicles. Data is expressed as mean ± S.D. ($n = 5$). Two-way ANOVA followed by Bonferroni comparisons test. Groups sharing the same letter (a, b, c) are not significantly different from each other. a vs. b $p < 0.05$

These dysfunctions are commonly present in pathologies in which CoQ10 is used as therapy. For example, CoQ10 supplementation improves response in patients with low ovarian reserve and has been associated with increased pregnancy rates in women undergoing in vitro fertilization procedures [19, 21]. Furthermore, CoQ10 administration improves ovarian response and ovulation rates in patients with polycystic ovarian syndrome [24].

CoQ10 is the only lipid-soluble antioxidant synthesized by the cell. It has a vital role in the electron transport chain where energy is obtained from dietary intakes. Here, we administered 5 mg/kg CoQ10 daily to rats, a dose equivalent to that recommended in humans (300 mg/day) that has no toxicity [37, 47]. We used tablets containing CoQ10 together with vitamin E since this combination improves CoQ10 absorption and retention by tissues, keeping the enzyme active for longer [48].

Literature about effects of CoQ10 supplementation on body weight have been inconsistent both in murine and human. Some authors showed that oral CoQ10 administration led to significant weight loss in rats [49]; however, no alteration in body weight was described by others [50]. In accordance with the latter, here, CoQ10 treatment did not alter body weight of control nor obese rats.

In our study, the lipid metabolic profile of obese rats showed an increase in serum triglycerides and total cholesterol levels due to an increase in LDL/VLDL cholesterol. CoQ10 administration did not alter those parameters in obese nor in control animals. Vast are the studies showing similar alterations in CAF-induced obese animals [51–53]. However, in our previous study we did not find alteration in serum cholesterol nor triglycerides after 60 days of CAF diet administration [14]. This difference may be due to the duration of the diet protocol; here animals were fed with CAF for 88 days. Those 28 days of difference may explain the differences observed in the lipid metabolism of the animals.

There are controversies regarding the effects of CoQ10 on these metabolites. Xu et al. showed decreased serum cholesterol and triglyceride levels after 12 weeks of CoQ10 treatment in mice [54]. On the other hand, the CoQ10 administration in hyper triglyceridemic patients did not modify triglycerides and total cholesterol levels [55]. Our results support those described by Mabuchi et al. since CoQ10 did not modify serum triglycerides nor cholesterol levels in our animal model.

Regarding the glucidic metabolic profile, we found that obese rats are hyperglycemic; in agreement with many authors working with CAF-induced obesity [15, 33, 45, 56, 57]. However, these authors described that their animals also showed glucose intolerance and insulin resistance. Here we found no alterations in glucose tolerance nor insulin resistance as consequence of obesity, in accordance with those described by Holemans et. al. that showed that CAF administration generates hyperglycemia without any alteration in the GTT and ITT curves [58]. Again, the effect of CoQ10 on the glucidic metabolism is controversial. In a mice model of obesity and type 2 diabetes, a decrease in glucose levels and a better response in the GTT were observed after CoQ10 treatment [54]; while in patients with those pathologies, CoQ10 administration did not modify blood glucose levels compared to those administered with placebo [59]. Our findings coincide with the latter, since hyperglycemia was not changed in animals that received CoQ10.

When the estrous cycles were evaluated, estrus and diestrus phases revealed shortening and prolongation in obese rats than control, respectively, similarly to those described previously by us and others [14, 60]. Moreover, here we show the restoration of the duration of the estrous cycle phases in obese animals after CoQ10 treatment, which has not been previously reported in the literature. The estrous cycle reflects the ovarian function in female mammals. It is characterized by changes in

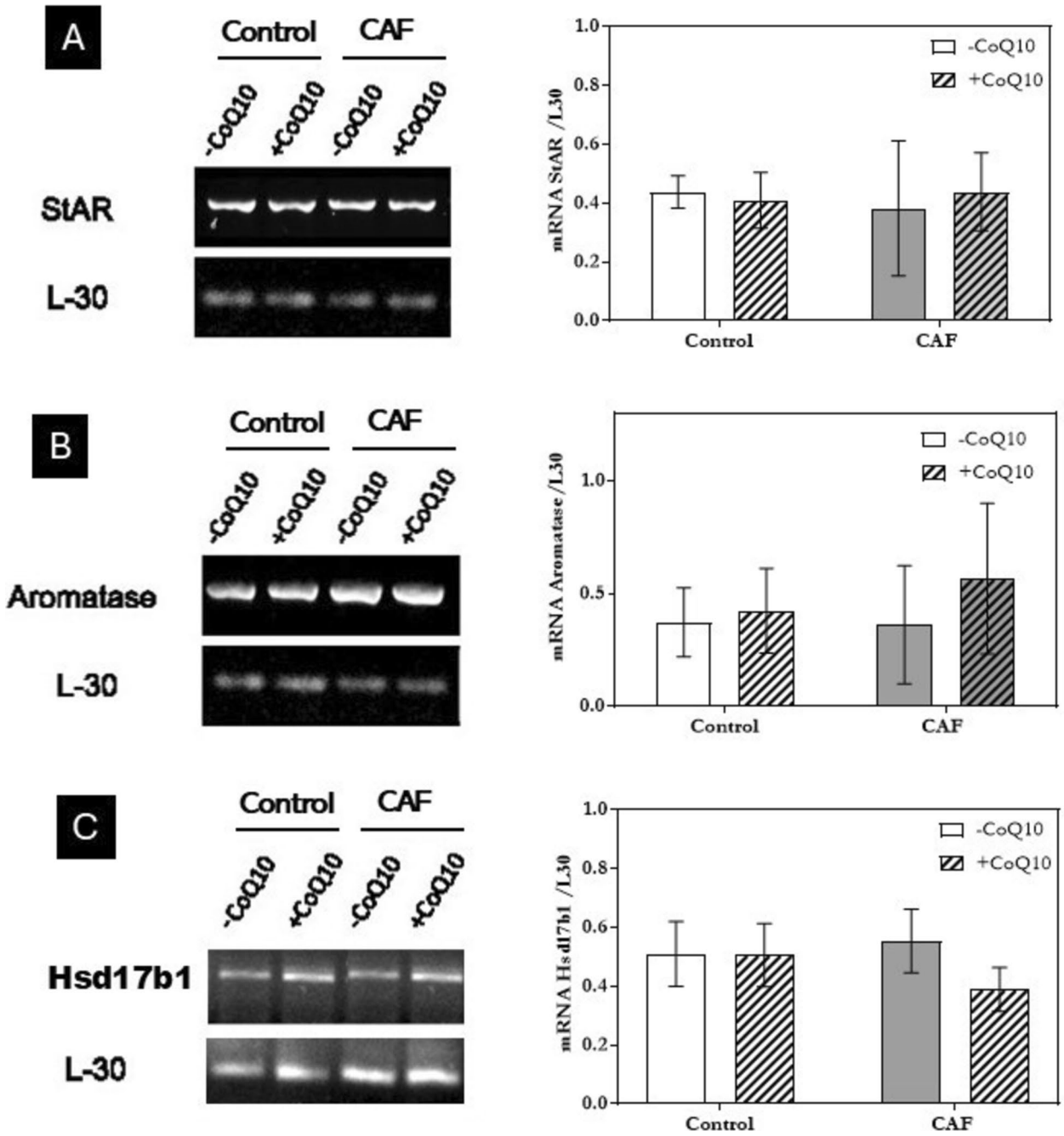

Fig. 8 Ovarian mRNA levels of StAR (**A**), Aromatase (**B**) and Hsd17b1 (**C**) in control and obese rats that were treated, or not, with Coenzyme Q10. Data is expressed as mean ± S.D. ($n = 5$). Two-way ANOVA followed by Bonferroni comparisons test. No significant differences between groups were detected

the levels of gonadotropins and, consequently, of steroid hormones, namely estrogen and progesterone. Our results show no alteration in serum estrone nor progesterone levels in obese, treated or not with CoQ10. However, obese rats showed increased serum estradiol levels, that were normalized after CoQ10 treatment. It was unexpected for us not to find variations in progesterone levels in obese rats, since they show fewer corpora lutea. One possible explanation is that animals were sacrificed during the estrus phase of the cycle, when progesterone levels are the lowest, so the sensitivity of the technique we used was not sufficient to detect any

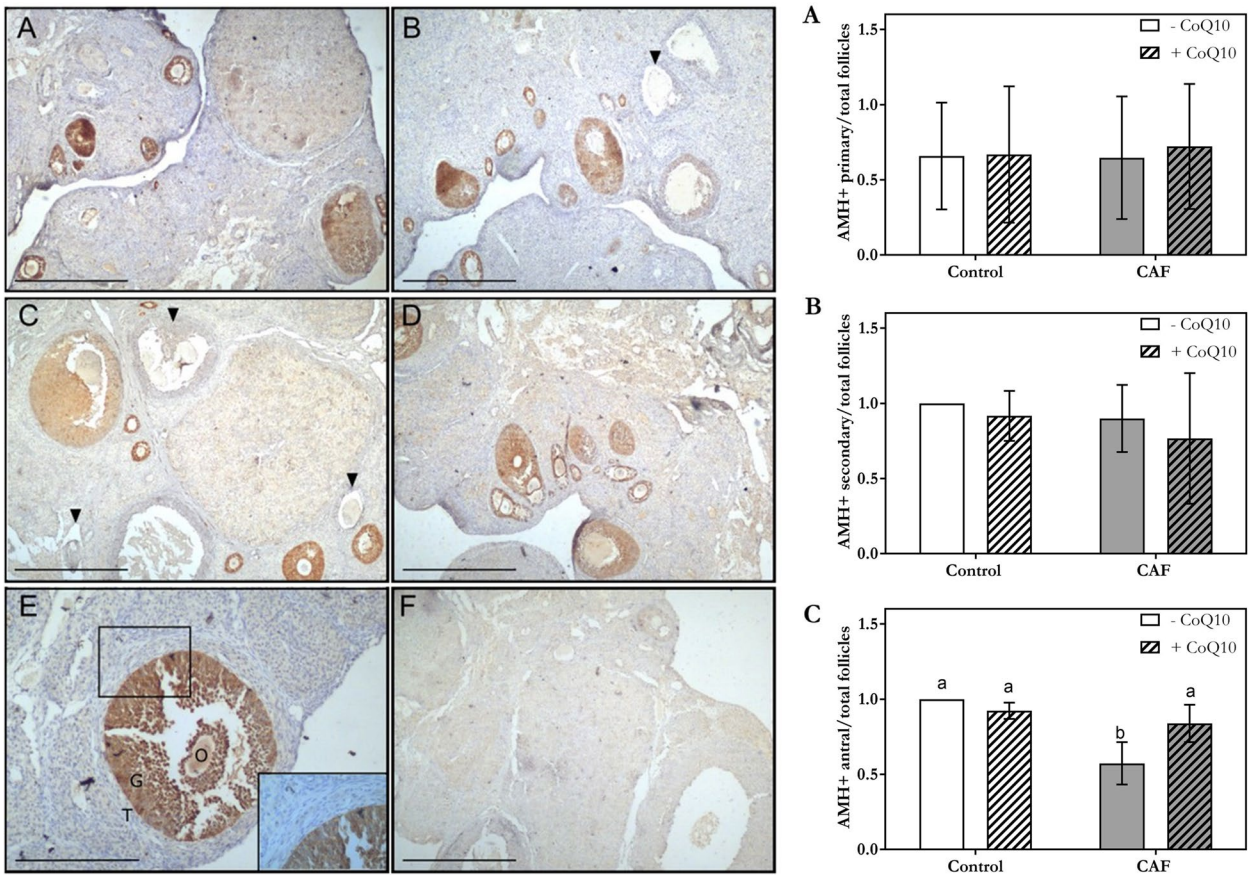

Fig. 9 Anti-Müllerian hormone (AMH) immunohistochemical staining in ovaries. Left panel: Representative sections showing AMH immunoreactivity (AMH+) in granulosa cells of primary, secondary, and antral follicles from Control (**A**), ControlQ10 (**B**), Obese (**C**) and ObeseQ10 (**D**) rats. Arrowheads shows AMH- follicles. (**E**) Antral follicle showing AMH staining in granulosa (G) but not in theca cells (T); O: oocyte. (**F**) Negative control. Scale bars: A-D and F = 500 µm; E = 20 µm. Right panel: Number of AMH+ primary (**A**), secondary (**B**) and antral (**C**) follicles relative to the total number of follicles in each stage. Data is expressed as mean ± S.D. ($n = 5$). Two-way ANOVA followed by Bonferroni comparisons test. Groups sharing the same letter (a, b, c) are not significantly different from each other. a vs. b $p < 0.001$

difference. New experiments are being designed to confirm this.

Regarding the effect of obesity on circulating levels of estradiol, there is much controversy because they vary depending on the sex, the moment of the female cycle in which the blood sample is taken, etc. In addition, although in females most of estradiol is synthesized by the ovary, the adipose tissue can contribute to the production of this hormone, to a greater or lesser extent, depending on the degree of adiposity exhibited. In fact, in our previous study in which we administered CAF for 60 days, obese rats showed decreased serum estradiol levels, were insulin resistant and glucose intolerant [14], unlike those described in the present work. These differences in the effect of CAF show that the body weight gain does not explain per se the changes in serum estradiol levels, but the latter depends on the metabolic profile. What we do show here is that estradiol levels are not increased due to a greater conversion of estrone to estradiol, since estrone levels were not altered in obese rats.

It has been shown that estradiol production is abolished by AMH [61]. Here, we found that in addition to showing higher estradiol levels, obese rats have fewer follicles synthesizing AMH; leading us to suggest that the decrease in AMH may cause the increase in estradiol levels in obese rats. It is worth mentioning that the technique used to measure AMH in this study is not quantitative (immunostaining) and therefore it is not possible to ensure that a smaller number of AMH + follicles imply decreased serum AMH levels. New experiments are being designed to confirm this hypothesis.

Moreover, it has been shown that AMH modulates follicular atresia. An increase in the number of atretic antral follicles was described in AMH-null mice [62], while in vivo AMH treatment prevents follicular atresia [63]. Here, we found that obese rats have fewer

AMH + follicles and an increased number of atretic antral follicles. This allows us to suggest that the decrease in AMH in obese rats may induce follicular atresia concomitantly with the increase in estradiol levels. Moreover, we show that the mechanism by which the estradiol synthesis is stimulated does not involve the induction of ovarian mRNA StAR nor Aromatase expression. All our suggestions are consistent with the fact that CoQ10 normalized the number of AMH + follicles as well as serum estradiol concentration and the number of atretic follicles in obese rats.

In conclusion, the findings of this study underscore the multifaceted impact of CAF-induced obesity on both metabolic and reproductive functions in rats. The administration of CAF over an 88-day period resulted in significant metabolic disturbances, including hyperglycemia, hypertriglyceridemia, and hypercholesterolemia, alongside notable reproductive disruptions such as reduced follicle numbers, increased follicular atresia, and altered estrous cycles.

However, our investigation revealed a glimmer of hope in the form of CoQ10 supplementation. While 5 mg/kg CoQ10 demonstrated positive effects on ovarian disruptions, it did not ameliorate the metabolic alterations induced by obesity. This highlights the complex interplay between metabolic and reproductive systems in obesity. We hypothesize that CoQ10's beneficial effects on ovarian health may be attributed to its well-documented antioxidant and anti-inflammatory properties. These effects could help counteract the oxidative stress and chronic inflammation typically associated with obesity, particularly at the ovarian level. Unlike systemic metabolic alterations, which involve complex, multi-organ pathways, the ovary may respond more directly to localized reductions in oxidative damage and inflammatory signaling. Additionally, CoQ10 may play a role in stabilizing mitochondrial function within ovarian cells. Obesity has been shown to impair mitochondrial dynamics, leading to disruptions in follicular development and steroidogenesis. By supporting mitochondrial bioenergetics, CoQ10 might specifically benefit ovarian function, even in the absence of broader metabolic improvements. New experiments are being conducted by us to confirm this.

In light of our findings, it becomes evident the need to assess higher doses of CoQ10 administered over different periods of time in order to determine if modifying these parameters achieves the normalization of obese metabolism, in addition to the ovarian alterations that the dose and duration evaluated here were capable of reversing.

Moving forward, it is evident that a holistic approach is essential in addressing the challenges posed by obesity-related reproductive complications. Combining CoQ10 supplementation with a balanced diet and increased physical activity presents a promising strategy for obese patients seeking pregnancy. Nonetheless, the persistence of metabolic abnormalities underscores the ongoing need for comprehensive therapeutic interventions.

In essence, this study reinforces the urgency of developing tailored therapies that address the intricate metabolic and reproductive intricacies of obesity. Only through concerted efforts can we hope to mitigate the adverse effects of obesity on reproductive health and offspring well-being, thus paving the way towards healthier outcomes for future generations.

Abbreviations

CAF	Cafeteria diet
CoQ10	coenzyme Q10
GTT	glucose tolerance test
ITT	insulin tolerance test
RIA	radioimmunoassay
P4	progesterone
E2	estradiol
CL	corpora lutea
AMH	Anti-Müllerian hormone

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13048-025-01604-7.

Additional file 1. Supplementary Table I. Nutritional information for each food included in the cafeteria diet. Data is expressed as g per 100g of food.

Additional file 2. Supplementary Figure 1. Aromatase immunohistochemical staining in ovaries from Ctrl and CAF rats. a: primary follicle. b: big antral follicle. c: small antral follicle and its amplification in d. e: inicial corpus luteum. f: corpus luteum. Scale bars 50 µm (a), 100µm (b, c, d, e and f).

Additional file 3.

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Authors' contributions

Gisela Belén Sarrible and María Victoria Bazzano handled rats and performed most of the experiments except RIA that was performed by María Guillermina Bilbao and provided by Julián Alberto Bartolomé, ELISA that was performed and provided by Rodrigo HernánDa Cuña and inmunohistochemistry that was performed by Melanie Neira. Data collection, analysis and interpretation of data were at Gisela Belén Sarrible, Caterina Koutsovitis and Evelin Mariel Elia's expenses. Conceptualization, funding acquisition, supervision, project administration and drafting the initial version of the manuscript were carried out by Evelin Mariel Elia. All authors critically reviewed and edited the manuscript, approving the final version to be published

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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