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Disturbed ovarian morphology, oestrous cycling and fertility of high fat fed rats are linked to alterations of incretin receptor expression

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ABSTRACT

Obesity is a major cause of infertility in females with a direct correlation between energy intake and reproductive dysfunction. To explore underlying mechanisms, disturbances in reproductive health and incretin/reproductive hormone receptor expression were studied in female Wistar rats fed a high-fat-diet for 20-weeks. Metabolic parameters and ovarian/adrenal gene expression were monitored along with estrous cycling and fertility upon mating. High-fat-feeding significantly increased body weight, plasma insulin and HOMA-IR, indicative of obesity and insulin resistance. Estrous cycles were prolonged compared to normal chow-fed rats, with 50 % having an average cycle length $\geq$ 7days. Reproductive outcomes revealed high-fat-diet reduced litter size by 48 %, with 16 % rats unable to achieve pregnancy. Furthermore, 80 % of the high-fat group took > 35 days to become pregnant compared to 33 % fed a normal-diet. Also, 35 % of pups born to high-fat-fed rats were eaten by mothers or born dead which was not observed with control rats. These changes were associated with downregulation of Amh, Npy2R and GcgR gene expression in ovaries with upregulation of InsR and Glp-1R genes. In adrenals, Glp-1R, GipR, Npy2R, InsR, GcgR, GshR and Esr-1 genes were upregulated. Histological analysis of high-fat-diet ovaries and adrenals revealed changes in morphology with significantly increased number of cysts and reduced adrenal capsule thickness. Circulating levels of insulin, testosterone and progesterone was significantly higher in high-fat group with reduced FSH levels in plasma. These data demonstrate that high-fat feeding disrupts female reproductive function and suggest important interactions between gut and reproductive hormones in ovaries and adrenals which merit further investigation.

1. Introduction

Obesity affects approximately half of the general population and is associated with female infertility caused by disrupted menstrual function, anovulation, miscarriage, and other related pregnancy complications [1]. More than 38 % of women over 20 years of age are classified as obese with related burden of reproductive dysfunction [2]. In addition, previous studies have observed less successful pregnancies and lower live birth outcomes in overweight women undergoing assisted reproduction [3,4]. Since Type 2 diabetes (T2D) and insulin resistance are linked with higher body mass index, substantial evidence reveals a correlation between female infertility and T2D. Polycystic ovarian syndrome (PCOS), a reproductive disorder associated with metabolic syndrome affects 10 % of women in their fertile years, which involves symptoms including reproductive abnormalities, altered androgen levels, hyperinsulinaemia, insulin resistance and increased risk of gestational diabetes [5,6].

Obesity is a multifactorial disorder with various causes, the most prominent of which are excessive food intake and sedentary lifestyle. Diets rich in fat consumed in excess of requirements make a major contribution to a chronic calorific surplus leading to adiposity and associated complications [7]. Studies performed in humans have suggested that diet is a potential key factor in modifying release of gonadotrophins and follicular maturation [8,9]. Further evidence for a link includes hyperinsulinemia, hyperandrogenaemia, altered gonadotrophin secretion and altered neuroregulation of the hypothalamic–pituitary–gonadal axis disrupting reproductive function in obese females [10]. Interestingly, gastric bypass surgeries, such as Roux-en Y (RYGB), promote weight loss but also result in substantial improvements in comorbidities including diabetes, cardiovascular disease, infertility and certain cancers [6,11].

Alterations in the post-surgery secretion of gut peptides, including glucagon-like peptide-1 (GLP-1), Peptide YY (PYY), oxyntomodulin and glucose-dependent insulinotropic polypeptide (GIP), are considered as a

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key driving force behind the amelioration of T2DM after surgery. Such an association casts the spotlight on the possibility that gut hormones also contribute directly to the beneficial effects of RYGB on fertility. Thus, these hormones may represent an important mediator and undetermined link between diet and reproductive dysfunction [6,12].

Consistent with this view, GLP-1 released from intestinal enteroendocrine L-cells in response to feeding has been suggested to be involved in oocyte maturation in PCOS [13]. Similarly, PYY released by L-cells along with GLP-1 and acting on Neuropeptide Y (NPY) receptors has been shown to delay the estradiol-induced LH surge in ovariectomized ewes [14,15]. Expression of the NPY receptor family in granulosa cells and ovary has been reported [16,17]. This could suggest that NPYR agonists play a role in ovarian development and related secretory function. Interestingly, evidence suggest that modulation of both GIPR and GLP-1R significantly suppresses progesterone synthesis in the presence of FSH and expression of many progesterogenic factors [18]. Finally, recent studies from our laboratory have demonstrated deranged estrous cycling and reproductive outcomes in global GIPR and GLP-1R knock-out mice [19].

Despite many published studies on obesity and its association with PCOS, there is poor documentation of how obesity alters the morphology and gut-hormone receptor expression in ovaries/adrenals, and how this translates to circulating reproductive hormone levels. Therefore, in the present study we evaluated the effects of feeding high-feeding Wistar rats on expression of gut hormone receptors in ovaries and adrenals. Furthermore, we assessed disturbances in estrous cycling and changes in circulating reproductive hormones together with effects on ovarian and adrenal morphology. Finally, we investigated whether changes in reproductive function translated to abnormal pregnancy outcomes. The results support the concept that high-fat feeding disrupts female reproductive function and that alterations of gut-hormone receptors in ovaries and adrenal may play an important role.

2. Materials and methods

2.1. Animal models

Female Wistar rats (Envigo Ltd, UK) were fed with either a regular rodent chow diet (10 % fat, 30 % protein, 60 % carbohydrate; 12.99 KJ/g) or a high fat diet (40 % fat, 43 % carbohydrate and 17 % protein; 26.15 kJ/g, Special Diet Services, UK) ad libitum, for 20 weeks starting at 4 weeks of age. Rats were housed individually in an air-conditioned room at 22 ± 2 °C with a 12 h light and 12 h dark cycle in the Biomedical and Behavioural Research Unit at University of Ulster, Coleraine. For the fertility and pregnancy outcome study, female rats fed respective diets for 27 weeks were mated with healthy lean males (8–10 weeks old) previously maintained on regular rodent chow diet. Mating pairs were kept together for four consecutive estrous cycles (16 days). Males were then removed, and female rats were monitored for pregnancy outcome. All animal experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

2.2. Assessment of stages of estrous cycle

Assessments of estrous cycle stages were carried out as described previously using vaginal smears taken every day from conscious rats between 11:00 am and 12:00 noon [20]. A sterile cotton-tipped swab wetted with distilled water wase gently and quickly introduced into the vaginal orifice; the introduction was relatively shallow (approximately 1 cm) to avoid excessive cervical stimulation and a consequent pseudo-pregnancy. Subsequently, the swab wase carefully rotated (one twist) against the vaginal wall. Afterwards, the collected sample of vaginal epithelial cells was placed on a glass slide, dried at 37 °C and observed under light microscope (Olympus IX51) with ×10 objective lens. Samples were collected and examined over a 16-day period and number of completed cycles of proestrus, oestrus, metestrus and dioestrus were computed for each rat.

2.3. Haematoxylin and eosin staining of ovaries and adrenals

After 20 weeks on high fat diet, ovaries and adrenals from both groups of rats were excised, immediately fixed, processed and subsequently embedded in paraffin wax using an automated tissue processor (Leica TP1020, Leica Microsystems, Nussloch, Germany), as described previously [21]. After rehydration using a series of ethanol solutions, the sections were exposed to haematoxylin solution for 5 min and rinsed with tap water, acid alcohol (0.25 % HCl, 50 % methanol) and again in tap water prior to staining with eosin for 5 min. Following rinsing with distilled water, sections were dehydrated using ethanol, dipped in histo-clear II for 2 min and mounted using DePeX mounting medium. The slides were then scanned and analysed using NanoZoomer digital pathology software (NDP.serve 3.3.30). The freehand line function was used to measure area of the ovaries while the ruler function was used to measure thickness of adrenal capsule and zona glomerulosa. Column graphs were generated using GraphPad PRISM (La Jolla, CA, USA; version 5).

2.4. Expression of genes in ovarian and adrenal

The mRNA expression of selected genes in ovaries and adrenals was assessed by qPCR using the primers listed in Table 1. mRNA was extracted from the tissues [21] using a RNeasy Mini Kit following manufacturer's instructions (Qiagen, UK). mRNA (150 ng) was reverse transcribed to cDNA using transcriptor first strand cDNA synthesis kit (Roche, Burgess Hill, UK). qPCR was performed on a Lightcycler 480 System (Roche, UK) using designed primers (Thermo Fisher Scientific).

2.5. Biochemical analysis and determination of hormone concentrations

Blood samples were collected after 18 weeks on high fat diet, from the cut tip on the tail vein of conscious rats into chilled fluoride/heparin micro-centrifuge tubes (Sarstedt, Numbrecht, Germany). Food was withheld 2 hrs previously. Fasting blood glucose was measured directly using a hand-held Ascencia Contour blood glucose metre (Bayer Healthcare, Newbury, UK). At the same time, HbA1c was assessed with commercially available A1c EZ 2.0 Portable Glycohemoglobin Analyzer kit (Wuxi BioHermes Bio & Medical Technology Co., Ltd.). Plasma insulin was assayed in the fasted blood samples by a modified dextran-

Table 1
List of rat primers used.

Gene symbol	Alias/common name	Primer sequence (5'-nt-3')
GLP-1R	Glucagon-like peptide 1 receptor	F-ATCAAAGACGCTGCCCTCAA R-CCACGCAGTATTGCATGAGC
GIPR	Gastric inhibitory polypeptide receptor	F-TGGAGATTGCGAGCAGGACT R-GAATCTGTCTCCGCCCTCTG
NPY2R	Neuropeptide Y2 receptor	F-GAACTGGACCTGCTTTGAGTG R-AAGAATTGCGTTGGCCCTTG
Hsd11b1	11-beta dehydrogenase 1	F-TGTCTCGGTAGGAGATGCTCA R-AGAGGCAACTTCCAGATGGC
INSR	Insulin, receptor	F-GCTACCTGGCCACTATCGAC R-AACTGCCCATTGATGACGGT
NPY1R	Neuropeptide Y1 receptor	F-AGGACTTCGCCACAAGATGG R-CCACACTGTCAGACCCGAAC
GHSR	Ghrelin Receptor	F-AGTACGACATGCTGTTGGGG R-GCAGGAAGGATGCTGTCACC
GCGR	Glucagon receptor	F-TAGACCCAGCAACCTGAGGA R-AGACATGACAGCACCACCAG
AMH	Anti-Mullerian hormone	F-CGCCCTAACCCTTCAACCAA R-CGTGAAACAGCGGGAATCAG
ESR1	Oestrogen receptor 1	F-GCCACTCGATCATTCGAGCA R-CCTGCTGGTTCAAAAGCGTC

coated charcoal radioimmunoassay [22]. Terminal plasma collected after 20 weeks on high fat diet was used to measure reproductive hormone levels, that were determined by the Ligand Assay and Analysis Core (Centre for Research in Reproduction, University of Virginia, USA).

2.6. Statistical analysis

Statistical analysis was performed using GraphPad PRISM (La Jolla, CA, USA; version 5). Data are presented as mean ± SEM for a given number of observations (n) as indicated in the Figures. All samples were numbered and blinded, and researchers were unblinded only when analyses were complete. Differences between groups were compared using one-way ANOVA or unpaired 2-tailed Student's t test as appropriate. Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Effects of high-fat diet on body weight, blood glucose, HbA1c and HOMA-IR

Body weight change over the duration of the study is shown (Fig. 1a). High-fat fed Wistar rats exhibited significantly increased ($p < 0.05$ to $p < 0.001$) body weight from week 5 to week 20 compared with chow-fed rats. Blood glucose was elevated after 5–7 weeks compared with normal chow fed rats (Fig. 1B). At 18 weeks, HbA1c and blood glucose were not significantly elevated (Fig. 1C,D) but HOMA-IR was increased providing evidence of insulin resistance (Fig. 1E).

3.2. Effects of high-fat diet on estrous cycling

Fig. 2 A shows representative images of estrous cycle stages for 5 consecutive days in control and high-fat fed rats. High-fat feeding resulted in estrous cycles with a mean duration of 4.9 ± 0.3 days which was significantly ($p < 0.05$) longer than controls at 4.1 ± 0.1 days (Fig. 2B). 50 % of the high-fat fed rats had irregular estrous cycle days whilst all control animals displayed regular cycling (Fig. 2C). A prolonged average cycle length of ≥ 7 days was considered as irregular.

3.3. Effects of high-fat diet on ovarian and adrenal morphology

Ovarian morphology in high-fat fed rats was characterised by increased size and cystic appearance compared to normal controls (Fig. 3A,B,C,D). The number of cysts (per mm^2 of ovary) significantly ($p < 0.01$) increased with high-fat regime compared to control (Fig. 3E). Although, not significant high-fat fed rats showed a trend of reduced antral follicle and corpus luteum changes compared to normal chow fed rats (Fig. 3F,G). Similarly, morphology of the adrenal gland showed irregularities (Fig. 4A). Capsule thickness was increased ($p < 0.001$), but no difference was observed in zona glomerulosa compared to controls (Fig. 4B,C).

3.4. Effects of high-fat diet on receptor gene expression in ovaries and adrenal

Ovarian expression of *Glp-1R* and *Insr* genes was significantly upregulated ($p < 0.05$ to $p < 0.001$) in high-fat fed rats compared to control. *Gipr*, *Npy1r, Gshr and Esr-1* showed trend of upregulation which was not significant. Expression of *Amh, Npy2R* and *GcgR* genes was significantly downregulated ($p < 0.01$) compared to control (Fig. 5). In adrenals expression of *Glp-1R, Gipr*, *Npy2R, Insr, GcgR, Esr-1*, *Gshr*, *and Amh* genes was significantly upregulated ($p < 0.05$ to $p < 0.001$) by high fat feeding Expression of *Npy1R* and *11b-Hsd* showed trend in upregulation but values were not significantly different from normal controls (Fig. 6).

3.5. Effects of high-fat diet on circulating insulin, testosterone, progesterone, estradiol, luteinizing hormone (LH) and follicle-stimulating hormone (FSH)

High-fat diet markedly ($p < 0.05$ to $p < 0.01$) increased circulating insulin, testosterone and progesterone (Fig. 7A,B,C). Plasma FSH was significantly ($p < 0.05$) lower in high-fat fed rats when compared to normal diet controls (Fig. 7F). Plasma estradiol and LH concentrations were similar in the two groups of rats (Fig. 7D,E).

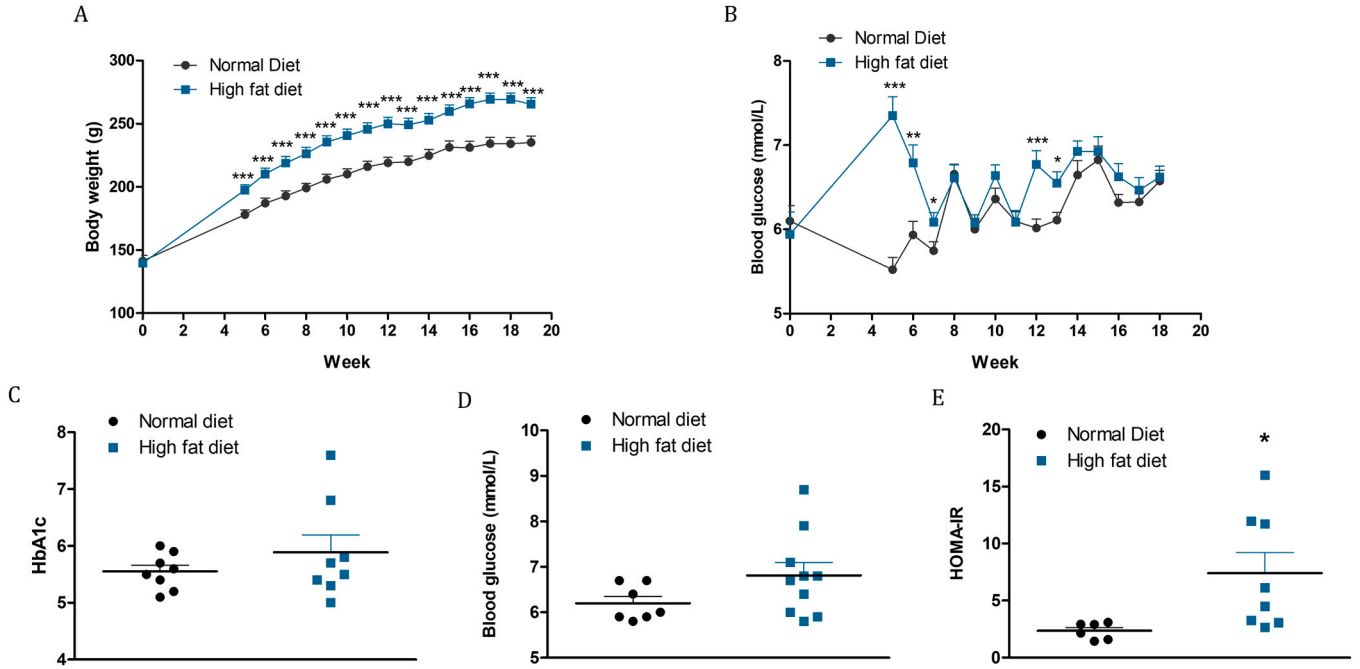

Fig. 1. Body weight, blood glucose, HbA1c and HOMA-IR. Effects of 18–20 weeks of high fat diet on (A) body weight and (B) 2hr-fasted blood glucose (C) glycated haemoglobin (HbA1c) (D) blood glucose and (E) HOMA-IR of Wistar rats. Values represent mean±SEM for 8 rats. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared with normal diet controls.

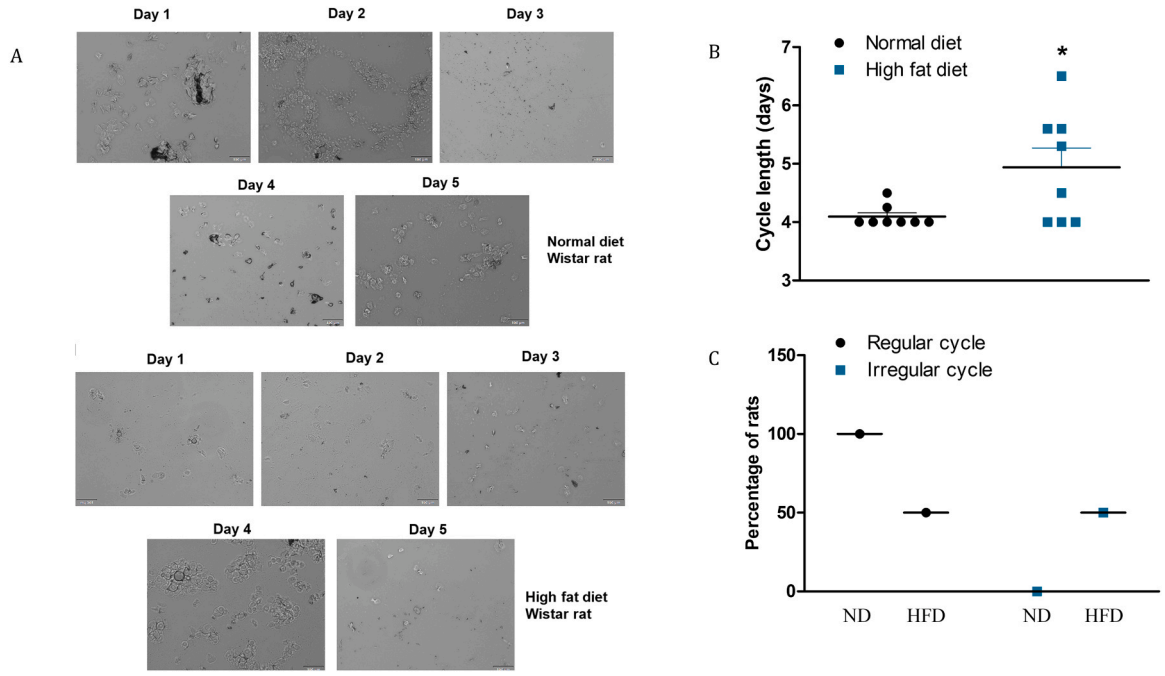

Fig. 2. Estrous cycle. Effects of 20 weeks of high fat diet on oestrous cycle analysis. (A) Estrous cycle representative images (B) Average estrous cycle lengths after 4–5 cycles for normal chow fed and high fat fed Wistar rats. Control rats showed regular 4–5 day cycles. (C) The proportion of rats with irregular cyclicity is significantly increased in high fat fed group. Prolonged average cycle length (≥ 7 days) was considered as irregular. Values represent mean±SEM for 6–8 rats. *$p < 0.05$, compared with normal diet controls.

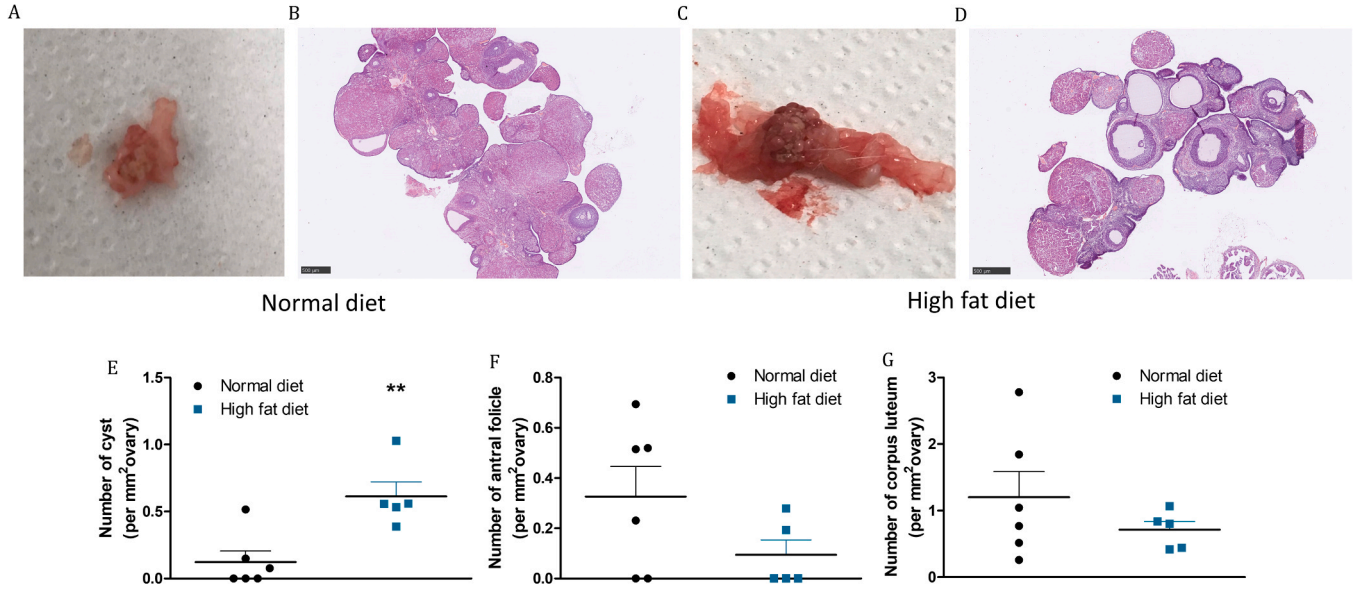

Fig. 3. Ovarian histological examination. Histological sections of ovaries of both normal diet and high fat diet Wistar rats were stained haematoxylin and eosin. (A-D) Representative images of ovarian morphology of normal diet controls and high-fat fed rats, (E) number of cysts (per mm^2 ovary), (F) number of antral follicles (per mm^2 ovary) and (G) number of corpus lutea (per mm^2 ovary). Values represent mean±SEM for 6–8 rats. **$p < 0.01$ compared with normal diet controls.

3.6. Effects of high-fat diet on reproduction outcomes

All of the normal control animals became pregnant and gave birth, whilst 16% of high-fat fed rats did not even get pregnant (Fig. 8A). The average number of days to parturition from the time that males and females were together for breeding was 38 ± 4 days for high-fat group and 32 ± 5 days for the control group (Fig. 8B). However, 80 % of high-fat fed rats took more than 35 days to give birth compared to 33 % for controls (Fig. 8C). This suggests prolonged gestation or decreased conception frequency in high fat fed rats. Reproductive outcomes showed a 48 % reduction in litter size compared to normal diet controls (Fig. 8D). In addition, 35 % of pups born to high-fat fed rats were eaten by their mothers or born dead whilst this phenomenon was not observed with control rats (Fig. 8E). Fig. 8F shows number of male and female litters born to control and high-fat groups.

4. Discussion

Despite previous research indicating a negative impact of maternal obesity on female fertility, the underlying mechanisms are poorly

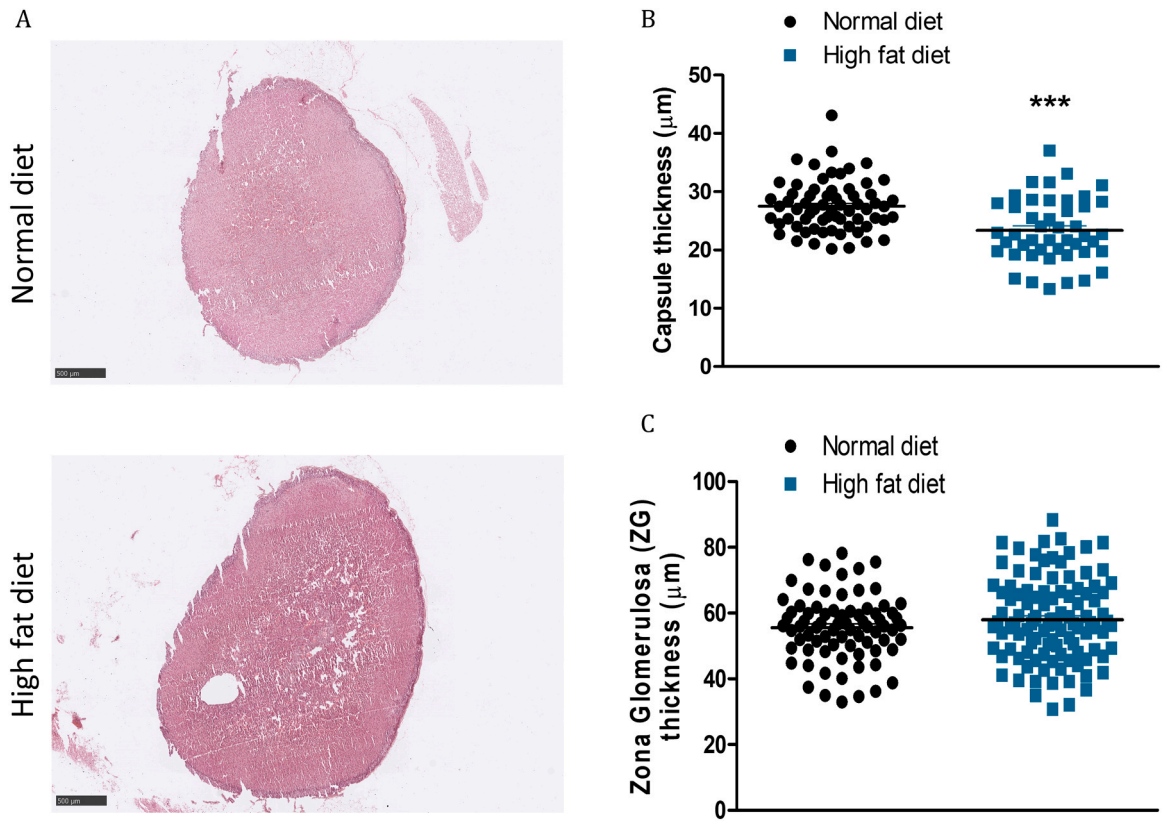

Fig. 4. Adrenal histological examination. Histological sections of adrenal of both normal diet (A) and high fat diet (B) Wistar rats after 20 weeks of dietary regime were stained with haematoxylin and eosin. (C) Capsule thickness (μm) and (B) thickness of zona glomerulosa (μm) were measured. * **$p < 0.001$ compared with normal diet controls. Values represent mean±SEM for 5–6 rats.

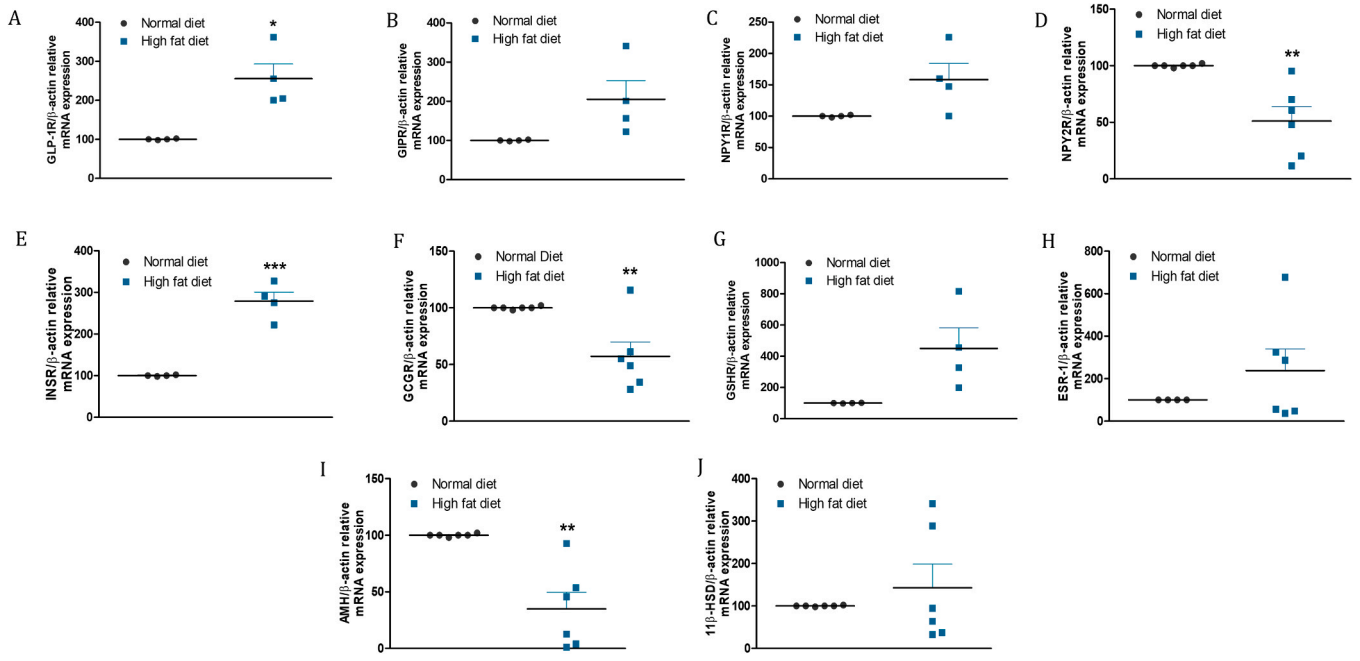

Fig. 5. Ovarian gene analysis. Effects of 20 weeks of high fat diet on relative mRNA expression (in %) in the ovaries of Wistar rats. (A) GLP-1R, (B) GIPR, (C) NPY1R, (D) NPY2R, (E) INSR, (F) GCGR (G) GSHR, (H) ESR-1, (I) AMH (Anti-Mullerian Hormone) and (J) 11B-HSD. Values represent mean±SEM for 4–6 rats. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared to controls animals.

understood [23]. To address this issue and provide a model for further work, the present studies was aimed at making a detailed investigation of the impact of high-fat feeding on estrous cycling, morphology of ovaries and adrenals, gut-hormone receptor expression, circulating reproductive hormones, fertility and pregnancy outcomes.

As expected, feeding female Wistar rats with high-fat diet for 20

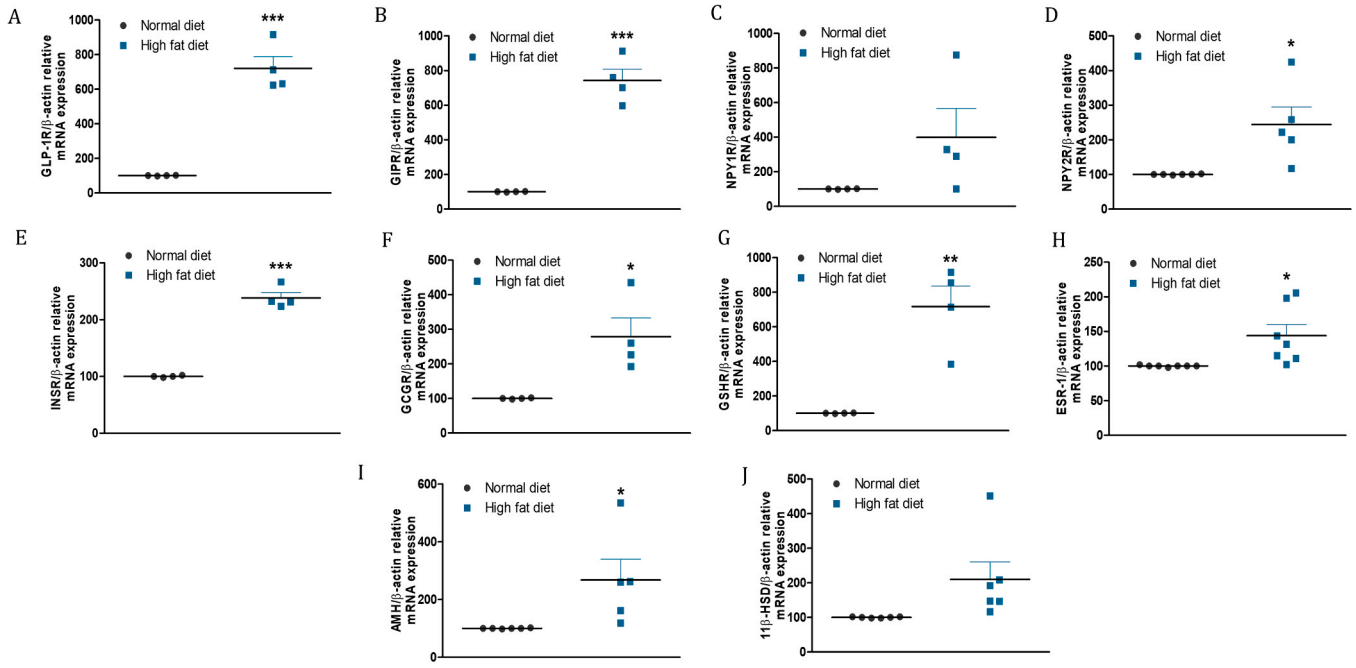

Fig. 6. Adrenal gene analysis Effects of 20 weeks of high fat diet on relative mRNA expression (in %) in the adrenal of Wistar rats. (A) GLP-1R, (B) GIPR, (C) NPY1R, (D) NPY2R, (E) INSR, (F) GCGR (G) GSHR, (H) ESR-1, (I) AMH (Anti-Mullerian Hormone) and (J) 11B-HSD. Values represent mean ±SEM for 4–6 rats. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared with normal diet controls.

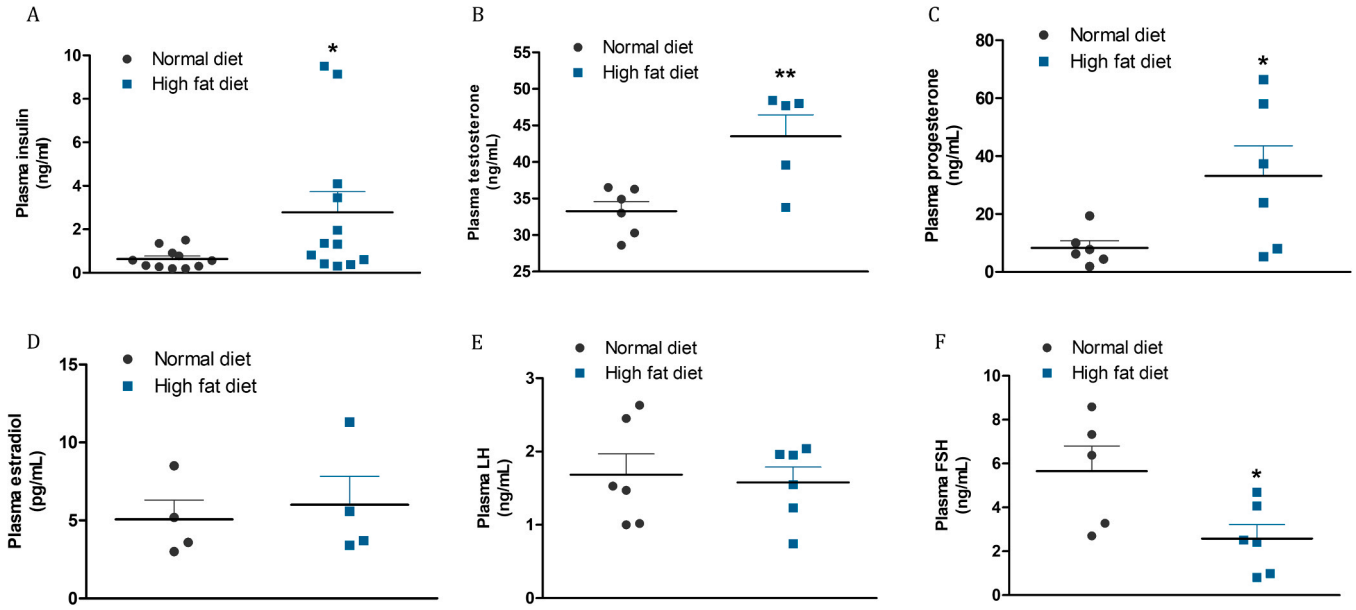

Fig. 7. Plasma hormone measurement. Effect of 20 weeks of high fat diet on hormone measurement in the plasma of normal chow fed control and high fat fed Wistar rats. (A) insulin, (B) testosterone, (C) progesterone, (D) estradiol, (E) luteinizing hormone (LH) and (F) follicle-stimulating hormone (FSH). Values represent as mean ± SEM for 5–6 rats. *$p < 0.05$ and **$p < 0.01$ compared with normal diet controls.

weeks from 4 weeks of age was associated with increased body weight and insulin resistance as indicated by HOMA-IR. Blood glucose and HbA1c were not elevated over time due to compensatory increases in circulating insulin. Interestingly, insulin resistance has been associated with incidence of multiple cysts, hyperandrogenism and anovulation in human females [24,25]. Similarly, ovarian cysts were noted in rats fed with high-fat diet, suggesting a strong link with insulin resistance and elevated insulin secretion which is heavily regulated by various gut hormones secreted in response to feeding.

These observations together with our other results demonstrate a broad range of important detrimental effects of high-fat feeding on reproductive function of female Wistar rats. During estrous cycle monitoring, half of the high-fat fed animals exhibited abnormal cycling with daily vaginal smears revealing longer cycle length compared to normal-diet controls. In mammals, pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus during the follicular (every 1–1.5 hrs) and luteal phase (every 2–4 h) regulate the estrous cycle and hence, studies have suggested that disrupted hypothalamic activity causes prolonged estrous cycling [26]. Hyperleptinemia resultant from expanded adipose stored stores might also contribute to

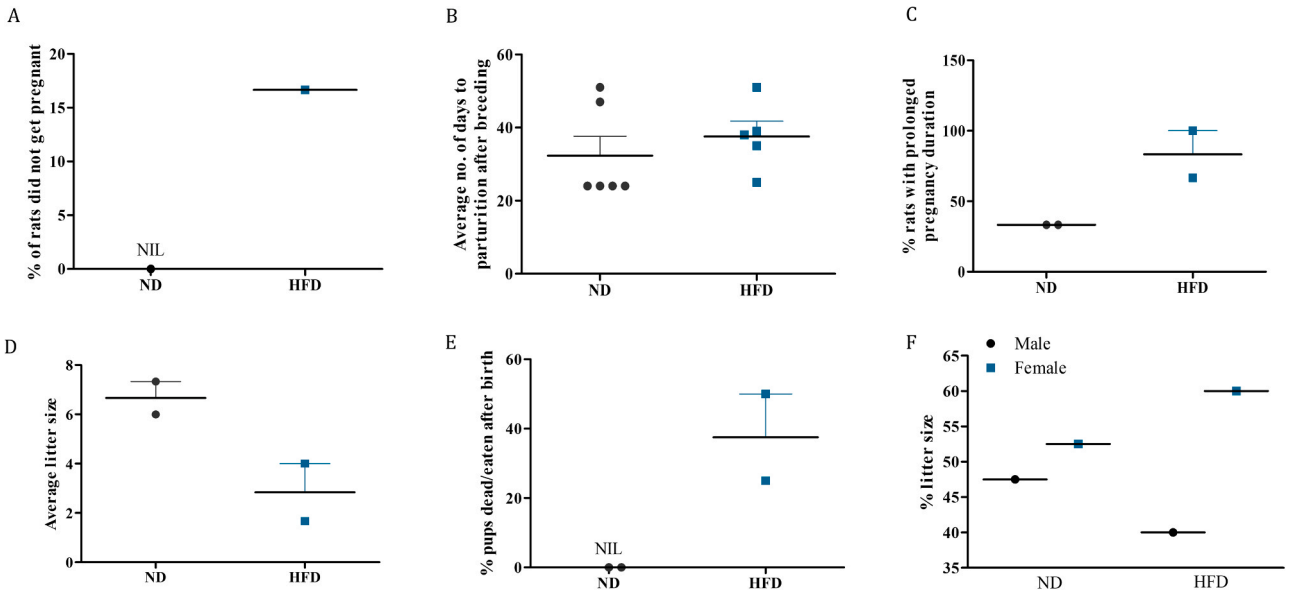

Fig. 8. Pregnancy outcome. Pregnancy outcome of female control and high-fat fed Wistar rats after (27 weeks on high-fat diet). (A) Average litter size, (B) percentage of rats not producing pups, (C) average number of days to parturition after kept together for breeding, (D) percentage of female rats with prolonged pregnancy duration (more than 35 days), (E) Percentage of pups born dead or eaten by mothers and (F) percentage litter (i.e., number of male and female pups born). Values represent as mean±SEM for 4 rats, ***$p < 0.001$ compared with normal diet controls.

hormonal disruption at the level of the hypothalamohypophysis axis [27]. Abnormal lipid profiles have also been suggested to play a role in female infertility affecting ovarian function and oocyte quality [28].

In addition to derangements of estrous cycling, high-fat fed rats displayed disturbed ovarian morphology characterised by formation of polycystic ovaries with reduced antral follicles and corpora lutea. These results are consistent with rodent models of PCOS [29,30] but changes in antral follicles and corpora lutea were less clear, with other studies suggesting an increase, no change or decrease in number [31,32].

The menstrual cycle in humans or the estrous cycle in rodents is controlled by the hypothalamic–pituitary–gonadal axis [33]. In the present study, high-fat feeding resulted in an increased circulating testosterone. This is consistent with studies in women demonstrating increased testosterone and dehydroepiandrosterone sulphate after consumption of high-fat diet which was decreased after switching to low-fat diet [34,35]. Elevated testosterone in PCOS is mainly associated with high levels of insulin [36]. Moreover, excess testosterone is promoted by increased LH stimulation from the pituitary [37]. Metformin, which mildly activates the GLP-1 system, is used in the management of PCOS and lowering testosterone [38,39]. As such, high-fat diet did not alter plasma LH and estradiol levels in our study. However, others have reported reduced LH levels after prolonged high-fat feeding for up to 180 days [40]. Consistent with other studies, we observed elevated levels of progesterone and reduced FSH in high-fat fed rats [41,42]. Interestingly, increased levels of insulin, testosterone, progesterone and reduced FSH contribute to prolongation of estrous cycle and reduced reproductive function.

In addition to ovarian hyperandrogenism, adrenal hyperplasia contributes to impaired fertility in females [43]. Our data suggest reduced adrenal capsule thickness post high-fat diet. Similar results were observed by Swierczynska et al. (2015) in C57BL6 mice. Adrenal capsule is populated with Gli1-positive progenitor cells which was depleted in high-fat fed rodents [44]. Furthermore, obesity is associated with altered circulating levels of plasma cortisol and aldosterone which may contribute to increased fat mass [45].

Quantification of key gut-hormone receptor mRNAs indicated that GLP-1R and INSR were upregulated, whilst NPY2R, GCGR and AMH were downregulated in ovaries. Adrenal expression of gut-hormone receptors including GLP-1R, GIPR, NPY2R, INSR and GCGR was upregulated by high-fat feeding. These changes are consistent with a possible role in the observed disturbances of reproductive function. This accords with our recently published data showing that genetic deletion of GLP-1R and GIPR significantly disturbed estrous cycling in mice [19]. Consistent with this, a recent study identified GLP-1R expression in rodent ovarian granulosa cells [13]. Additionally, the same study proposed involvement of GLP-1/GLP-1R axis in contributing to oocyte maturation in PCOS [13]. Exendin-4 administration leads to an increase in corticosterone in rodents, suggesting an involvement of central GLP-1 receptors in the hypothalamus regulating adrenal secretions. Interestingly, others have struggled to evidence expression of GLP-1R in adrenal cortex and further studies are required to solve the discrepancy [46,47]. A few studies have been conducted to evaluate GIP and its receptor levels in females with obesity related PCOS. With regards to adrenal function, GIP receptors directly activate steroidogenesis in rodents stimulating plasma glucocorticoid levels in mice [48]. These differences in gut-hormone receptor mRNA levels between the normal diet and high-fat fed groups is interesting and requires further investigation in both humans and rodents.

In the present study, insulin receptor expression was upregulated in adrenal and ovaries by high fat feeding. Previous studies have revealed a role for insulin in follicular development and suppressing estradiol secretion [49,50]. Insulin has also been shown to stimulate basal and FSH or LH-induced secretion of progesterone in addition to promoting proliferation in rat thecal cell [51,52]. Many publications support expression of glucagon receptor in ovaries, but few studies have explored the crosstalk between insulin and glucagon receptors in reproduction [53]. We also investigated gene expression of anti--Müllerian hormone (AMH), which regulates folliculogenesis in PCOS. AMH mRNA levels were significantly decreased in ovaries of high-fat fed rats. Interestingly, it has been reported that AMH gene polymorphism was associated with levels of androgen and LH levels in PCOS with insulin resistance [54]. Our data also revealed decreased mRNA levels of NPY2R in ovaries. NPY2 receptors are important for satiety/body weight regulation and may play a role in diet-induced irregularities of female reproduction [55].

In agreement with previous studies, pregnancy outcomes were significantly impacted by high fat feeding [56,57]. High-fat fed rats had difficulties in conceiving, becoming pregnant and litters delivered were

frequently eaten by mothers. Others have also reported increased pup mortality in rats fed with high-fat diet [58,59]. Although limited information exists for humans, our preliminary rodent data suggest that gut hormones and their receptors are associated with disturbances in reproductive function, including altered estrous cycle length, reproductive hormone levels, and pregnancy outcomes. Changes in appetite-controlling gut hormones are likely to affect female fertility and warrant further detailed investigation.

Taken together, the present observations along with previously published literature provide evidence that prolonged consumption of high-fat diet severely disrupts female reproductive function. It appears that gut hormones may play an important role in, for example, the preservation of ovarian morphology [6,12,15,19,60]. Given that hyperinsulinemia and insulin resistance are classic features of obesity, diabetes and PCOS, our data suggest that gut hormone receptor modulation might represent a novel means for treatment of female reproductive dysfunction.

CRediT authorship contribution statement

DK, AS and RCM contributed to conduct/data collection, analysis and writing of the manuscript. DK, RCM and PRF contributed to study design, analysis and writing of the manuscript. All authors approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. No potential conflict of interests relevant to this article were reported.

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