REPRODUCTION
RESEARCH

Maternal nutrition and reproductive functions of female and male offspring

Ziya Kalem[1], Müberra Namlı Kalem[2], Elvan Anadol[3], Batuhan Bakırarar[4], Canan Yılmaz[5], Çiğdem Elmas[6], Perihan Yalçınkaya[6], Halil Ruso[1] and Timur Gürgan[1]

[1]Gurgan Clinic IVF and Women Health Center, Ankara, Turkey, [2]Department of Obstetrics and Gynecology, Liv hospital Ankara, Ankara,Turkey, [3]Gazi University, Laboratory Animal Breeding and Experimental Researches Center, Ankara, Turkey, [4]Department of Biostatistic, Ankara University, Ankara, Turkey, [5]Department of Biochemistry, Gazi University, Ankara, Turkey and [6]Department of Histology and Embryology, Gazi University, Ankara, Turkey

Correspondence should be addressed to M Namlı Kalem; Email: muberranamli@hotmail.com

Abstract

This study aimed to investigate the effects of maternal unhealthy nutrition on the reproductive functions of female and male adult offspring. This was an animal study carried out with 24 virgin female Wistar rats (dams) and their male and female offspring. Rats were divided standard diet (SD) or cafeteria diet (CD) groups, after 10 weeks of feeding, all rats were paired with a Wistar stud male, and each group was again divided into CD and SD groups during the pregnancy and lactation periods. After birth, six female and six male pups in each group, were subjected to study. Following 3 weeks of lactation, the pups were fed with SD for 8 weeks and killed when they were considered adult at 11th week for analysis. Primordial and antral follicle counts, serum anti-Mullerian hormone (AMH) and phosphatase and tensin homolog (PTEN) in the oocyte cytoplasm were examined to evaluate ovarian function, and E-cadherin and integrin-β1 levels were examined in endometrial tissues for the evaluation of endometrial receptivity in female offspring. Sperm analysis was performed in male offspring. In groups in which the dams were fed CD, primordial follicular pool, PTEN, and endometrial receptivity were reduced. In contrast, AMH and the number of antral follicles were not changed. In male offspring, the testicles were smaller, testosterone production decreased, AMH increased and the number and function of sperm were not changed. Sperm analysis results were not changed. All negative effects on reproductive functions were more apparent in groups fed with the CD during the pregestational period.

Reproduction (2018) **156** 353–364

Introduction

The environment encountered during early life has important effects on health trajectories later in life (Szyf *et al*. 2009). Most adult-onset health problems are caused by the intrauterine life origin in the 'early life programming' phenomenon, which is defined in the Barker hypothesis (Barker 1995). Maternal nutrition is an important factor in this programming as epigenetic factors are known to play important roles in later wellbeing of men (Alfaradhi & Ozanne 2011). Obesogenic feeding is a major problem and is more common during reproductive age when compared to advanced ages among humans (WHO 2017), leading to adverse outcomes during later stages of life for both mothers and offspring (Reynolds *et al*. 2013). There are numerous studies indicating that several reproductive functions can be altered *in utero* under the influence of maternal nutrition. Several studies have documented the effects of obesogenic maternal feeding on female offspring and showed that the ovarian reserve diminishes due to increased lipid peroxidation in rats (Aiken *et al*. 2016, Chan *et al*. 2015, Lane *et al*. 2015, Dupont *et al*. 2012, Connor *et al*. 2012). Similarly, male rats were shown to have reduced fertility due to increased oxidative stress levels exerted by maternal overnutrition and obesity (Rodriguez-Gonzales 2015).

In this study, for the first time, we investigated the effects of obesogenic nutrition during pregestational, pregnancy and lactation periods on the reproductive abilities of adult male and female offspring. The cafeteria diet (CD) was chosen to be the obesogenic diet as it is high in simple sugars and saturated fat and is the most frequent form of unhealthy diet at the present time (Sampey *et al*. 2011). In order to evaluate the female reproductive ability, we analysed the primordial and antral follicle counts as well as the cytoplasmic expression of phosphatase and tensin homolog (PTEN) in the oocytes.

The primary aim of the study was to investigate the effect of maternal obesogenic nutrition on the reproductive function of the male and female of the offspring. The secondary aim of the study was to

© 2018 Society for Reproduction and Fertility
ISSN 1470–1626 (paper) 1741–7899 (online)
https://doi.org/10.1530/REP-18-0070
Online version via www.reproduction-online.org
Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI

compare the effects of obesogenic diet during the pregestational, gestational and lactation periods. It is previously reported that PTEN is among the potential preserver of the ovarian reserve (Reddy *et al*. 2010, Ernst *et al*. 2017). In cases of PTEN deficiency, the whole of the primordial follicle pool expresses premature activation resulting in depletion of the ovarian reserve (Reddy *et al*. 2010, Novella-Maestre *et al*. 2015). In addition to ovarian marker PTEN, well-documented endometrial receptivity markers E-cadherin and integrin-β1 were evaluated (Zhang *et al*. 2013). On the male side of the study, we evaluated the male reproductive function by analysing the sperm concentration and sperm motility.

Materials and methods

Animals and experimental design

This is a cross-over study investigating the effect of two different nutritional diets during pregestational, gestational and lactation periods on Wistar rats and their male and female pups.

There were two types of diet in this study: Standart rat diet (SD) (Korkuteli Food Industry, Turkey) : 74.4% carbohydrate, 18.2% protein, 7.4% lipid, 15.9 kJ/g metabolic energy. Cafeteria diet (CD) including biscuits, potato crisps, crackers and nut chocolate: 54.1% carbohydrate, 14.1% protein and 32.8% lipid, 22.4 kJ/g metabolic energy.

At the beginning of the study, 24 virgin female Wistar rats (120–125 g) were obtained from Harlan Laboratories (The Netherlands), maintained with a 12-h light/darkness cycle and fed a standard rat diet *ad libitum*. The animals were randomly divided equally into two nutritional groups. Rats in group 1 were fed a SD, and group 2 were fed a CD throughout 10 weeks, along this period body weights of the animals were measured between 09:00 and 10:00 h daily.

After 10 weeks of feeding, all rats were paired with a Wistar stud male, and mating was confirmed by the appearance of a vaginal plug. Each group was again divided into CD and SD groups during the pregnancy and lactation periods. While group 1A continued to be fed with SD, group 1B were subjected to be fed with CD. On the other hand, while group 2A were fed with SD, the group 2B continued to be fed with CD. Consequently, there were four different nutrition groups consisting of six rats in each group and each group were fed as they were fed during pregnancy (Table 1).

After birth, all offspring were weighed, and the litter size was standardised to 12 offspring per litter. From each group, six offspring from each gender were selected based on their weights that are closest to the median among the group for the next phase of the study. Consequently, there were 24 male and 24 female pups. The pups were fed with SD for 8 weeks following the lactation period. The pups were considered to be adult once they were 11 weeks old.

In order to time the culling of the animals, vaginal cytologic examination was performed to determine the stage of the oestrus cycle in all adult female offspring. Vaginal secretions were collected with a plastic pipette filled with 10 IU normal saline (NaCl 0.9%) by embedding the tip into the vagina. Vaginal fluid was dropped on glass slides. The unstained material was evaluated under the light microscope (Leica CME Microscope, 1349522X; Leica; 40× objective lens), as described by Marcondes *et al*. (2002).

At the end of the experiment, all adult offspring at 11th week were killed under ketamine (30 mg/kg body weight (BW)) and xylazine (6 mg/kg BW) anaesthesia. Blood samples were drawn intracardially into plain tubes without heparin in proestrus for endocrine and biochemical analyses, and serum were separated by centrifugation at 3000 rpm for 10 min and stored at −80°C until analysis. The uterus, ovaries, testicles, retroperitoneal tissues, and gonadal fat pads were weighed. After dry weights of the tissues were measured, tissues were first fixed for 72 h in a 10% neutral formaldehyde solution for light microscopic examination and paraffin blocks were obtained using routine procedures.

All the female rats studied on the proestrus stage of the estrous cycle and all experimental procedures were carried out in the morning and kept same across groups in order to avoid circadian influence of endocrine profiles.

Hormonal analysis

Serum follicle-stimulating hormone (FSH), luteinising hormone (LH), AMH, testosterone and oestradiol levels were estimated by enzyme-linked immunosorbent assay. FSH, LH and AMH analyses were performed using an ELISA kit (SunRed; SunRed Biotechnology Company, Shangai China) and testosterone and oestradiol levels were determined by an ELISA kit (Oxford; Serotec Ltd, UK). Investigators who were blinded to the sample groups performed all analyses.

Analyses of ovarian and uterine tissues

Ovarian and endometrial sections measuring 5 µm thick were taken from prepared paraffin blocks. Although full-thickness sections were acquired from the ovaries, endometrial tissues were selected for immunohistochemical (IHC) staining when the uterine blocks were prepared. Every one out of four ovarian sections were used for follicular counting and stained

Table 1 Diet of mother rats and offspring in the different study groups.

	Pre-pregnancy (10 weeks)	**Pregnancy** (3 weeks)	**Lactation** (3 weeks)	**Off-spring** (*n* = 6 female + 6 male after lactation (8 weeks))
Group 1 (Dams)				
1a (*n* = 6)	SD	SD	SD	SD
1b (*n* = 6)	SD	CD	CD	SD
Group 2 (Dams)				
2a (*n* = 6)	CD	SD	SD	SD
2b (*n* = 6)	CD	CD	CD	SD

Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI

with haematoxylin and eosin. IHC staining was performed on ovarian sections with anti-PTEN antibodies, whereas endometrial sections were subjected to IHC staining with anti-integrin-β1 and anti-E-cadherin antibodies. For IHC, six random sections were selected for each rat and one central and five peripheral regions were evaluated (36 regions in total for each rat).

Haematoxylin and eosin staining

Sections obtained from the ovaries were maintained in an incubator at 37°C overnight and were then incubated for an additional 1 h at 57°C to facilitate deparaffinisation. After incubating in xylol for 5 min twice, the sections were dehydrated by washing a graded alcohol series (100, 96, and 80%) for 10 min each wash and were then washed with running water twice for 10 min each to complete dehydration. Sections were then incubated in haematoxylin solution for 10 min and washed again under running water for 10 min. Next, sections were dipped 2–3 times in a mixture of 70% alcohol plus three drops of glacial acetic acid, washed in running water for 10 min and stained with eosin solution for 5–10 min. Finally, sections were washed in running water for 10 min, dehydrated through a graded alcohol series (100, 96 and 80%) and washed with xylol for 15 min twice. Images were taken and evaluated on a Leica DCM 4000 computer-aided imaging system (Leica).

Ovarian follicle counting

The sections were stained with haematoxylin and eosin, and follicles were counted based on the following criteria: Primordial follicles were defined as an oocyte surrounded by a layer of squamous (flattened) granulosa cells. Primary follicles possessed an oocyte surrounded by a single layer of cuboidal granulosa cells. Secondary follicles were surrounded by more than one layer of cuboidal granulosa cells, with no visible antrum. Early antral follicles have emerging antral spaces, and antral follicles possessed a clearly defined antral space.

IHC staining for PTEN, integrin-β1 and E-cadherin

Ovarian sections and endometrial sections were incubated at 37°C overnight and then at 57°C for 1 h to facilitate deparaffinisation. After incubation in xylol for 5 min three times, the sections were dehydrated by incubation in a graded ethanol series (100, 96, and 80%) for 3 min each and then washed with distilled water twice for 5 min each. Sections were fixed in EDTA buffer in a microwave at 750W for 10 min to ensure that the receptor sites are uncovered from formaldehyde. After cooling to 25°C, tissues on cross sections were circled with a Pap pen. The tissues were then washed with PBS 3 times for 3 min each followed by treatment with 3% H_2O_2 in order to block the endogenous peroxidase activity. Next, slides that were washed with PBS 3 times were treated with Ultra-V block in order to prevent any unwanted interactions. The tissues were then subjected to either PTEN primary antibody (for ovarian tissues, ab31392, Abcam) or integrin-β1 (for endometrial tissues, bs-04862, lot: AF09128-545; Abcam) and E-cadherin (for endometrial tissues, bs-10009R, lot: 9I02V8; Abcam) at 4°C overnight. After incubation, microscope slides (washed with PBS) were treated with biotinated secondary antibody to achieve primary antibody link. Slides (washed with PBS) were then treated with streptavidin peroxidase for 20 min in order to achieve enzyme and biotin link. Finally, nuclear staining was achieved using 4′,6-diamino-2-phenolindole (DAPI). Haematoxylin was used as background staining, and the slides were closed using Entellan followed by image analysis using Leica DCM 4000 (Germany) computed-aided imaging system.

Evaluation of PTEN, integrin-β1 and E-cadherin expressions in ovarian and endometrial sections

PTEN, integrin-β1 and E-cadherin expressions were scored using an immunoreactive scoring scale and evaluated by two researchers who did not have any prior knowledge of the groups of rats. Accordingly, six zones (one central and five peripheral) were selected from ovarian tissue sections (5 µm thickness) and subjected to IHC staining with anti-PTEN antibodies. The HSCORE, defined below, was used to evaluate the immunoreactive density in these zones. The HSCORE was determined by the following formula: HSCORE ¼ Pi (i + 1), where 'i' is the intensity of labelling with a value of 0, 1, 2 or 3 (none, weak, moderate or strong) and Pi is the percentage of labelled cells for each intensity, within a range of 0–100%. The rate of positive cells was scored by the extent of immunostaining and was assigned to one of the following categories: 0 (0%, no positive cells), 1 (≤30% positive cells), 2 (30–60% positive cells) and 3 (>60% positive cells).

Analyses of testicles and sperm

Both the testes were weighed after removal of adipose tissue and dissected using blades and tweezers in 4 mL MOPS-buffered cell culture medium (G-MOPS; Vitorlife, Denmark) without oil overlay. Subsequently, an aliquot of the resulting suspension was used for quantifying the sperm parameters (i.e., concentration, motility and progressive motility) using Makler Counting Chambers (10 µm depth; Sefi Medical Instruments). The term 'total motility' was used to define spermatozoa expressing any type of sperm motility. The term 'progressive motility' was used to define spermatozoa that are swimming in a linear pattern with fast forward type of motility. All motility measurements were performed at room temperature (25°C) by repeating the measurement of each sample twice.

All procedures that involved the use of animals were approved by the Gazi University Animal Experiments Local Ethics Committee (approval no: G.U: ET 15.064; Turkey).

Statistical analysis

All statistical analyses were performed using SPSS for Windows 11.5 (SPSS Inc.). Compatibility of data with the normal distribution was examined graphically and with the Kolmogorov–Smirnov test. For quantitative variables, means ± standard deviations (S.D.s) and medians (minimum–maximum) were used, whereas numbers (percentages) were used for categorical variables. For determination of statistically significant differences between categories of

Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI

a qualitative variable with two categories, Student's *t*-tests were used if the normal distribution assumption was met; if not, Mann–Whitney *U* tests were used. For determination of statistically significant differences between categories of a qualitative variable with more than categories, one-way ANOVA was used if the normal distribution assumption was met; if not, Kruskal–Wallis tests were used. In cases in which the normal distribution assumptions were provided by both variables, Pearson's correlation coefficient was used; however, where at least one variable did not provide this assumption, Spearman's correlation coefficient was used to evaluate the relationships between two qualitative variables. For evaluation of the relationship between two quantitative dependent variables, paired *t*-tests were used if the assumption of normal distribution was provided, and the Wilcoxon sign-ranks test was used in cases in which the assumptions were not provided. Linear regression tests were used to examine the effects of independent variables on quantitative dependent variables. Differences with *P* values of less than 0.05 were considered statistically significant.

Power analysis

When the mean ± standard deviation values for the group variable categories 1A, 1B, 2A, 2B were found to be 55.62 ± 9.35, 47.17 ± 7.48, 15.62 ± 5.92, 12.67 ± 4.56 for the promodial follicle variable, the sample size of 96 rats at the significance level of 0.05 with using one-way ANOVA test for power analysis, the power of the study was found to be 0.99.

Results

Findings in dams and neonatal offspring

At the beginning of the study, no statistically significant differences were found between the initial weights of the dams in the groups ($P=0.644$). A statistically significant difference was found between the group variables category (1A, 1B, 2A, 2B) for the weight gain of the mother rats ($P=0.014$). These double-subgroups were examined with Mann–Whitney *U* tests, and the differences between groups 1B and 2A were statistically significant ($P=0.008$). The means ± S.D.s values of groups 1A, 1B, 2A and 2B were 95.83 ± 8.09, 123.33 ± 17.84, 83.33 ± 12.57 and 91.17 ± 12.22 respectively.

No statistically significant differences in the number of newborn offspring were found between groups ($P=0.769$). The means ± S.D.s values of the number of offspring in groups 1A, 1B, 2A and 2B were 10.20 ± 2.59, 7.80 ± 5.81, 7.00 ± 4.36 and 9.40 ± 5.55 respectively.

Statistically significant differences in weights of the newborn offspring were found between groups ($P<0.001$). These double-subgroups were examined with Mann–Whitney *U* tests, and the differences between groups 1A and 2A ($P<0.001$), groups 1A and 1B ($P<0.001$), groups 2A and 1B ($P<0.001$), groups 2A and 2B ($P=0.001$) and groups 1B and 2B ($P<0.001$) were found to be statistically significant. The means ± S.D.s values of the weights of offspring in groups 1A, 1B, 2A and 2B were 5.76 ± 0.64, 6.68 ± 0.23, 4.93 ± 0.61 and 5.95 ± 1.58 respectively.

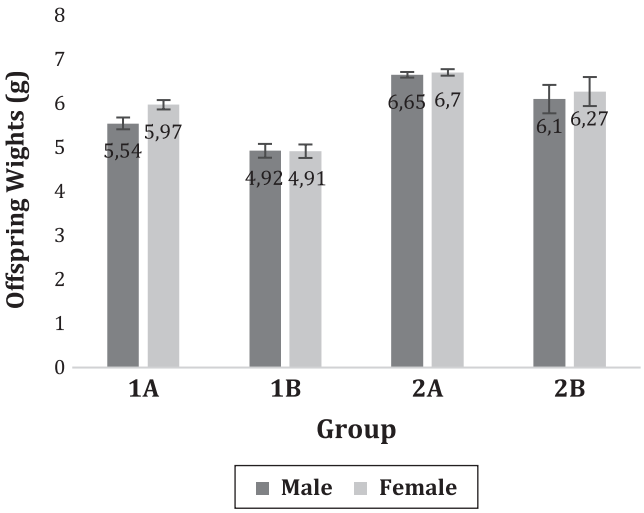


Figure 1 Newborn offspring weights, according to the groups, separated by sex.

Newborn offspring weights included this study were shown in Fig. 1 graphically separated by sex (Fig. 1). There was no statistically significant relationship between the sex of the offspring (male, female) and the group (1A, 1B, 2A, 2B) variable ($P=0.809$). Male and female numbers were 25 (49.0%) and 26 (51.0%) in 1A group, 8 (38.1%) and 13 (61.9%) in 1B group, 17 (50.0%) and 17 (50.0%) in 2A group, 21 (50.0%) and 21 (50.0%) respectively.

Findings in adult female offspring

Weights of the female offspring in the adult period; measurements of ovarian, retroperitoneal and gonadal adipose tissues and hormonal statuses are shown in Table 2.

Ovarian results

The numbers of primordial follicles and antral follicle were shown in Fig. 2. No statistically significant differences in the number of antral follicles were found between groups ($P=0.691$). Significant differences in the number of primordial follicles were found between groups ($P<0.001$). These double-subgroups were examined with Mann–Whitney *U* tests, and the differences between groups 1A and 2A ($P<0.001$), groups 1A and 2B ($P<0.001$), groups 2A and 1B ($P<0.001$) and groups 1B and 2B ($P<0.001$) were statistically significant.

The results of the IHC staining of the ovarian tissue with PTEN primary antibody are shown in Fig. 3. PTEN immunoreactivity was observed in the oocytes, granulosa cells and theca cells of the follicles at every

Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI

Table 2 Comparison of weight, gonadal weight, gonadal and retroperitoneal adipose tissues and hormones in female offspring at 11th weeks.

	Female 1A ($n=6$)		Female 1B ($n=6$)		Female 2A ($n=6$)		Female 2B ($n=6$)		*P* value
	Mean ± S.D.	Median (min–max)	Mean ± S.D.	Median (min–max)	Mean ± S.D.	Median (min–max)	Mean ± S.D.	Median (min–max)	
Total body weight (g)	183.05 ± 9.56	180.80 (172.00–198.00)	174.08 ± 12.89	173.50 (156.00–191.00)	189.50 ± 6.41	189.50 (181.00–198.00)	189.65 ± 13.02	188.50 (177.00–213.00)	0.069[a]
Ovarium (g)	0.07 ± 0.02	0.07 (0.04–0.09)	0.08 ± 0.02	0.08 (0.05–0.10)	0.07 ± 0.01	0.07 (0.05–0.08)	0.06 ± 0.01	0.06 (0.05–0.10)	0.359[a]
Ovarium/total body weight (%)	0.04 ± 0.01	0.04 (0.02–0.05)	0.04 ± 0.01	0.04 (0.03–0.06)	0.03 ± 0.01	0.03 (0.03–0.04)	0.03 ± 0.01	0.03 (0.03–0.04)	0.107[a]
Uterus (g)	13.04 ± 1.12	12.06 (11.20–13.64)	12.78 ± 2.12	12.84 (11.90–14.22)	14.25 ± 2.14	14.04 (12.60–14.64)	13.68 ± 3.12	14.46 (11.20–13.64)	0.685[a]
Uterus/weight	0.07 ± 0.01	0.07 (0.07–0.08)	0.07 ± 0.01	0.08 (0.06–0.08)	0.08 ± 0.01	0.07 (0.07–0.08)	0.07 ± 0.01	0.07 (0.06–0.08)	0.732[a]
Retroperitoneal adipose tissue (g)	0.86 ± 0.34	0.81 (0.52–1.48)	0.45 ± 0.14	0.41 (0.26–0.67)	0.77 ± 0.12	0.73 (0.68–0.98)	1.47 ± 0.85	1.02 (0.79–2.75)	0.002[b]
Retroperitoneal adipose tissue/total body weight (%)	0.47 ± 0.17	0.44 (0.30–0.78)	0.26 ± 0.09	0.23 (0.16–0.40)	0.41 ± 0.07	0.39 (0.35–0.54)	0.77 ± 0.44	0.53 (0.45–1.43)	0.003[b]
Gonadal adipose tissue (g)	1.32 ± 0.26	1.29 (0.97–1.68)	0.79 ± 0.22	0.80 (0.56–1.00)	1.23 ± 0.20	1.22 (1.01–1.52)	2.31 ± 0.50	2.41 (1.47–2.96)	0.001[b]
Gonadal adipose tissue/total body weight (%)	0.73 ± 0.17	0.72 (0.49–0.95)	0.45 ± 0.12	0.45 (0.33–0.60)	0.65 ± 0.10	0.66 (0.52–0.77)	1.22 ± 0.29	1.31 (0.77–1.58)	<0.001[a]
LH (mIU/mL)	0.31 ± 0.07	0.31 (0.20–0.42)	0.31 ± 0.07	0.31 (0.23–0.39)	0.31 ± 0.07	0.33 (0.20–0.38)	0.26 ± 0.08	0.25 (0.14–0.36)	0.561[a]
FSH (mIU/mL)	5.67 ± 0.37	5.65 (5.23–6.12)	7.13 ± 1.05	6.90 (6.23–9.18)	8.35 ± 0.83	8.35 (7.57–9.18)	6.83 ± 0.83	6.68 (5.73–8.23)	0.001[b]
AMH (ng/mL)	4.40 ± 0.44	4.35 (3.99–5.16)	4.87 ± 0.64	4.85 (3.97–5.83)	4.09 ± 0.78	4.29 (2.78–4.82)	4.05 ± 1.36	3.69 (2.41–5.71)	0.358[a]
Oestradiol (pmol/L)	62.20 ± 31.87	58.80 (32.90–99.50)	137.93 ± 27.50	131.00 (116.90–190.70)	63.17 ± 37.42	65.35 (22.10–107.80)	148.17 ± 48.87	147.05 (91.90–198.60)	0.001[b]
Testosterone (ng/mL)	2.65 ± 1.05	2.65 (1.48–3.83)	3.44 ± 0.62	3.45 (2.59–4.17)	4.27 ± 0.77	3.96 (3.63–5.65)	0.61 ± 0.24	0.64 (0.26–0.90)	<0.001[a]

[a]One-way ANOVA, [b]Kruskal–Wallis test.

Figure 2 Number of primordial follicles and antral follicles according to the groups.

stage of follicular development. As the follicles grew, PTEN expression increased in granulosa cells, but decreased in the oocyte cytoplasm. Compared with groups 1A and 1B, PTEN expression was significantly decreased in the oocyte cytoplasm in groups 2A and 2B. This finding was consistent with the decreasing number

Figure 3 Results of IHC staining with PTEN primary antibody of ovarian tissues. Primordial follicles (red arrows), primary follicle (PF), secondary follicle (SF), early antral follicle (EAF), antral follicle (AF), antrum (A), oocyte cytoplasm (white arrows), theca cells (black arrows), corpus luteum (CL).

Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI

of primordial follicles in groups 2A and 2B (Table 3). Additionally, there were no significant differences in PTEN expression in the oocyte cytoplasm when groups 1A and 1B were compared ($P=0.512$). Statistically significant differences were observed between groups 1A and 2A ($P<0.001$), groups 1A and 2B ($P<0.001$), groups 1B and 2A ($P<0.001$) and groups 1B and 2B ($P<0.001$) when subgroups were compared in pairs for PTEN staining.

Moreover, there was a statistically significant correlation between the amount of PTEN staining in the oocyte cytoplasm and the number of primordial follicles ($P<0.001$, $r=0.759$). This relationship was not observed between PTEN and the number of antral follicles ($P=0.847$, $r=0.042$).

Primordial follicle number was used as a dependent variable, and group (1A, 1B, 2A and 2B) was used as the independent variable. When linear regression analysis was performed, the model was found to be statistically significant ($P<0.001$). The group variable corresponded to 79.5% of the change in the number of primordial follicles, and the model was calculated as the number of primordial follicles = 72.875 + (−16.042) × group. Thus, the number of primordial follicles decreased by 16.042 units as the group number changed.

Next, PTEN staining was used as a dependent variable, and group (1A, 1B, 2A and 2B) was used as the independent variable. When linear regression analysis was performed, and the model was found to be statistically significant ($P<0.001$). The group variable corresponded to 54.9% of the change in PTEN, and the model was calculated as PTEN = 3.333 + (−0.514) × group. Thus, the PTEN staining value decreased by 0.514 units as the group number changed.

Endometrial results

The results of IHC staining of endometrial tissues with anti-integrin-β1 and anti-E-cadherin antibodies are shown in Figs 4 and 5. The expression levels of the endometrial receptor markers E-cadherin and integrin-β1 were significantly decrease in groups 2A and 2B compared with those in groups 1A and 1B (Table 3). Additionally, no significant differences in E-cadherin and integrin-β1 expression were observed in the comparison of groups 1A and 1B ($P=0.358$, $P=0.528$, respectively).

Statistically significant differences in E-cadherin staining were observed between groups 1A and 2A ($P<0.001$), groups 1A and 2B ($P<0.001$), groups 1B and 2A ($P<0.001$) and groups 1B and 2B ($P<0.001$) when subgroups were compared in pairs. Moreover, significant differences in integrin-β1 staining were observed between groups 1A and 2A ($P<0.001$), groups 1A and 2B ($P<0.001$), groups 1B and 2A ($P<0.001$) and groups 1B and 2B ($P<0.001$).

There was a positive moderate correlation between the results of E-cadherin and integrin-β1 staining in all groups, and this relationship was statistically significant ($P<0.001$, $r=0.655$). In the evaluation of the relationship between E-cadherin and integrin-β1, a statistically significant positive correlation was found in group 1A ($P=0.032$, $r=0.358$). No statistically significant differences were observed between the staining results of E-cadherin and integrin-β1 in groups 1B, 2A and 2B ($P=0.062$, $r=0.314$; $P=0.201$, $r=0.218$; and $P=1.000$, $r=0.000$, respectively).

Next, E-cadherin was taken as the dependent variable, and group (1A, 1B, 2A and 2B) was taken as the independent variable. When linear regression analysis was performed, the model was found to be statistically significant ($P<0.001$). The group variable corresponded to 56.1% of the change in E-cadherin, and the model was calculated as E-cadherin = 3.292 + (−0.508) × group. Notably, as the groups were changed (in the order of 1A, 1B, 2A, 2B), E-cadherin decreased by 0.508 units.

Finally, integrin-β1 was set as the dependent variable, and group (1A, 1B, 2A, 2B) was set as the independent variable. When linear regression analysis was performed, the model was found to be statistically significant ($P<0.001$). The group variable corresponded to 52.4% of the change in integrin-β1, and the model was calculated as integrin-β1 = 3.264 + (−0.497) × group. We found that as the groups were changed (in the order of 1A, 1B, 2A, 2B), integrin-β1 decreased by 0.497 units.

Findings in adult male offspring

Weights of the adult male offspring; measurements of testicles, retroperitoneal tissues and gonadal adipose tissues and hormonal statuses are shown in Table 4.

Sperm count, progressive motility and total motilities were shown in Fig. 6 according to the groups. No statistically significant differences in sperm count were found between groups ($P=0.072$). There were no statistically significant differences in total motility and progressive motility of sperm were found between groups ($P=0.853$ and $P=0.162$ respectively). No statistically significant differences in progressively motile sperm count (PMSC) variable were found between groups ($P=0.068$).

Discussion

This study was carried out to investigate whether maternal feeding affected the fertility potential of adult offspring. Compared with SD-fed dams in pregestational, gestational and lactation periods, the number of primordial follicles showing a pool of reserve follicles in the ovary and the level of PTEN expression were decreased in adult female offspring in CD-fed dams. Similarly, E-cadherin and integrin-β1 expression levels were decreased in endometrial tissues. In male offspring, AMH values increased, testosterone levels decreased and sperm number and function were not changed.

Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI

Table 3 Comparison of PTEN staining results in ovarian tissues and IHC staining with integrin and cadherin in endometrial tissues.

	1A ($n=36$)		1B ($n=36$)		2A ($n=36$)		2B ($n=36$)		
	Mean ± S.D.	Median (min–max)	Mean ± S.D.	Median (min–max)	Mean ± S.D.	Median (min–max)	Mean ± S.D.	Median (min–max)	
E-cadherin staining	2.75 ± 0.44	3.00 (2.00–3.00)	2.42 ± 0.50	2.00 (2.00–3.00)	1.58 ± 0.55	2.00 (1.00–3.00)	1.33 ± 0.48	1.00 (1.00–2.00)	<0.001[b]
Integrin β1 staining	2.72 ± 0.45	3.00 (2.00–3.00)	2.42 ± 0.50	2.00 (2.00–3.00)	1.61 ± 0.65	2.00 (1.00–3.00)	1.33 ± 0.48	1.00 (1.00–2.00)	<0.001[b]
PTEN staining	2.78 ± 0.42	3.00 (2.00–3.00)	2.47 ± 0.51	2.00 (2.00–3.00)	1.58 ± 0.60	2.00 (1.00–3.00)	1.36 ± 0.49	1.00 (1.00–2.00)	<0.001[b]

[a]One-way ANOVA, [b]Kruskal–Wallis test.

Dams and neonatal offspring

There were no differences in the number and weight of the offspring between SD-fed and CD-fed dams in our study. Few previous studies have evaluated the effects of feeding during the pregestational period and during pregnancy on the number of offspring in rodents. In studies of newborn weight, diet-induced maternal overweight has been shown to increase foetal growth (Gaccioli *et al*. 2013, Sferruzzi-Perri *et al*. 2013). Conversely, Desai *et al*. (2014) reported that maternal HFD has no effect on the weight of the offspring (Desai *et al*. 2014).

Adult female offspring

In our study, maternal CD feeding was found to increase the retroperitoneal and gonadal adipose tissues in female offspring. The differences between groups were related to feeding of the CD during the pregestational period, not during pregnancy. In previous studies, obesogenic nutrition during the periconceptional period, pregnancy and lactation have been reported to increase offspring adipose tissue; however, there are no studies comparing feeding during the pregestational period and during pregnancy (Samuelsson *et al*. 2008, Howie *et al*. 2009, Lane *et al*. 2015). Analysis of the differences in FSH, oestradiol and testosterone levels between groups has shown that there is deterioration in the steroidogenesis balance in the CD-fed group. There were no differences in groups in terms of AMH levels, as expected based on the observation that the number of antral follicles showing functional ovarian reserve did not differ between groups.

Ovaries

In rat studies, the negative effects of maternal feeding abnormalities on offspring ovarian reserve have been reported both maternal undernutrition and diet-induced maternal obesity (Bernal *et al*. 2010, Cheong *et al*. 2014). The results of our study revealed that maternal feeding of rats in pregestational, pregnancy and lactation periods did not affect the number of antral follicles in the ovaries of adult offspring. However, the number of primordial follicles was altered. The number of primordial follicles is determined in foetal life and forms the reserve follicle pool, whereas the number of antral follicles indicates the functional pool of follicles during maturity (Pepling *et al*. 2006, Pankhurst 2017). Accordingly, it is reasonable that the number of primordial follicles is affected by changes in foetal life, as described in the literature (Skinner *et al*. 2005, Maheshwari & Fowler 2008). However, the distribution of the decrease in the number of primordial follicles was remarkable in our study. Specifically, the number of primordial follicles was highest in the offspring of

Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI

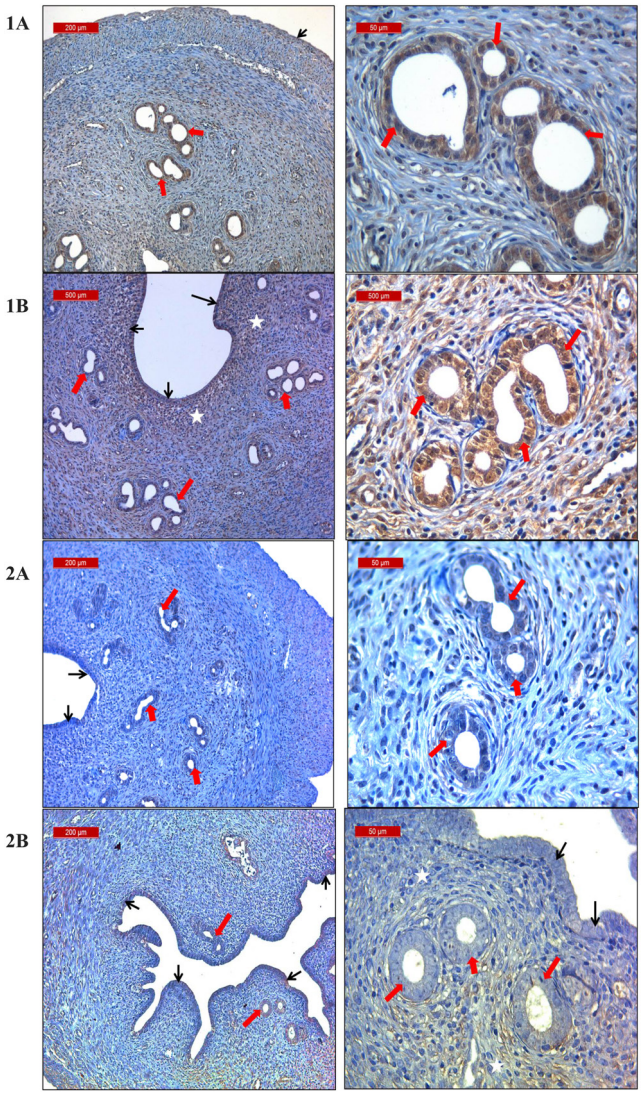


Figure 4 Results of IHC staining with integrin-β1 primary antibody of endometrium tissues. The endometrial glandular epithelium is demonstrated with red arrows, the surface epithelium is shown with black arrows, and stromal cells are shown with white stars.

dams fed an SD throughout the study. Additionally, the number of primordial follicles were reduced in the group fed with SD during the pregestational and with CD during pregnancy and lactation periods. The group fed with CD during pregestational period and SD during pregnancy and lactation showed lower number of primordial follicles than the aforementioned group. The lowest number of primordial follicles was observed to be on the group that fed always with CD during all the periods. This indicates that group fed with CD during the pregestational period exhibited less ovarian reserve even if fed with normal diet during the pregnancy and lactation periods.

PTEN is a potential protector of the ovarian reserve (Reddy *et al*. 2008, Ernst *et al*. 2017). Premature activation occurs in the whole primordial follicle pool in the case of PTEN deficiency because PTEN controls follicle activation through the FOXO3A signalling pathway (Reddy *et al*. 2008). This causes premature ovarian follicular depletion and premature ovarian insufficiency (Castrillon *et al*. 2003). In our study, a relationship was also observed between the primordial follicle number and PTEN staining in the oocyte cytoplasm when PTEN immunoreactivity results of the ovary tissues were evaluated, in accordance with previous studies (Castrillon *et al*. 2003, Reddy *et al*. 2008, 2010). As in primordial follicle counting, PTEN was observed to be the highest in rats fed normally during the pregestational period and pregnancy and the lowest in rats fed a CD during the pregestational period and the pregnancy.

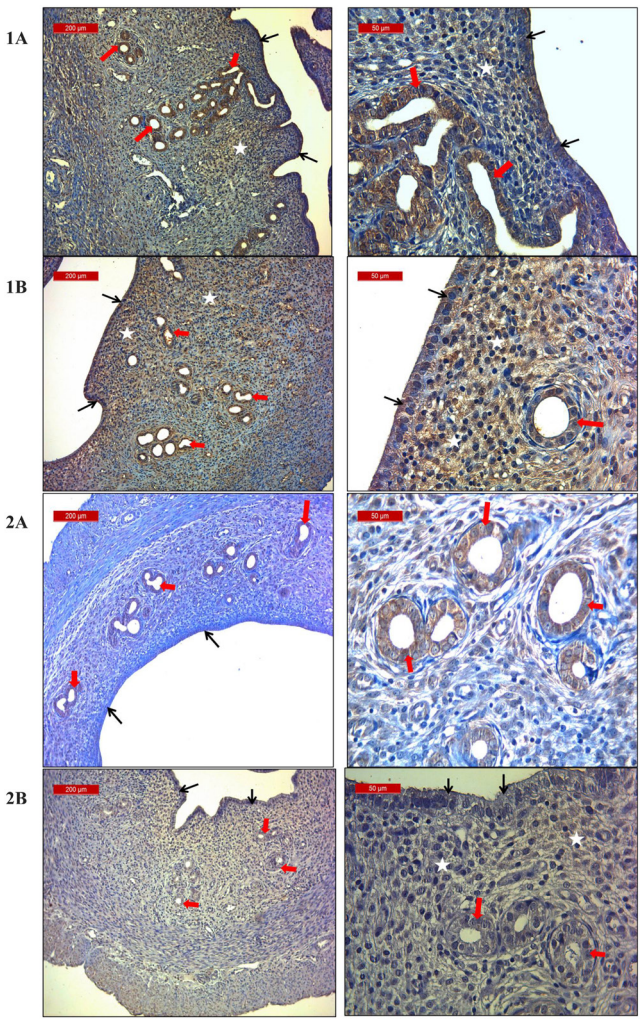


Figure 5 Results of IHC staining with E-cadherin primary antibody of endometrium tissues. The endometrial glandular epithelium is demonstrated with red arrows, surface epithelium is shown with black arrows, and stromal cells are shown with white stars.

Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI

Table 4 Comparison of weight, gonadal and retroperitoneal adipose tissues and hormones of male offspring at 11th weeks.

	Male 1A ($n=6$)		Male 1B ($n=6$)		Male 2A ($n=6$)		Male 2B ($n=6$)		*P* value
	Mean ± S.D.	Median (min–max)	Mean ± S.D.	Median (min–max)	Mean ± S.D.	Median (min–max)	Mean ± S.D.	Median (min–max)	
Total body weight (g)	211.67 ± 9.58	209.00 (202.00–230.00)	209.67 ± 21.62	209.00 (180.00–241.00)	214.17 ± 10.15	212.50 (203.00–232.00)	214.17 ± 21.54	213.50 (178.00–241.00)	0.958[a]
Testicle weight (g)	1.65 ± 0.11	1.64 (1.53–1.81)	1.25 ± 0.08	1.27 (1.12–1.33)	1.84 ± 0.11	1.85 (1.69–1.96)	1.32 ± 0.12	1.35 (1.14–1.46)	<0.001[a]
Testicle/total body weight (%)	0.78 ± 0.01	0.77 (0.73–0.86)	0.60 ± 0.05	0.60 (0.52–0.66)	0.86 ± 0.05	0.87 (0.80–0.92)	0.62 ± 0.04	0.61 (0.58–0.68)	<0.001[a]
Ret. adipose tissue (g)	0.49 ± 0.19	0.49 (0.18–0.71)	0.83 ± 0.16	0.88 (0.58–1.00)	0.83 ± 0.38	0.76 (0.42–1.42)	1.18 ± 0.49	1.11 (0.63–1.79)	0.015[a]
Ret. adipose tissue/ total body weight (%)	0.39 ± 0.07	0.40 (0.28–0.47)	0.23 ± 0.07	0.24 (0.10–0.30)	0.38 ± 0.16	0.37 (0.20–0.61)	0.54 ± 0.18	0.52 (0.35–0.78)	0.005[a]
Gonadal adipose tissue (g)	1.43 ± 0.12	1.39 (1.32–1.58)	1.79 ± 0.28	1.73 (1.54–2.33)	1.78 ± 0.31	1.90 (1.27–2.05)	1.90 ± 0.33	1.93 (1.41–2.26)	0.042[a]
Gonadal adipose tissue/ total body weight (%)	0.84 ± 0.09	0.83 (0.74–1.01)	0.69 ± 0.01	0.70 (0.55–0.79)	0.84 ± 0.17	0.91 (0.55–0.99)	0.90 ± 0.21	0.90 (0.65–1.27)	0.144[a]
LH (mIU/mL)	0.18 ± 0.02	0.17 (0.16–0.20)	0.27 ± 0.08	0.23 (0.20–0.37)	0.25 ± 0.06	0.25 (0.18–0.34)	0.31 ± 0.09	0.29 (0.23–0.46)	0.006[b]
FSH (mIU/mL)	2.27 ± 1.78	1.76 (0.51–4.62)	5.79 ± 1.56	5.76 (3.84–7.68)	4.50 ± 2.45	4.96 (0.79–7.18)	6.03 ± 1.24	5.82 (4.46–8.12)	0.007[a]
AMH (ng/mL)	1.42 ± 0.27	1.41 (1.12–1.76)	3.45 ± 0.50	3.31 (2.94–4.18)	4.15 ± 1.10	4.43 (2.80–5.34)	4.59 ± 0.18	4.54 (4.41–4.89)	<0.001[a]
Testosterone (ng/mL)	4.02 ± 2.58	3.00 (1.75–8.67)	3.70 ± 1.14	3.33 (2.63–5.56)	3.44 ± 1.35	3.56 (1.84–5.10)	0.42 ± 0.30	0.38 (0.06–0.88)	0.002[a]

[a]One-way ANOVA, [b]Kruskal–Wallis test. Ret., retroperitoneal

Endometrium

Interstitial and extracellular matrix interactions occur during embryonic implantation through cell-surface adhesion molecules. Well-known surface adhesion molecules E-cadherin and integrin-β1 were selected as endometrial receptivity markers in our study. In the literature, both of these molecules have been evaluated in the process of implantation in mice (Larue *et al*. 1994, Riethmacher *et al*. 1995, Brakebusch *et al*. 1997, Basak *et al*. 2002).

In our study, IHC staining of E-cadherin and integrin-β1 in endometrial tissues showed that both molecules showed similar expression, consistent with PTEN staining in the ovary. Expression levels in endometrial tissues were highest in the offspring of dams fed a SD throughout the study; moderate in the group fed an SD during the pregestational period and a CD during pregnancy and lactation; low in the group fed a CD during the pregestational period and a SD during pregnancy and lactation and lowest in the group always fed a CD during all periods. A positive correlation was found between E-cadherin and integrin-β1 in rats fed a SD throughout the study; however, this relationship was not observed in the other groups.

There are studies on maternal nutrition and offspring's reproductive function in the literature (Chavatte-Palmer *et al*. 2014, Rodríguez-González *et al*. 2014, Aiken *et al*. 2016). While gonadal functions were evaluated in these studies, endometrial receptivity was not analysed.

To our knowledge, this is the first study investigating the relationship between offspring endometrial receptivity and maternal nutrition.

Adult male offspring

In the literature, maternal obesity before and during pregnancy has been reported to increase offspring obesity risk (O'reilly & Reynolds 2013). In our study, the weights of adult male offspring were not changed in different maternal feeding groups. Increased retroperitoneal and gonadal adipose tissues in rats fed a CD were consistent with the literature (Drake & Reynolds 2010, Alfaradhi & Ozanne 2011).

Testis weights of male offspring were lower in groups fed a CD during pregnancy. Feeding the mother CD during the pregestational period did not affect testicular weight. However, sufficient data to support these findings could not be obtained from the literature. Some studies on maternal obesity and male offspring reproduction have been performed; however, these studies primarily focused on the semen parameters and sperm quality rather than testicular properties coupled with semen analysis and sperm quality. (Ramlau-Hansen *et al*. 2007, Rodríguez-González *et al*. 2014).

Testosterone, oestrogen and LH are important components of male sexual development and fertility.

Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI

Decreased testosterone levels in male animals and increased oestrogen and LH levels have been shown to have negative effects on sexual maturity, fertility and reproductive ageing (Chen *et al*. 2002, Mann & Lutwak-Mann 2012). In our study, LH and FSH levels increased, and testosterone levels decreased in male offspring rats in groups fed a CD during the pregestational period and pregnancy. Oestrogen levels were not examined. The results obtained in this study showed the negative effects of maternal obesogenic feeding on the hormonal profile of adult male offspring.

In our study, AMH levels increased in the CD groups. In males, AMH is prenatally and postnatally produced by Sertoli cells (Sharpe *et al*. 2003) and involved in foetal development and male and female sexual differentiation (Munsterberg & Lovell-Badge 1991). AMH regulates Leydig cell androgen steroidogenesis in testes (Laurich *et al*. 2002). The serum levels of AMH remain elevated in pre-puberty but decreases rapidly in transition to adulthood. It has been suggested that AMH levels has role in male type behaviour and cognitive development of the male offspring (Pankhurst & McLennan 2012, Wittmann & McLennan 2013, Morgan *et al*. 2017). Based on these, the implications of findings in our study merits further discussion. In our study, testosterone levels decreased in groups with increased AMH, suggesting that our rats in the CD-fed group still maintained high AMH and low testosterone profiles during the prepubertal period. Although endocrine maturation may be delayed in these groups. We did not examine whether high AMH levels caused behavioural changes in rats in this study.

Sperm analysis

According to the literature, maternal obesity in pregnancy and lactation increase oxidative stress in the testicles and sperm of male offspring and cause premature ageing in reproductive capacity (Rodríguez-González *et al*. 2014, Bautista *et al*. 2017). Similar results were obtained for paternal obesity (Fullston *et al*. 2015). In our study, groups did not exhibit any differences in terms of the number and motility of sperm, in contrast to the results of previous studies in the literature. Apparently, male gamete counts and properties did not show drastic changes across the groups when compared with the gamete properties of the female offspring. Therefore, we speculate that defects caused during the pre-natal period on the male offspring can be compensated during the post-natal life. This is due to the fact that sperm cells are produced in the post-natal life, whereas, follicles can not be produced during post-natal life. The reason for the inconsistencies between previous research and our study results may be related to the lack of statistical significance due to the inclusion of few rats in each group. Thus, it is clear that further studies with more number of rats are needed; however, in this study, we were limited by the rules established by the ethical committee of our institution.

In this study, the effects of maternal feeding during the pregestation, pregnancy and lactation periods on the reproductive capacity of both female and male adult offspring were described for the first time. The CD, which was chosen as an example of obesogenic nutrition, is consistent with the reality of everyday life. Primordial oocytes and PTEN in the oocyte cytoplasm were examined to evaluate ovarian function, and E-cadherin and integrin-β1 levels were examined in endometrial tissues for the evaluation of endometrial receptivity in female offspring. As a limitation, similarly, testicular function could be better studied if tissue examination of Sertoli cell function was performed in testicular tissues. We did not investigate if the decreased testosterone and increased AMH levels cause negative effects on male sexual behaviour. This is an important point for future studies in the near future.

In summary, this study showed that maternal feeding with a CD during pregestation, pregnancy, and lactation periods, had negative effects on the reproductive ability of adult offspring. In females, the oocyte pool is developed in intrauterine life and plays a lifelong role in the female reproductive system. Whereas spermatozoa production in males is a process that starts after puberty continues throughout life. Consistent with this, primordial follicle count and endometrial receptivity of female offspring were decreased, although the number of antral follicles and AMH levels were not changed. In male offspring, the testicles were smaller, gonadal adipose tissues increased, testosterone production decreased, and AMH increased. However, the number and function of sperm were not changed. Further studies are needed to investigate whether the decrease in testosterone level and increase in AMH level led to changes in male behaviour patterns.

In conclusion; this is the first study in the literature investigating the effects of unhealthy nutrition on the reproductive functions of both male and female rats. All negative effects on reproductive functions were more apparent in groups fed with the CD during the pregestational period. Although dams fed with the CD during the pregestational period were fed with a SD during pregnancy, the negative effects on offspring reproductive

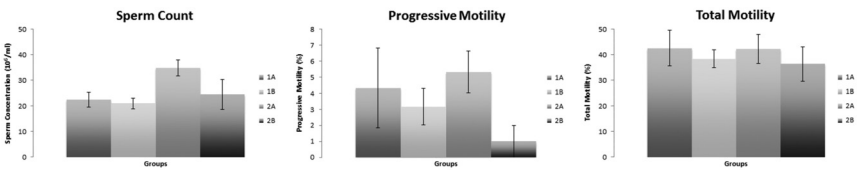


Figure 6 Sperm counts, progressive motility and total motility data according to the groups.

Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI

phenotype were maintained. Thus, consumption of unhealthy foods during the preconceptional period can negatively influence the reproductive system of the adult offspring, at least as much as unhealthy feeding during pregnancy and lactation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Ethics committee approval

Ethics committee approval was received for this study from the ethics committee of Gazi University School of Medicine (No: 15.064).

Author contribution statement

Ziya Kalem, Muberra Namlı Kalem, Elvan Anadol, Canan Yılmaz, Çigdem Elmas, Perihan Yalcinkaya, Halil Ruso, Batuhan Bakirarar, Timur Gurgan. Concept – Z K, M N K, E A; Design – M N K, Z K, E A; Supervision – T G; Materials – E A, C Y, C E, P Y, B B, H R; Writer – Z K, M N K.

References

Aiken CE, Tarry-Adkins JL, Penfold NC, Dearden L & Ozanne SE 2016 Decreased ovarian reserve, dysregulation of mitochondrial biogenesis, and increased lipid peroxidation in female mouse offspring exposed to an obesogenic maternal diet. *FASEB Journal* **30** 1548–1556. (https://doi.org/10.1096/fj.15-280800)

Alfaradhi MZ & Ozanne SE 2011 Developmental programming in response to maternal overnutrition. *Frontiers in Genetics* **2** 1–13.

Barker DJ 1995 Fetal origins of coronary heart disease. *BMJ* **311** 171–174. (https://doi.org/10.1136/bmj.311.6998.171)

Basak S, Dhar R & Das C 2002 Steroids modulate the expression of alpha4 integrin in mouse blastocysts and uterus during implantation. *Biology and Reproduction* **66** 1784–1789.

Bautista CJ, Rodríguez-González GL, Morales A, Lomas-Soria C, Cruz-Pérez F, Reyes-Castro LA & Zambrano E 2017 Maternal obesity in the rat impairs male offspring aging of the testicular antioxidant defence system. *Reproduction, Fertility and Development* **30** 75–81.

Bernal AB, Vickers MH, Hampton MB, Poynton RA & Sloboda DM 2010 Maternal undernutrition significantly impacts ovarian follicle number and increases ovarian oxidative stress in adult rat offspring. *PLoS ONE* **5** e15558. (https://doi.org/10.1371/journal.pone.0015558)

Brakebusch C, Hirsch E, Potocnik A & Fassler R 1997 Genetic analysis of beta1 integrin function: confirmed, new and revised roles for a crucial family of cell adhesion molecules. *Journal of Cellular Science* **110** 2895–2904.

Castrillon DH, Miao L, Kollipara R, Horner JW & DePinho RA 2003 Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science* **301** 215–218. (https://doi.org/10.1126/science.1086336)

Chan KA, Tsoulis MW & Sloboda DM 2015 Early-life nutritional effects on the female reproductive system. *Journal of Endocrinology* **224** 45–62 (https://doi.org/10.1530/JOE-14-0469)

Chavatte-Palmer P, Dupont C, Debus N & Camous S 2014 Nutritional programming and the reproductive function of the offspring. *Animal Production Science* **54** 1166–1176.

Chen H, Hardy MP & Zirkin BR 2002 Age-related decreases in Leydig cell testosterone production are not restored by exposure to LH in vitro. *Endocrinology* **143** 1637–1642. (https://doi.org/10.1210/endo.143.5.8802)

Cheong Y, Sadek KH, Bruce KD, Macklon N & Cagampang FR 2014 Diet-induced maternal obesity alters ovarian morphology and gene expression in the adult mouse offspring. *Fertility and Sterility* **102** 899–907. (https://doi.org/10.1016/j.fertnstert.2014.06.015)

Connor KL, Vickers MH, Beltrand J, Meaney MJ & Sloboda DM 2012 Nature, nurture or nutrition? Impact of maternal nutrition on maternal care, offspring development and reproductive function. *Journal of Physiology* **590** 2167–2180. (https://doi.org/10.1113/jphysiol.2011.223305)

Desai M, Jellyman JK, Han G, Beall M, Lane RH & Ross MG 2014 Maternal obesity and high-fat diet program offspring metabolic syndrome. *American Journal of Obstetrics and Gynecology* **211** 237.e1.

Drake AJ & Reynolds RM 2010 Impact of maternal obesity on offspring obesity and cardiometabolic disease risk. *Reproduction* **140** 387–398. (https://doi.org/10.1530/REP-10-0077)

Dupont C, Cordier AG, Junien C, Mandon-Pepin B, Levy R & Chavatte-Palmer P 2012 Maternal environment and the reproductive function of the offspring. *Theriogenology* **78** 1405–1414. (https://doi.org/10.1016/j.theriogenology.2012.06.016)

Ernst EH, Grøndahl ML, Grund S, Hardy K, Heuck A, Sunde L, Franks S, Andersen CY, Villesen P & Lykke-Hartmann K 2017 Dormancy and activation of human oocytes from primordial and primary follicles: molecular clues to oocyte regulation. *Human Reproduction* **32** 1684–1700. (https://doi.org/10.1093/humrep/dex238)

Fullston T, McPherson NO, Owens JA, Kang WX, Sandeman LY & Lane M 2015 Paternal obesity induces metabolic and sperm disturbances in male offspring that are exacerbated by their exposure to an 'obesogenic' diet. *Physiological Reports* **3** e12336. (https://doi.org/10.14814/phy2.12336)

Gaccioli F, White V, Capobianco E, Powell TL, Jawerbaum A & Jansson T 2013 Maternal overweight induced by a diet with high content of saturated fat activates placental mTOR and eIF2alpha signaling and increases fetal growth in rats. *Biology of Reproduction* **89** 96. (https://doi.org/10.1095/biolreprod.113.109702)

Howie GJ, Sloboda DM, Kamal T & Vickers MH 2009 Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. *Journal of Physiology* **587** 905–915. (https://doi.org/10.1113/jphysiol.2008.163477)

Lane M, Zander-Fox DL, Robker RL & McPherson NO 2015 Peri-conception parental obesity, reproductive health, and transgenerational impacts. *Trends in Endocrinology and Metabolism* **26** 84–90.

Larue L, Ohsugi M, Hirchenhain J & Kemler R 1994 E-cadherin null mutant embryos fail to form a trophectoderm epithelium. *PNAS* **91** 8263–8267. (https://doi.org/10.1073/pnas.91.17.8263)

Laurich VM, Trbovich AM, O'Neill FH, Houk CP, Sluss PM, Payne AH, Donahoe PK & Teixeira J 2002 Müllerian inhibiting substance blocks the protein kinase A-induced expression of cytochrome P450 17α-hydroxylase/C17–20 lyase mRNA in a mouse Leydig cell line independent of cAMP responsive element binding protein phosphorylation. *Endocrinology* **143** 3351–3360. (https://doi.org/10.1210/en.2001-211352)

Maheshwari A & Fowler PA 2008 Primordial follicular assembly in humans–revisited. *Zygote* **16** 285–296. (https://doi.org/10.1017/S0967199408004802)

Marcondes FK, Bianchi FJ & Tanno AP 2002 Determination of the estrous cycle phases of rats: some helpful considerations. *Brazilian Journal of Biology* **62** 609–614. (https://doi.org/10.1590/S1519-69842002000400008)

Mann T & Lutwak-Mann C 2012 *Male Reproductive Function and Semen: Themes and Trends in Physiology, Biochemistry and Investigative Andrology*. Berlin: Springer Science & Business Media.

Morgan K, Ruffman T, Bilkey DK & McLennan IS 2017 Circulating anti-Müllerian hormone (AMH) associates with the maturity of boys' drawings: does AMH slow cognitive development in males? *Endocrine* **57** 528–534.

Munsterberg A & Lovell-Badge R 1991 Expression of the mouse anti-mullerian hormone gene suggests a role in both male and female sexual differentiation. *Development* **113** 613–624.

Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI

Novella-Maestre E, Herraiz S, Rodriguez-Iglesias B, Diaz-Garcia C & Pellicer A 2015 Short-term PTEN inhibition improves in vitro activation of primordial follicles, preserves follicular viability, and restores AMH levels in cryopreserved ovarian tissue from cancer patients. *PLoS ONE* **10** e0127786. (https://doi.org/10.1371/journal.pone.0127786)

O'reilly JR & Reynolds RM 2013 The risk of maternal obesity to the long-term health of the offspring. *Clinical Endocrinology* **78** 9–16.

Pankhurst MW 2017 A putative role for anti-Müllerian hormone (AMH) in optimising ovarian reserve expenditure. *Journal of Endocrinology* **233** 1–13. (https://doi.org/10.1530/JOE-16-0522)

Pankhurst MW & McLennan IS 2012 Inhibin B and anti-Müllerian hormone/Müllerian-inhibiting substance may contribute to the male bias in autism. *Translational Psychiatry* **2** e148. (https://doi.org/10.1038/tp.2012.72)

Pepling ME 2006 From primordial germ cell to primordial follicle: mammalian female germ cell development. *Genesis* **44** 622–632. (https://doi.org/10.1002/dvg.20258)

Ramlau-Hansen CH, Nohr EA, Thulstrup AM, Bonde JP, Storgaard L & Olsen J 2007 Is maternal obesity related to semen quality in the male offspring? A pilot study. *Human Reproduction* **22** 2758–2762. (https://doi.org/10.1093/humrep/dem219)

Reddy P, Liu L, Adhikari D, Jagarlamudi K, Rajareddy S, Shen Y, Du C, Tang W, Hämäläinen T, Peng SL *et al.* 2008 Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. *Science* **319** 611–613. (https://doi.org/10.1126/science.1152257)

Reddy P, Zheng W & Liu K 2010 Mechanisms maintaining the dormancy and survival of mammalian primordial follicles. *Trends in Endocrinology and Metabolism* **21** 96–103.

Reynolds RM, Allan KM, Raja EA, Bhattacharya S, McNeill G, Hannaford PC, Sarwar N, Lee AJ, Bhattacharya S & Norman JE 2013 Maternal obesity during pregnancy and premature mortality from cardiovascular event in adult offspring: follow-up of 1,323,275 person years. *BMJ* **347** f4539–f4549 (https://doi.org/10.1136/bmj.f4539)

Riethmacher D, Brinkmann V & Birchmeier C 1995 A targeted mutation in the mouse E-cadherin gene results in defective preimplantation development. *PNAS* **92** 855–859. (https://doi.org/10.1073/pnas.92.3.855)

Rodríguez-González GL, Vega CC, Boeck L, Vázquez M, Bautista CJ, Reyes-Castro LA, Saldana O, Lovera D, Nathaneilsz PW & Zambrano E 2015 Maternal obesity and overnutrition increase oxidative stress in male rat offspring reproductive system and decrease fertility. *International Journal of Obesity* **39** 549–556.

Sampey BP, Vanhoose AM, Winfield HM, Freemerman AJ, Muehlbauer MJ, Fueger PT, Newgard CB & Makowski L 2011 Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet. *Obesity* **19** 1109–1117. (https://doi.org/10.1038/oby.2011.18)

Samuelsson AM, Matthews PA, Argenton M, Christie MR, McConnell JM, Jansen EH, Piersma AH, Ozanne SE, Twinn DF, Remacle C *et al.* 2008 Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance. *Hypertension* **51** 383–392. (https://doi.org/10.1161/HYPERTENSIONAHA.107.101477)

Sferruzzi-Perri AN, Vaughan OR, Haro M, Cooper WN, Musial B, Charalambous M, Pestana D, Ayyar S, Ferguson-Smith AC, Burton GJ *et al.* 2013 An obesogenic diet during mouse pregnancy modifies maternal nutrient partitioning and the fetal growth trajectory. *FASEB Journal* **27** 3928–3937. (https://doi.org/10.1096/fj.13-234823)

Sharpe RM, McKinnell C, Kivlin C & Fisher JS 2003 Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction* **125** 769–784. (https://doi.org/10.1530/rep.0.1250769)

Skinner MK 2005 Regulation of primordial follicle assembly and development. *Human Reproduction Update* **11** 461–471. (https://doi.org/10.1093/humupd/dmi020)

Szyf M 2009 The early life environment and the epigenome. *Biochimica et Biophysica Acta* **1790** 878–885. (https://doi.org/10.1016/j.bbagen.2009.01.009)

Wittmann W & McLennan IS 2013 The bed nucleus of the stria terminalis has developmental and adult forms in mice, with the male bias in the developmental form being dependent on testicular AMH. *Hormones and Behavior* **64** 605–610. (https://doi.org/10.1016/j.yhbeh.2013.08.017)

World Health Organization 2017 *Obesity and Overweight factsheet.* WHO Factsheet. Accessible from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Zhang S, Lin H, Kong S, Wang S, Wang H, Wang H & Armant DR 2013 Physiological and molecular determinants of embryo implantation. *Molecular Aspects of Medicine* **34** 939–980. (https://doi.org/10.1016/j.mam.2012.12.011)

Received 8 February 2018
First decision 13 March 2018
Revised manuscript received 5 July 2018
Accepted 13 July 2018

Downloaded from Bioscientifica.com at 12/10/2020 10:39:57AM
via FT-GAZI