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Maternal Overweight Disrupts the Sexual Maturation of the Offspring

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Abstract

The aims of the present work were to study the effect of maternal overweight and obesity on the ovarian reserve, follicular development, and ovulation of the offspring and to assess whether this maternal condition alters oocyte integrity. To this end, female offspring from rats fed standard (OSD) or cafeteria (OCD) diet were used. Body weight, vaginal opening, and estrous cycle were recorded and ovaries were obtained on the day of the second estrus. In addition, ovarian weight, ovulation rate (measured by the number of oocytes within oviducts), follicular development (determined by histology), and oocyte integrity were examined. The OCD were divided into 2 groups: offspring from rats with 17% and 28% of overweight (OCD17 and OCD28, respectively). Both OCD groups showed higher body weight, but OCD28 also exhibited early vaginal opening and higher ovarian weight and glycemia at euthanasia compared with OSD. Both OCD17 and OCD28 had lower number of primordial and primary follicles, and only OCD28 exhibited lower number of antral follicles, all compared with OSD rats. In addition, both OCD17 and OCD28 had higher ovulation rate than controls, and OCD28 had lower number of healthy oocytes, which, in turn, exhibited morphological alterations such as larger perivitelline space and zona pellucida than those of control animals. These results suggest that maternal overweight may severely affect the reproductive ability of the offspring, likely as a result of altering the organogenesis.

Keywords

maternal overweight, offspring, sexual maturity, ovary

Introduction

Obesity has increased in recent years, particularly in children and adolescents, and is considered a global epidemic, that is, as the most important noncommunicable chronic diseases. Maternal overnutrition may induce multiple pathologies both in the mother and in the offspring. Obese or overweight women may have many diseases such as diabetes and hypertension and have high probability of reproductive disorders, including menstrual dysfunction, anovulation, gestational diabetes, adverse pregnancy, preeclampsia, miscarriage or premature births, complex deliveries, subfertility, ovarian polycystic syndrome, and cancer.[1-6] These disorders may be the result of a disruption in the hypothalamic–pituitary–ovarian axis, affecting different reproductive processes. The risk of these diseases has a direct relation to the degree of overweight or severity of obesity.[7] These maternal disorders result in a detrimental genetic, hormonal, and biochemical environment for the embryo or fetus.[3,5] It is now known that maternal obesity severely affects the offspring phenotype by altering the fetal programming in various systems, including reproduction.[8] Moreover, some of these effects seem to occur before gestation by acting on the role and/or integrity of the gametes.[9]

Studies in rodent obesity models have examined the negative impact of obesity on oocyte recruitment, competence, quality and maturity, fertilization rate, and embryo quality. However, some results, especially those in humans, are conflicting.[9-11] In obese women, the intraovarian environment is altered because they have high levels of insulin, glucose, triglycerides, leptin, and some proinflammatory factors in both blood and follicular fluid.[12] In female rats, pubertal timing and subsequent ovarian function are influenced by the nutritional status in utero, with both maternal caloric restriction and maternal high-fat nutrition, resulting in early pubertal onset.[13] Connor et al[14] found that pups from high-fat-fed dams exhibit early puberty and irregular estrous cycles by having a prolonged and persistent estrus. All these data indicate that a poor quality of nutrition, by either excess or restriction, can disrupt the ovarian

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function, especially the development and quality of oocytes. Therefore, the aims of the present work were to study the effect of maternal overweight and obesity on the ovarian reserve, follicular development, and ovulation of the offspring and to assess whether this maternal condition is able to alter oocyte integrity.

Methods

Animals

Immature female Sprague Dawley rats aged 21 days were purchased from the School of Veterinarian Sciences of Buenos Aires University, Argentina. Animals were maintained under controlled conditions of light (12-hour light/12-hour darkness), temperature (22°C), and humidity, with free access to the respective diets and water. All animals were handled according to the Guiding Principles for the Care and Use of Research Animals, and all protocols were approved by the Institutional Committee of the School of Medicine of Buenos Aires University by resolution 2167/13.

Experimental Design

Mothers. At 22 days of age, female rats were divided randomly into 2 groups, as described previously[15] (1) control animals fed with a standard diet (SD) containing 11.4% fat, 71.4% carbohydrates, and 17.2% protein (18.4 kJ/g) and (2) diet animals fed with a high-fat palatable (cafeteria) diet (CD) containing 30% fat, 52.8% carbohydrates, and 17.2% protein (24.1 kJ/g). The latter included SD and the following products in the alternate form: chocolate, peanuts, cheese, snacks, cookies, sausages, peanut butter, condensed milk, and butter. Cafeteria feeding from weaning mimics the eating habits of many people from an early age and is effective in inducing obesity without impacting on reproductive success, as described previously.[16] When CD animals attained either 15% or 30% overweight, both SD and CD rats were mated with control males on the day of proestrus, which is determined by the examination of vaginal smears. Pregnancy was confirmed by the presence of sperm cells in vaginal smears. The litter size was adjusted to 8 pups per litter to ensure both adequate and uniform nutrition. The SD and CD diets were supplied continuously until weaning of their offspring, including pregnancy and lactation. Food intake of mothers was monitored daily and changes in body weight were monitored 3 times a week.

Offspring. At 21 days of age, all female offspring were weaned, fed with an SD, and inspected daily to record the body weight, vaginal opening, and estrous cycle. From the day of vaginal opening, vaginal smears were examined daily to identify the estrous cycle. Blood-glucose level was measured by the One-Touch Ultra reagent strips and a glucometer OneTouch Ultra (Johnson & Johnson Cia, Milpitas, California) in samples obtained from the tail vein at weaning and euthanasia. All animals (8-10 per group) were killed by decapitation in the afternoon of the estrous day of the second estrous cycle because (1) the first cycle is very irregular, (2) the second cycle is not completely regular, but rats retain the follicular reserve almost completely, and (3) the transport of oocytes through the ampullary segment takes minutes, and all the oocytes reach the oviducts 12 hours after ovulation and remain there for at least 2 days.[17,18] Both ovaries and femurs were obtained and used. All chemicals were purchased from Sigma-Aldrich (St Louis, Missouri).

Ovarian Histology and Follicle Counting

After assessing the ovulation rate and weighing both ovaries, 1 ovary was fixed in neutral-buffered formalin, while the other was frozen at −80°C to be used in other studies. Ovaries were processed as indicated previously.[19] The ovarian sections were serially sectioned at 6-µm thickness, mounted onto glass slides, and some of them were stained with hematoxylin and eosin and analyzed using an Olympus CX21 microscope (Olympus Corp, Tokyo, Japan). Ovarian follicles were analyzed for every 10 sections and only follicles containing an oocyte were counted. Each corpus luteum was followed through consecutive sections to ensure that it was counted only once. Ten sections from the same ovary and 8 to 10 ovaries from different animals of the same group were analyzed in each group. Follicles were counted and classified as primordial (Po), primary (Pi), preantral (PA), or antral (A), as described previously.[19] Primordial follicles were characterized as oocytes surrounded by a single layer of flattened granulosa cells. Primary follicles were characterized as oocytes surrounded by a single layer of cuboidal granulosa cells. Preantral follicles were characterized as oocytes surrounded by 2 or more layers of cuboidal granulosa cells with no visible antrum. Antral follicles were identified by the presence of an antrum. The abundance of each type of follicle or corpus luteum was normalized by the total ovarian area in the section, as reported previously.[19-21] The area of ovary was measured with ImageJ (version 1.42q) and expressed per 10 mm^2.

Ovulation Rate

Immediately after euthanasia, ovaries and its accompanying oviducts were immediately removed and the oviducts were flushed with hyaluronidase (1 mg/mL in saline), using a 30-gauge needle. The number of oocytes was counted by means of a stereoscopic microscope.[22]

Oocyte Integrity

To study whether maternal overweight induces changes in the oocyte integrity in the offspring, after the treatment with hyaluronidase, the denude oocytes were (1) observed and photographed to evaluate the morphological characteristics using an Olympus CX21 microscope with Samsung ES55 camera (Samsung, Seoul, South Korea), as described previously[23,24] and (2) stained with Hoechst-33258 (HO, 10 µg/mL) and propidium iodide (PI, 50 µg/mL) to identify live and dead oocytes, using a fluorescence microscope Nikon Eclipse E200 with Nikon DS-Fi1 camera (Nikon Instruments Inc, NY, USA). Hoechst-33258

is a blue dye that fluoresces in contact with nucleic acids, whereas PI is a red dye that fluoresces in contact with nucleic acids but only when the cell membrane becomes permeable. Microscope images and levels of fluorescence intensities were observed and quantified using ImageJ software, as described previously.[25] Briefly, each oocyte was analyzed by its fluorescence intensity. After establishing a base value of intensity, the oocytes were classified according to the fluorescence intensity based on its response to HO or PI using ImageJ analysis software. Different levels of fluorescence were observed (low, moderate, or high). After this quantitative analysis, an overlay of images taken with the filters corresponding to each dye (Imaging Software NIS Elements, version 3.22.00) confirmed the results obtained. This last analysis yielded 3 different oocyte populations: (1) blue nuclei indicating living cells, (2) intense red nuclei indicating dead cells, and (3) bright red or Fuchsia nuclei which represents hyperresponsiveness to both dyes indicating dead or damaged cells.

Cytotoxicity Assays

To study whether maternal overweight was able to cause some genetic alteration, the bone marrow of each animal was used to evaluate clastogenic effects using the comet assay as described previously.[26,27] To this end, both femurs were extracted to obtain a suspension of bone marrow cells by flushing with phosphate-buffered saline, using a 1-mL syringe with a 21-guage needle. The alkaline comet assay was also used to quantify the damage in the DNA in oocytes as a measure of possible damage in germ cells.[27-29] The presence of comets was observed by fluorescence microscopy (Nikon Eclipse E200 at 100× magnification with photo camera Nikon DS-Fi1). At least 150 cells were analyzed, with 2 slides scored per animal. The DNA damage in the cells was analyzed by measuring the length of DNA migration and the percentage of migrated DNA, using the software Comet Assay Software Project (CASP 1.2.2).

Statistical Analysis

All data are expressed as mean ± standard error of the mean (SEM). Differences between the 2 groups were analyzed using Student *t* test. Comparisons between more than 2 groups were performed using 1-way analysis of variance (ANOVA) with Dunnett multiple comparison test or 2-way ANOVA with Bonferroni posttest, depending on the assay. Levene test and a modified Shapiro-Wilk test were used to assess the homogeneity of variances and normal distribution, respectively. Differences between groups were considered significant when $P < .05$.

Results

Body and Ovarian Weight, Vaginal Opening, and Estrous Cycle

Figure 1 shows the pattern of changes in the body weight of SD and CD rats (Figure 1A) and their offspring (Figure 1B). After CD was offered, rats gradually increased the intake of cafeteria food, while they reduced the intake of SD. Before they became pregnant, the intake by CD animals was 28% more than that in controls, and 44.4% of it corresponded to CD. Considering the overweight of the CD rats when they became pregnant (day 0), and to relate the effects observed in the offspring to the different degree of overweight of their respective mothers, CD rats and their offspring were divided into 2 groups: rats with 17% (with ranges of 15%-19%) and 28% (with ranges of 25%-31%) overweight (CD17 and CD28, respectively) and offspring from CD17 and CD28 rats (OCD17 and OCD28, respectively). At 30 days of age, both CD groups showed significantly greater weight gain than controls (Figure 1A), but these rats exhibited normal estrous cycles and no differences were found in the overall litter size when they gave birth (data not shown).

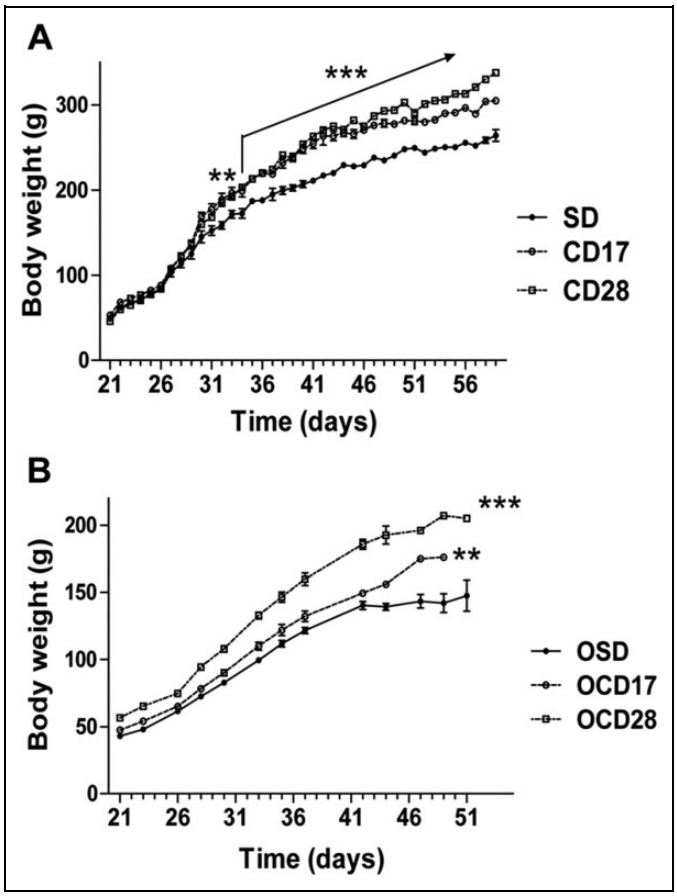

Figure 1. Pattern of changes in body weight in rats fed with a standard (SD) or cafeteria (CD) diet (A) and in their offspring (B). According to the overweight reached on day 0 of gestation, CD animals and their offspring were divided into 2 groups: rats with 17% (CD17) and 28% (CD28) overweight and offspring from CD17 (OCD17) and CD28 (OCD28) rats. Each point represents the mean ± standard error of the mean (SEM) for 8 to 10 animals per group. $^{**}P < .01$ and $^{***}P < .001$ versus the respective control (2-way analysis of variance [ANOVA] and Bonferroni posttest).

Regarding the offspring, OCD showed different patterns of weight gain and size. Figure 1B shows that both the OCD groups were heavier than the OSD group from weaning (21 days of age), though this difference was only significant

Table 1. Systemic and Reproductive Parameters Measured in the Offspring From Rats Fed Standard or Cafeteria Diet.[a,b]

	Body Weight (g)		Length (cm)	Ovarian Weight	Vaginal	Second	Glycemia (mg/dL)	
	Day 21	Day 49	Day 49	(Euthanasia Day)	Opening (Days)	Estrus (Days)	Weaning Day	Euthanasia Day
OSD	43.0 ± 1	142 ± 7	17.1 ± 0.1	28 ± 1	36 ± 0.5	44 ± 1	147 ± 3	125 ± 5
OCD17	47.4 ± 1	176 ± 3[c]	19.0 ± 0.1[d]	33 ± 2	33 ± 0.5	41 ± 2	155 ± 3	133 ± 4
OCD28	56.5 ± 1[e]	207 ± 2[e]	19.5 ± 0.1[d]	41 ± 3[e]	33 ± 0.2[d]	43.0 ± 0.6	150 ± 3	145 ± 5[c]

Abbreviations: ANOVA, analysis of variance; OCD, female offspring from rats fed cafeteria diet; OSD, female offspring from rats fed standard diet; SEM, standard error of the mean.

[a]According to the overweight reached by the mothers on day 0 of gestation, OCD was divided into 2 groups: 17% (OCD17) and 28% (OCD28). Each point represents the mean ± SEM for 8 to 10 animals per group.

[b]Ovarian weight, vaginal opening, and second estrus were analyzed using 1-way ANOVA and Dunnett multiple comparison test. Bodyweight, length, and glycemia were analyzed using 2-way ANOVA and Bonferroni posttest.

[c]$P < .01$.

[d]$P < .05$.

[e]$P < .001$ versus OSD.

for OCD28 ($P < .001$; Table 1). However, at 49 days of age, this day was the last day that we still had animals from all groups, both OCD group rats were significantly heavier and larger than the OSD group (Table 1). Table 1 also shows the results obtained regarding glycemia and other reproductive parameters in the offspring. We found that both OCD17 and OCD28 had higher ovarian weight and exhibited an early vaginal opening than OSD, though these differences were only significant in OCD28. The latter also exhibited a higher serum concentration of glucose than controls at euthanasia, although these levels did not reach diabetic values.[30,31]

Follicular Growth

Histological examination of the different growing follicles during sexual maturity is an excellent method to assess the reproductive development and ovarian function. Figure 2 shows photomicrographs and quantitative results obtained by histological analysis of small (Figure 2A-D) and A (Figure 2E-H) follicles from ovarian sections of each group of rats. The offspring from both CD groups exhibited a lower number of Po (OCD17: 8.8 ± 0.9, OCD28: 6 ± 1) and Pi (OCD17: 5.6 ± 0.2, OCD28: 6.3 ± 0.6) follicles than OSD (Po: 14 ± 1, Pi: 10.2 ± 0.8). No differences were found with PA follicles (Figure 2A-D). Unlike that observed with small follicles, only OCD28 exhibited a lower number of A follicles (2.8 ± 0.3) than control animals (4.7 ± 0.3), without changes in those from OCD17 (Figure 2E-G).

Ovulation Rate

To study the effect of maternal overweight on the ovulation rate, both the number of corpora lutea in the ovarian sections and the number of ovulated oocytes were counted. We found that the offspring from both CD groups clearly had a higher ovulation rate than control animals, which was evidenced by (1) the number of corpora lutea expressed per mm^2 (OSD: 2.7 ± 0.4, OCD17: 4.3 ± 0.3, OCD28: 4.9 ± 0.6; Figure 3A-D) and (2) the number of oocytes present within oviducts expressed per rat (OSD: 7 ± 1, OCD17: 12 ± 1, OCD28: 11.5 ± 0.6; Figure 3E).

Oocyte Integrity

After oocytes were denuded of cumulus cells by gentle mechanical pipetting, different morphological characteristics were determined to examine the changes caused by maternal obesity on oocyte integrity. Only oocytes from OCD28 were analyzed. OCD28 oocytes displayed greater size and perimeter in parallel with greater zona pellucida thickness and perivitelline space than those recovered from OSD (Figure 4A, B). Moreover, some oocytes from OCD28 were deformed and exhibited irregular membranes (Figure 4A).

Figure 4C shows the presence of oocytes after staining with HO (blue) and PI (red orange) and the merged image of oocytes from both OSD and OCD28. Healthy cells were identified as cells with positive staining to HO and negative to PI. Quantitative analysis showed that OCD28 exhibited a lower percentage of healthy oocytes (55 ± 5, $P < .05$) than OSD (93 ± 5). The figure also shows the irregularity in the membrane in OCD28 oocytes with positive PI staining (Figure 4C, arrow).

Cytotoxicity Assays

Neither bone marrow nor oocytes from OCD28 exhibited significant differences in the percentage of DNA in the tail as compared with those of OSD (data not shown).

Discussion

In the present work, we showed that diet-induced maternal overweight clearly impacts on the sexual development of the offspring, particularly on the reproductive system, leading to lower follicular reserve, less follicular development, higher ovulation rate, and featured changes in oocyte morphology. It is worth noting that all the changes observed in the offspring were caused exclusively by the high-fat diet in mothers as the offspring were fed with SD after weaning.

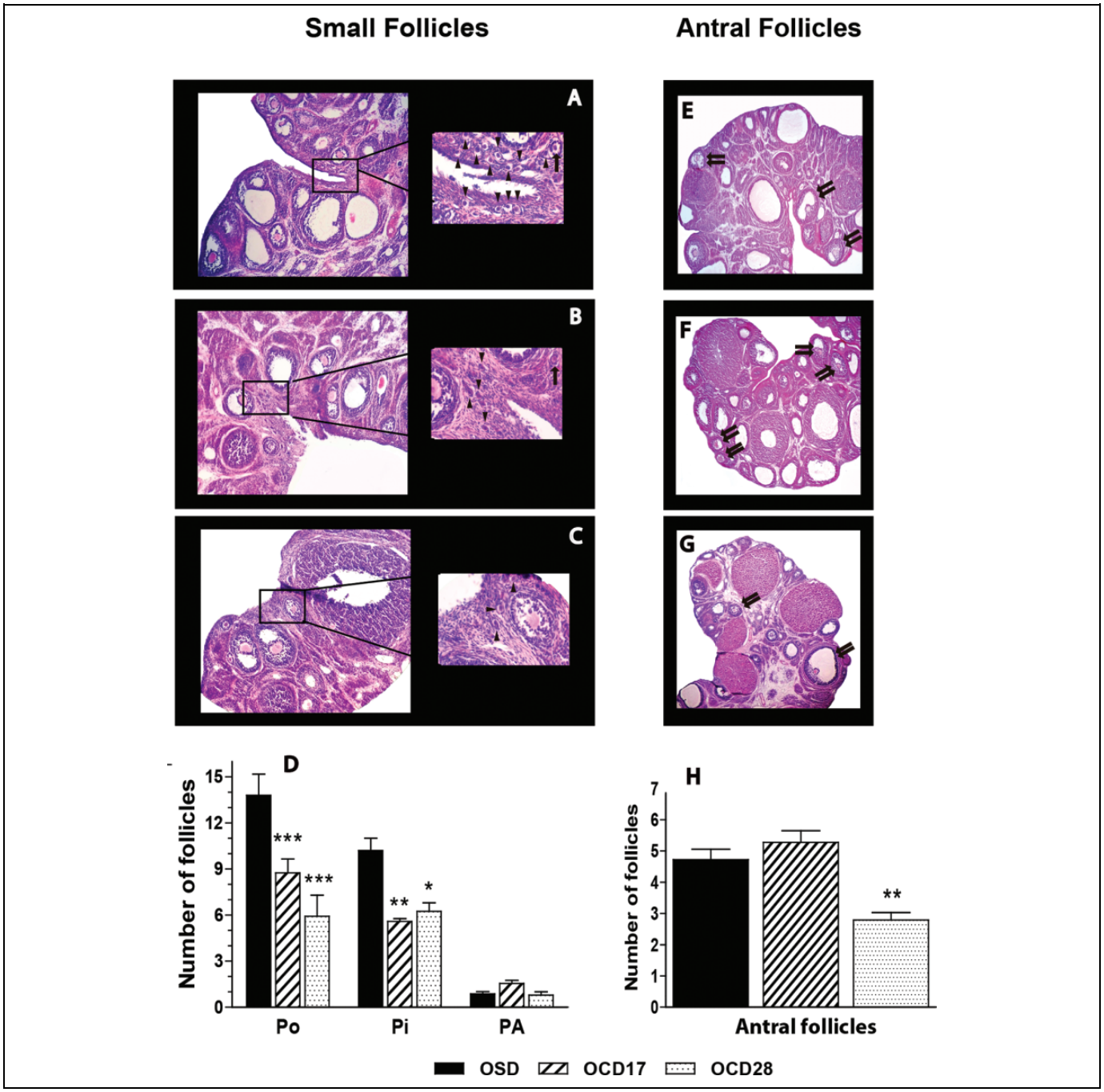

Figure 2. Number of small (A-D) and antral (E-H) follicles in the offspring from rats fed standard (OSD) or cafeteria (OCD) diet, the latter of which were divided into 2 groups according to the overweight reached by the mothers on day 0 of gestation: 17% (OCD17) and 28% (OCD28) of overweight. A-C and E-G, Histological appearance of ovarian sections from OSD (A), OCD17 (B), and OCD28 (C). D and H, Quantitative morphometric analysis of ovarian sections for the different stages of small and antral follicles, respectively. Values are expressed as the number of follicles per 10 mm^2. Data represent the mean $\pm$ SEM for 8 to 10 ovaries from different animals with the same treatment, and each value represents the mean of 10 sections. *$P < .05$, **$P < .01$, and ***$P < .001$ versus OSD (2-way ANOVA and Bonferroni posttest). Po indicates primordial follicles; Pi, primary follicles; PA, preantral follicles. Arrowhead indicates Po; arrow, Pi; double arrows, antral follicles (magnification $\times$40 and $\times$400).

Despite the differences in biological models, the increase in the ovarian weight and ovulation rate in both groups of OCD compared with OSD is consistent with other reports. Minge et al[32] found an increase in both parameters and a delayed embryonic development in mice fed with a high-fat diet. Likewise, and using mice as well, Aiken et al[33] found higher body weight in female offspring from rats fed an obesogenic diet than in those from rats fed a control diet, but found no

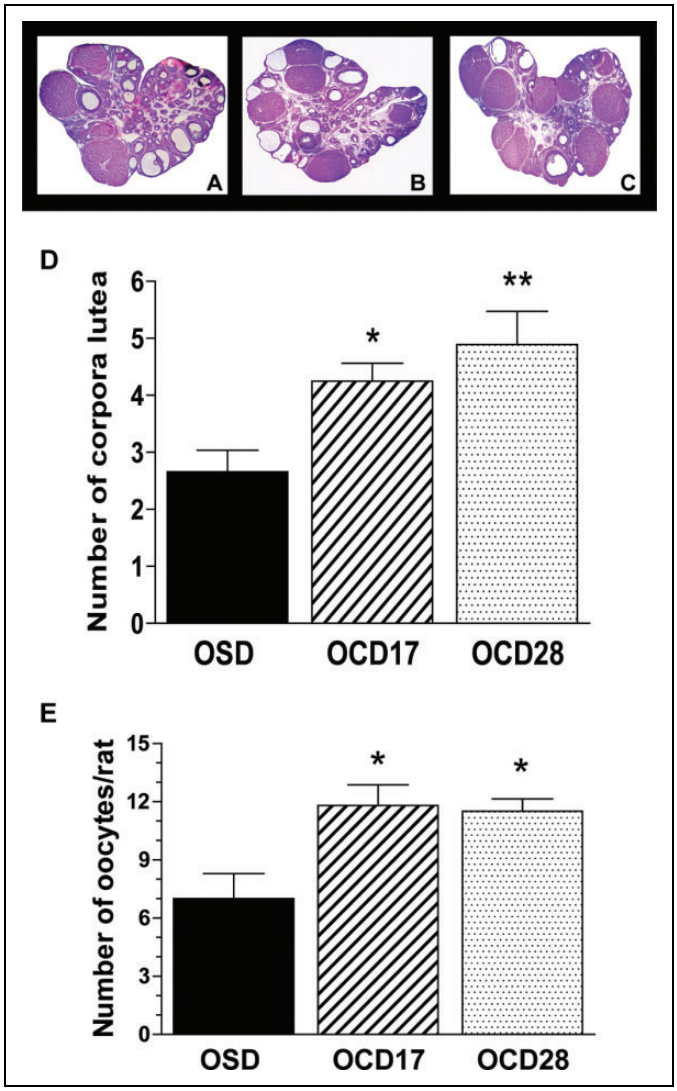

Figure 3. Number of corpora lutea (A-D) and ovulation rate (E) in the offspring from rats fed standard (OSD) or cafeteria (OCD) diet, the latter of which were divided into 2 groups according to the overweight reached by the mothers on day 0 of gestation: 17% (OCD17) and 28% (OCD28) overweight. A-C, Histological appearance of ovarian sections from OSD (A), OCD17 (B), and OCD28 (C). D, Quantitative morphometric analysis of ovarian sections for the corpora lutea expressed as the number of corpora lutea per 10 mm^2. Data represent the mean $\pm$ standard error of the mean (SEM) for 8 to 10 ovaries from different animals with the same treatment, and each value represents the mean of 10 sections (magnification $\times$40). E, Ovulation rate expressed as the number of oocytes present within oviducts per rat. Each bar represents the mean $\pm$ SEM of 8 to 10 animals, whose values were obtained as the sum of both ovaries from each animal. *P < .05 and **P < .01 versus OSD (1-way analysis of variance [ANOVA] and Dunnett posttest).

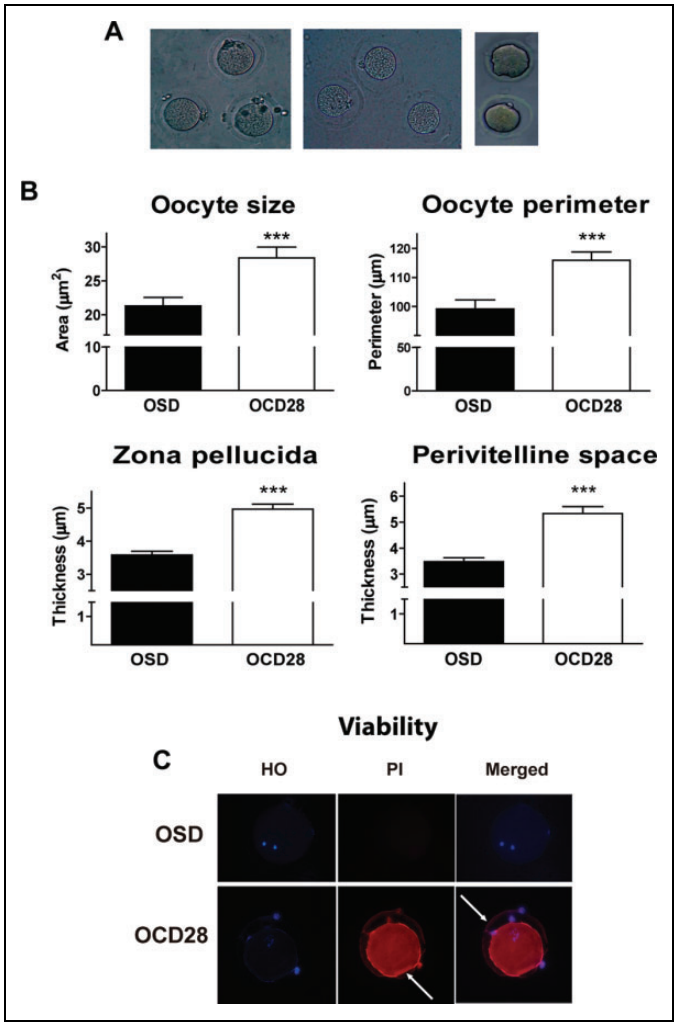

Figure 4. Examination of different morphological parameters (A-B) and viability (C) of the oocytes from the offspring from rats fed standard (OSD) and cafeteria (OCD) diet, whose mothers had reached 28% of overweight on day 0 of gestation (OCD28). A, Representative images of oocytes from OSD and OCD28. B, Quantitative analysis of the morphologic parameters. Each bar represents the mean $\pm$ standard error of the mean (SEM) of 60 to 65 oocytes from different offspring from mothers with the same diet. ***P < .001 versus OSD (2-way analysis of variance [ANOVA] and Bonferroni posttest; magnification $\times$400). C, Representative images of oocytes stained with Hoechst (HO), propidium iodide (PI), and merged. Arrow indicates irregularity of the membrane (magnification $\times$400).

differences in the ovarian weight between both the groups. Unlike our biological model, mothers were fed an obesogenic diet only during pregnancy. However, the rats used in the present work were fed a high-fat diet from 22 days of age (weaning) until weaning of their offspring, including pregnancy and lactation. This may also explain why Aiken et al[33] found no changes in the follicular reserve in young adult females.

OCD, especially OCD28, clearly displayed an early vaginal opening and a higher serum concentration of glucose (at euthanasia) compared with controls. Early onset of puberty induced by obesity has also been described in different species, including humans. Sánchez-Garrido et al[34] evaluated the onset of puberty by observing the vaginal opening in rats fed with different nutritional and timing manipulations and found that overfeeding during lactation, achieved by a small litter (4 pups per litter), exhibited advanced puberty in approximately 2 days. Likewise, they observed no differences in the glucose levels of

any of the groups evaluated at vaginal opening. Differences between these last results and the increase observed in our rats may be due to the fact that we measured glucose levels on the day of euthanasia. Using rats, other authors have also found that the offspring from high-fat-fed mothers[13,14] or offspring fed high-fat diet postweaning[14,35] display an early puberty onset and irregular estrous cycles. Since our animals were killed on the afternoon of the estrous day of the second estrous cycle, we did not expect to find irregular cycles. Regarding glycemia, we did not study the offspring after they reached the second estrus. However, OCD28 exhibited differences in body weight, size, and glycemia, at euthanasia, despite receiving an SD after weaning. Although we estimate that these values correspond to nondiabetic values, this transgenerational phenomenon makes the offspring susceptible to metabolic syndromes such as glucose intolerance, diabetes, and/or insulin resistance in the future, as described by other authors.[3] An interesting work using a biologic model similar to ours showed that CD feeding leads to the development of obesity and insulin resistance but not to higher plasma glucose levels.[36] Furthermore, other authors have described that children born to obese mothers have a high risk for obesity and diabetes.[37,38]

In our study, both the body weight and size of pups from the CD groups were higher than those from the SD group. Luzzo et al[39] found that 14.5-day embryos from high-fat-fed mice were smaller than those from controls. However, they did not study these offspring at birth or after birth. Connor et al[14] found that the female offspring of high-fat-fed dams were lighter at birth than the offspring of control dams. However, at weaning, these pups were significantly heavier than the offspring of control dams. In this study, we found no differences in the body weight of offspring at birth between the different groups, but pups of CD mothers were significantly heavier than those of controls from day 12 of age (data not shown) until killing. These results indicate that, despite the differences in the maternal nutrition, maternal overfeeding induces overweight in the offspring, including the risks involved in this condition.

In addition to showing early puberty and an increase in body weight and serum glucose, the offspring of high-fat-fed mothers had a smaller number of immature follicles, including Po follicles. These results are consistent with those obtained by Cheong et al[40], who, using an experimental mouse model, found a reduced number of Po, A, and Graafian follicles in the ovaries of offspring intrauterinely exposed to a high-fat diet. These authors also observed that these offspring had increased expression of some genes, including *Gdf9*, a member of the transforming growth factor β, which is involved in follicular growth and folliculogenesis.[41-43] These authors did not study the ovulation rate. However, in the present work, the ovulation rate, measured by both the number of oocytes within oviducts and corpora lutea in ovarian sections, was increased in the offspring from obese dams. Despite the difference in the biological model, these results are consistent with previous studies. Wang et al[44] reported that high-fat-induced obesity stimulates the activation of Po follicles and promotes the development and maturity of ovarian follicles. These authors also found that rats fed with high fat display a lower number of Po follicles and a greater number of developing follicles and corpora lutea in parallel with a greater number of atretic follicles. Thus, these authors suggested that a high-fat nutrition accelerates the rate of follicle loss, which may be the cause leading to the earlier sexual maturity and declined fertility. Anyway, all these studies clearly reflect that maternal overnutrition impacts on the reproductive capacity in both the mothers, as indicated by Wang et al,[44] and their offspring, particularly by depleting the follicular reserve. Taken together, these data indicate that both rats fed with high fat and their offspring may undergo (1) early puberty, (2) higher rate of follicle loss, which means less follicular reserve, and (3) risk of premature ovarian aging.[35] Conversely, other works have found that rodents fed with calorie restriction (1) exhibit delayed onset of puberty and reproductive capacity,[45,46] (2) show twice the number of Po follicles than their age-matched controls,[47] and(3) maintained the cyclicity when 80% of their age-matched controls were acyclic.[47,48] Thus, some authors suggested that caloric restriction might preserve and prolong the reproductive ability.[48] Based on these data, it is reasonable to suggest that the formation of the ovarian follicular reserve and the reproductive life span are markedly vulnerable to the calorie and lipid content of the food diet.

The fact that the offspring from rats with overweight exhibited a lower number of follicles but a higher ovulation rate was surprising, because it seems that maternal obesity inhibits the formation of the follicular pool but stimulates follicular depletion by increasing the ovulation rate. However, some authors have demonstrated that the activation of Po follicles depends on some spatial determinants. Hirshfield[49] reported that depletion of the ovarian reserve results in increased follicle loss. Initial studies stated that the rate of recruitment of dormant follicles into the growing pool was inversely correlated with the size of the resting follicle pool.[49,50] Gaytan et al[51] stated that resting follicle crowding is a key factor influencing the rate of recruitment into the growing pool and that resting follicles would be the source of local inhibitory signals that decrease the probability of initiating growth in the neighboring resting follicles. Moreover, these authors suggested the existence of a threshold in follicle density that accelerates follicle activation. Thus, our findings seem to be consistent with these results.

Further studies are necessary to investigate the mechanism/s by which a high-fat diet alters the follicular count, the follicular development, and the ovulation rate. However, it is reasonable to suspect that a high-fat diet may alter the expression of various genes involved in the formation of the follicular pool as well as in the follicular development and ovulation. Studies are in progress in our laboratory to examine the genes that may be either activated or inhibited in the offspring from CD-fed dams.

Finally, oocytes from OCD28 were larger and exhibited larger perivitelline space and zona pellucida than those from controls. These results seem to contrast with those obtained by Jungheim et al[3] who found more apoptotic preovulatory ovarian follicles and smaller and fewer mature oocytes in obese mice than in controls. It is worth mentioning that these authors used adult obese mice (mice fed with a high-fat diet) and that

follicular growth was induced by high doses of gonadotropin, which may be involved in a hormonal response inhibited in control and obese animals. However, the results of both works suggest that both obese animals and their offspring suffer changes in the oocyte morphology. Successful fertilization depends on many factors, including oocyte quality, and in turn, oocyte quality depends on several factors such as the age of the animal, the ovarian morphology, environmental factors, the body condition or diet, and others related to the breed of the animals.[52,53] Thus, the lower percentage of viability observed in our study could be due to the changes found in the oocyte morphology such as irregular plasma membrane, greater perivitelline space, and zona pellucida (Figure 4). The mechanism involved in these effects still needs to be elucidated, though it is probable that the reduction in viability percentage is due to a greater apoptosis rate, as observed by other authors.[44,54-57] However, more studies regarding this issue are in progress in our laboratory.

In conclusion, we found that female offspring from obese rats showed early puberty, reduction in the follicular reserve, higher number of ovulated oocytes, and a significant percentage of these oocytes with altered morphology. All these effects suggest that maternal overweight may severely affect the reproductive ability of the offspring, likely as a result of altering the organogenesis, including that at the reproductive level.

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