# Removal of mouse ovary fat pad affects sex hormones, folliculogenesis and fertility

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## **Abstract**

Research

As a fat storage organ, adipose tissue is distributed widely all over the body and is important for energy supply, body temperature maintenance, organ protection, immune regulation and so on. In humans, both underweight and overweight women find it hard to become pregnant, which suggests that appropriate fat storage can guarantee the female reproductive capacity. In fact, a large mass of adipose tissue distributes around the reproductive system both in the male and female. However, the functions of ovary fat pad (the nearest adipose tissue to ovary) are not known. In our study, we found that the ovary fat pad-removed female mice showed decreased fertility and less ovulated mature eggs. We further identified that only a small proportion of follicles developed to antral follicle, and many follicles were blocked at the secondary follicle stage. The overall secretion levels of estrogen and FSH were lower in the whole estrus cycle (especially at proestrus); however, the LH level was higher in ovary fat pad-removed mice than that in control groups. Moreover, the estrus cycle of ovary fat pad-removed mice showed significant disorder. Besides, the expression of FSH receptor decreased, but the LH receptor increased in ovary fat pad-removed mice. These results suggest that ovary fat pad is important for mouse reproduction.

#### **Key Words**

- adipose tissue
- ovary fat pad
- ▶ folliculogenesis
- sex hormones
- ▶ female fertility

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## Introduction

The normal folliculogenesis is a basis of high-quality oocyte formation. Follicle development starts during neonatal life when primordial follicles are formed in rodents. After that, these primordial follicles develop, in turn, to primary, secondary, tertiary and eventually Graafian follicles (McGee & Hsueh 2000).

Throughout these reproductive periods of the female, sex hormones secreted by the corresponding organs of the hypothalamic-pituitary-gonadal axis (HPG axis) play important roles (Veldhuis et al. 2009). Folliclestimulating hormone (FSH) is the major stimulator of follicle development. It is important for the development

of primary and secondary follicles to the antral stage, and more importantly, further promotes antral follicular development. Luteinizing hormone (LH) can induce ovulation of Graafian mature follicles. The LH surge that stimulates ovulation also initiates meiotic resumption, and these two events are coordinated such that by the time the oocyte is ovulated, it has completed the maturation processes necessary to produce a fertilizable egg (DiLuigi et al. 2008). Besides, estrogens play important roles in the intraovarian regulation of folliculogenesis by acting on specific ovarian receptors. Estrogen receptor alpha-specific deletion in theca cells can lead to premature ovarian failure (Lee et al. 2009). Moreover, estrogens can also induce uterine cell proliferation during the late follicular phase of the menstrual cycle (Hsueh et al. 2015). According to follicular developmental stage and gonadotropin dependence, preantral follicle stage (including primordial, primary, and secondary follicles) is named as the gonadotropin-responsive phase and the other stages (including antral and ovulation stages) are named as gonadotropin-dependent phase (McGee & Hsueh 2000, McNatty et al. 2007, Orisaka et al. 2009). These three sex hormones coordinate precisely with gonadotropin-releasing hormone (GnRH) and progesterone to regulate the female reproductive process. The levels of these hormones change regularly in an estrous cycle, which lasts for different lengths of time in different species (for humans, 29.1 days of a menstrual cycle and mouse 4–5 days of an estrous cycle) (Chiazze et al. 1968, Miller & Takahashi 2013, Häggström 2014).

As we all know, fat is important for energy storage, organ protection and body temperature maintenance. Adipose tissue can secrete many immune regulators and adipokines that transport to the rest of the organism through microcirculation and the general blood circulation, which participates in inflammation regulation and obesity-dependent metabolic diseases (Ouchi et al. 2011). Therefore, adipose tissue can be viewed as an immunologic organ (Schaffler et al. 2007). Fat from obese mice can produce TNFα, an important inflammatory factor for its role in autoimmune disorders (Hotamisligil et al. 1993). Leptin is another hormone secreted by adipocytes, which can regulate the body fat stores and help the organism attain a balanced state between energy output and intake (Halaas et al. 1995). Besides, leptin can influence the ovary function and developing embryo through its interaction with gonadotropins and the hormones that control their synthesis (Chehab et al. 1996, 1997). However, with the improvement of our living standards, some obesity-related problems such as type 2 diabetes and cardiovascular disease become more and more popular, in which fat exerts negative effects. That is the two faces of fat, which were described thoroughly by Powell (2007).

Adipose tissue distributes mainly in the subcutaneous region and is also found in visceral compartments. As for the female reproductive system, including ovary, oviduct and uterus, a large mass of fat adheres to these organs in mouse of childbearing age under normal physiological conditions. Recently, it was reported that brown adipose tissue transplantation can ameliorate polycystic ovary syndrome and improve fertility (Yuan et al. 2016). Adipose tissue may play an important role in the control of the function of adjacent structures through its paracrine secretions. For example, perivascular adipose tissue can affect the vascular inflammation response (Guzik et al. 2007). It is believed that the white adipose tissue attached to viscera can produce some bioactive mediators involved in distinct metabolic and inflammatory profiles (Drouet et al. 2012). More importantly, the adipose tissue of the adult and the placenta of developing fetus can secrete estrogen in humans and a number of higher primates (Byers et al. 2012). On the other hand, the neighboring two things usually are functionally relevant in evolutionary terms, such as promoter for gene transcription, molecule chaperon for protein expression regulation, granulosa cells and oocytes, mammary ducts and fat pad.

As the white fat can secrete adipokines that are implicated in angiogenesis, which can enhance the formation of capillaries, it has been reported that angiogenesis is important for ovarian function and the dominant follicle selection (Berisha *et al.* 2000, Fenzl & Kiefer 2014). Besides, some adipokines involved in metabolism and secreted by adipocytes also play important roles in this process (Rosen & Spiegelman 2006). Thus, it seems reasonable to hypothesize that ovary fat pad composed of white adipose tissue, which distributes around the ovary, may play important roles in ovary function and female reproduction. We suppose that ovary fat pad may function as a paracrine organ and can affect the ovarian function and eventually fertility by affecting the secretion and function of sex hormones.

In our research, we studied the roles of ovary fat pad in female mouse reproduction and found that ovary fat pad-removed mice displayed subfertility and reduced follicular development. Further study identified that the levels of three important sex hormones (estrogen, FSH and LH) and their corresponding receptors expression were affected. Besides, the estrus cycle showed disorder. We conclude that ovary fat pad may be an indispensable

tissue, which can ensure the ovary follicular development stays on the rails through its effect on the hormone secretion regulation, and eventually guarantees the female reproductive process.

#### Materials and methods

All chemicals and media were purchased from Sigma Chemical Company except for a few specifically mentioned below.

#### **Animals and treatments**

Six-week-old ICR mice care and handling were conducted according to rules promulgated by the Ethics Committee of the Institute of Zoology, Chinese Academy of Science. To remove the ovary fat pad, adipose tissue surrounding the ovary must be gently pulled to avoid detachment of small pieces of ovary and then removed surgically as cleanly as possible, with no injury to the ovary. In this process, only the adipose tissue was operated on and the ovary was never touched with surgical instruments. As for control groups, the same incisions were made at the same position over the muscle layer and then sutured after the same exposure time in air to the experimental groups. After that, the muscle and surface layers were sutured carefully. After surgery, all mice were cared for at least one week to recover. When it comes to fertility analysis, mice recovered from surgery were mated with adult ICR males, and one female was caged with one male. After pregnancy, the number of offspring was recorded to conduct the statistical analysis.

### Ovulation detection and estrus cycle analysis

To collect and count the ovulated MII (metaphase II) oocytes, mice were superovulated by injection of 10 IU of pregnant mare's serum gonadotropin (PMSG) at the estrus stage (Tarin *et al.* 2002, Kon *et al.* 2014), followed 46–48 h later by 10 IU of human chorionic gonadotropin (hCG). The MII oocytes were recovered from the oviducts 13–14 hours after hCG, and the cumulus cells were dispersed with 1 mg/mL hyaluronidase. After three washes in M2 medium, oocytes were counted under a microscope and then the statistical analysis was conducted.

Vaginal cytology was used to follow estrous cycles, as previously described (Byers *et al.* 2012). The uterine stages of estrus, proestrus, metestrus and diestrus were assessed on the basis of the cell types observed in vaginal smears.

### Blood collection and hormone analysis

As the general levels of FSH, LH and estrogen are all relatively high at proestrus (Miller & Takahashi 2013), we selected mice at this stage to detect their hormone levels after a recovery period. Briefly, the mice were anesthetized by 2,2,2-tribromoethanol (Sigma-Aldrich 75-80-9) injection, and then blood was sampled by removing the eyeballs. All blood samples were clotted at 4°C for overnight. Low-speed centrifugation (876g for 20 min) was used to separate the serum, and the supernatant was then stored at -20°C for further analysis.

Estrogen FSH and LH measurements were conducted at a commercial laboratory (Kemei Co., Beijing, China). The assay sensitivity was <0.5 pg/mL (estrogen), 0.25 IU/mL (FSH), 0.2 IU/mL (LH) and the intra- and interassay coefficients of variation were <10% and <15.2% (estrogen), 2.2–2.5% and 3.7–8.7% (FSH), 10% and 15% (LH), respectively. Each serum sample was measured in duplicate.

### Real-time quantitative PCR analysis

One ovary was ground in TRIzol Reagent (Ambion 94204) and extracted by organic solvents (chloroform and isopropyl alcohol). Then, the primary product was treated with DNase (Qiagen) to remove the genome DNA, and the quality of total RNA was assessed by detecting the 260/280 and 260/230 absorbance ratio and agarose gel electrophoresis. After that, the RNA was reverse transcribed with cDNA synthesis kit (Invitrogen). All primers used in our research were designed on the primer 5.0 software and the predicted amplified product was blasted on NCBI to ensure the specificity of the primers.  $\beta$ -Actin was used as a control gene to correct the cDNA level of samples (Gong et al. 2015). The primers used are as follows. β-Actin  $(154 \, bp)$ : 5'-GGCTGTATTCCCCTCCATCG-3' (sense); 5'-CCAGTTGGTAACAATGCCATGT-3' (anti-sense). Fshr (FSH receptor)  $(229 \, bp)$ : 5'-CCCAACCATGGCTTAGAAAA-3' (sense); 5'-GATCCCCAGGCTGAGTCATA-3' (anti-sense). Lhr (LH receptor) (232bp): 5'-CTGAAAACTCTGCCCTCCAG-3' (sense); 5'-AATCGTAATCCCAGCCACTG-3' (anti-sense). Roche LightCycler 480 II system and ABI StepOne/ StepOnePlus system were used to quantify the relative expression levels of RNA. The qRT-PCR was performed with 10 µL final reaction volumes with 1 µL of cDNA, 5 μL of SYBR Premix (Takara Biotechnology (Dalian) Co., Ltd.), 3 µL of nuclease-free water and 0.5 µL of each primer set mentioned previously. PCR was conducted using the

following cycle parameters: 10 min at 95°C, and 35 threestep cycles of 15s at 95°C, 20s at 60°C and 30s at 72°C. The cDNA form control group was used as a standard for quantitative correction. All cDNA samples were applied in dilution of 1:100 to obtain the results within the range of standard. To avoid potential contaminations, the blank control was set, in which the cDNA template was replaced by the nuclease-free water. To guarantee the absence of primer dimers and the specificity of PCR products, all PCR products were separated on 1% agarose gel and visualized using Bio-Rad ChemiDoc XRS+ system. We removed the value of samples, which separated with abnormal bands. Each reaction was performed in triplicates, and the outliers (C<sub>t</sub> value) were excluded based on GraphPad Prism 5 software. Threshold cycle C<sub>t</sub> was used to carry out the analysis of transcript, and the relative gene expression was calculated by Pfaffl method (Pfaffl 2001).

# Hematoxylin and eosin staining and quantification of ovarian follicles

Hematoxylin and eosin staining was performed as described previously (Liang *et al.* 2015). Ovaries were obtained from ovary fat pad-removed and control group mice, which were treated with PMSG for 48 h. After killing, the ovary was fixed in 4% formaldehyde overnight, and then dehydrated with a graded ethanol series and embedded in paraffin. Paraffin-embedded ovaries were cut into sections of 8-µm thickness and mounted on glass slides. After drying at 48°C for overnight, paraffin sections were deparaffinized in xylene, hydrated by a graded alcohol series and stained with hematoxylin and eosin for histological analysis.

To characterize different follicle developmental stages, all follicles were divided into four classes. Briefly, based on the well-accepted standards established by Peterson and Peters (Pedersen & Peters 1968, Jiang 2015), the primordial follicles were identified as type 1-type3a, primary follicles (type 3b-type 4), secondary follicles (type 5), early antral follicles (type 6-type 7) and Graafian follicles (type 8). In particular, the primordial follicles refer to those follicles having a compact oocyte surrounded by a single layer of flattened granulosa cells (Johnson *et al.* 2004). In each section, only those follicles that contained oocytes with clearly visible nuclei were counted.

## Statistical analysis

All experiments were repeated at least three times. Data were evaluated by Student's *t*-test and

expressed as mean $\pm$ s.e.m. through GraphPad Prism 5 software (GraphPad). P < 0.05 was considered statistically significant.

## **Results**

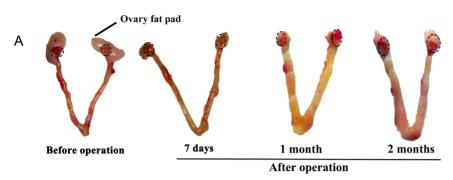
# Ovary fat pad-removed mice displayed subfertility and ovulated less matured oocytes

To study the roles of ovary fat pad in mouse reproduction, we removed this adipose tissue by surgical operation and only an incision at the same position was made with no lesion left. As shown in Fig. 1A, little fat adjacent to ovary was observed after different times of recovery (one week, 1 month and 2 months). After one week of recovery, these females were mated with adult males and the results showed that ovary fat pad-removed female mice were significantly subfertile, generating  $5.33 \pm 1.09$ (n=6) offspring per female, compared with  $16.8\pm1.66$ (n=5) offspring for control groups (Fig. 1B). Besides, the recovery time-dependent effect on reproduction after removal of ovary fat pad was also studied. We examined the fertility of ovary fat pad-removed mice recovered for one month and two months, respectively. As shown in Fig. 1A and B, although there was no sufficient adipose tissue regenerated around the ovary, we found that as recovering time extended, the treatment group displayed an increased trend on fertility. However, ovary fat padremoved mice still showed subfertility compared with the control group (for one month, control group vs treatment group:  $17.67 \pm 2.40$ , n=4 vs  $8.25 \pm 1.65$ , n=4, P<0.05; for 2 months, control group vs treatment group:  $14.67 \pm 0.67$ ,  $n=4 \text{ vs } 9.25 \pm 1.55, n=4$ ).

The subfertility could be due to a small number of mature oocytes ovulated for fertilization. Therefore, we counted the ovulated metaphase II (MII) oocytes after PMSG and hCG treatment and found that ovary fat pad-removed mice ovulated less MII oocytes than the control (19.64 $\pm$ 6.72, n=22 vs 8.35 $\pm$ 6.64, n=23; P<0.05) (Fig. 1C).

# The follicular development was abnormal in ovary fat pad-removed mice

Depending on the above results, we naturally speculated that the subfertility could be due to ovarian dysfunction, which leads to the inhibition of follicular development process. As shown in Fig. 2B, there was no significant difference in primordial follicle pool between control and experimental groups. However, the ovary fat



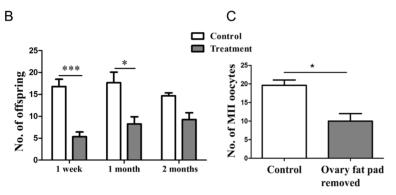
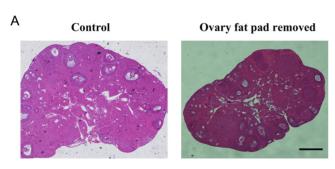
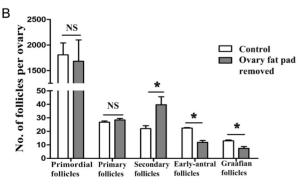


Figure 1
Ovary fat pad-removed mice displayed subfertility and ovulated less mature oocytes. (A) Adipose tissue around ovary did not reproduce obviously in ovary fat pad-removed mouse after 1-week, 1-month and 2-month recovery. The broken circle showed the ovary. (B) Breeding assay showed subfertility of the ovary fat pad-removed mice. \*P<0.05; \*\*\*P<0.001. (C) The number of MII oocytes ovulated *in vivo* decreased significantly after hormone treatment in ovary fat pad-removed mice. P<0.05. A full colour version

of this figure is available at http://dx.doi.





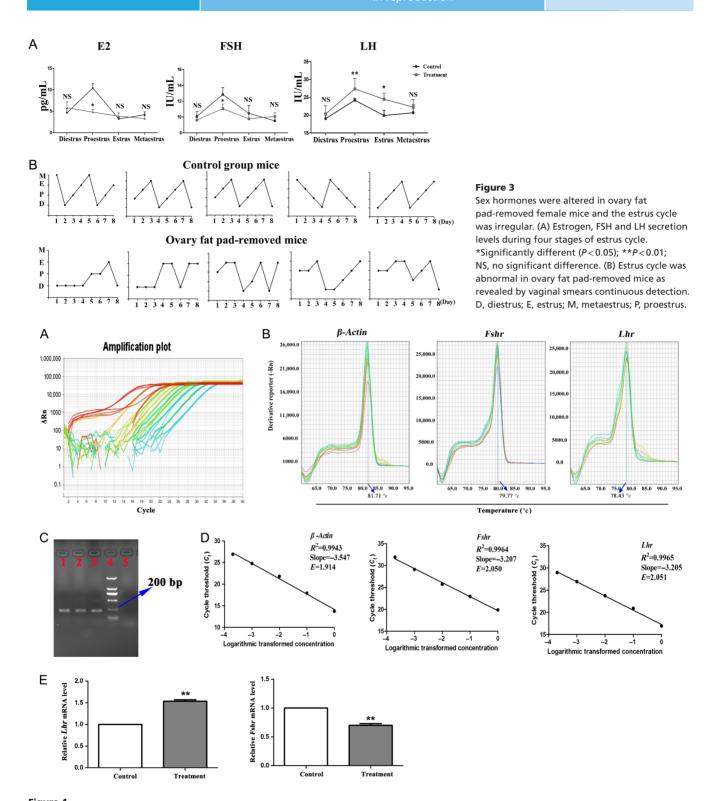
**Figure 2** The follicular development was inhibited in ovary fat pad-removed mice. (A) Histology of ovarian sections from ovary fat pad-removed mouse and control group mouse. Less-matured follicles were observed in experimental group. Bar= $500\,\mu m$ . (B) The quantifications of numbers of different types of follicles per ovary from ovary fat pad-removed group and control group. \*Significantly different (P<0.05); NS, no significant difference. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-16-0174.

pad-removed mice displayed a follicle development block at growing follicle stage, with less follicles developed to early antral follicles and eventually Graafian follicle stage (Fig. 2A and B). Specifically, a high proportion of follicles were blocked at secondary follicle stage (control group  $22.01\pm2.21$ , n=4 vs experimental group  $39.70\pm5.83$ , n=5; P<0.05).

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# The sex hormone levels are disordered and the estrus cycle is irregular in ovary fat pad-removed mice

As a large portion of follicles was blocked at growing stage especially secondary follicle stage, whereas follicle development was closely related to sex hormone regulation, we then measured the estrogen, FSH and LH levels at different stages of estrus cycle between ovary fat pad-removed mice and control group. We found that the levels of all these three sex hormones displayed abnormality, estrogen and FSH levels decreased especially at proestrus in experimental group (estrogen: control group  $10.43 \pm 1.02 \,\mathrm{pg/mL}$ ,  $n=4 \,\mathrm{vs}$  experimental group  $4.81 \pm 0.59 \,\text{pg/mL}$ , n=4, P < 0.05; FSH: control group  $12.18 \pm 1.06 \text{ IU/mL}$ , n=4 vs experimental group  $11.06 \pm 0.60 \text{ IU/mL}$ , n = 4, P < 0.05) (Fig. 3A). However, for LH, the level in ovary fat pad-removed mice displayed a rising trend (LH: control group 24.20 ± 0.56 IU/mL, n=4 vs experimental group  $27.37 \pm 2.91 \text{ IU/mL}$ , n=4, P < 0.01) (Fig. 3A).



FSH and LH receptors expression was affected after the ovary fat pad was removed in female mice. (A) Amplification plot of real-time qPCR for  $\beta$ -actin, fshr and lhr. (B) Melting curve of real-time qPCR for β-actin, fshr and lhr. The melting temperatures were as follows: β-actin, 81.71°C; Fshr, 79.77°C; Lhr, 78.43°C. (C) Agrose gel electrophoresis was used to detect the specificity of real-time qPCR products. Lane 1–5 represented fshr, Ihr, β-actin, DNA marker (500 bp) and negative control, respectively. (D) Determination of real-time qPCR efficiencies for β-actin, fshr and lhr. C, value vs relative logarithmic transformed cDNA concentration was calculated. The corresponding efficiencies were calculated according to the equation:  $E = 10^{(-1/slope)}$ . (E) Relative Fshr and Lhr mRNA level was decreased and increased, respectively, in female mice after ovary fat pad was removed. \*\*Significantly different (P<0.01). A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-16-0174.

**Table 1** Statistical analysis for amplification efficiency.

	Slope			R <sup>2</sup>			E		
Gene	1	2	3	1	2	3	1	2	3
β-Actin	-3.547	-3.510	-3.539	0.9943	0.9933	0.9958	1.914	1.927	1.916
Fshr	-3.207	-3.317	-3.187	0.9964	0.9969	0.9974	2.050	2.002	2.060
Lhr	-3.205	-3.192	-3.228	0.9965	0.9955	0.9968	2.051	2.057	2.041

Besides, we also detected the estrus cycle and found that ovary fat pad-removed mice displayed estrus disorder and prolonged period at some stages of estrus cycle (Fig. 3B). However, in control group, one estrus cycle is regular four days. Mice with prolonged estrus cycle also take averagely a long time to be mated with male mice because the male always mates with the female at a right stage of estrus cycle (data not shown).

# The hormone receptors expression was altered in ovary fat pad-removed mice

According to the abnormal hormone levels, we further studied the corresponding sex hormone receptors expression pattern. To unify the mice into the same estrus stage, they were treated with PMSG at estrus and after 48h, the ovaries were gathered to extract RNA for reverse transcription. We firstly confirmed the specificity of amplification and found that the melt curve showed a single peak and the product-specific melting temperatures were as follows:  $\beta$ -actin, 81.71°C; Fshr, 79.77°C; Lhr, 78.43°C (Fig. 4A and B). Besides, gel electrophoresis was also used to detect the specificity of RT-PCR products, and the lane of negative control group showed no obvious bands (Fig. 4C). Then, the RT-qPCR efficiencies were calculated from the relative standard curves for different primers based on the ABI StepOne/StepOnePlus system (Fig. 4D and Table 1). After real-time quantitative PCR analysis, we found that the level of Fshr decreased significantly (control group 1 vs experimental group  $0.70\pm0.03$ , n=3, P<0.01), but the *Lhr* level increased significantly (control group 1 vs experimental group  $1.535 \pm 0.04$ , n=3, P<0.01) (Fig. 4E).

## **Discussion**

In the present study, we investigated the role of ovary fat pad in female reproduction and found that adipose tissue surrounding the ovary is important for ovary function and folliculogenesis, and thus, female fertility.

The positive or negative feedback regulation mechanisms of sex hormones play important roles in

folliculogenesis, whereas adipose tissue is closely related with sex hormones. Androgens can regulate body fat distribution in humans, and extensive tissues including adipose tissue can biosynthesize estrogen in higher primates (Simpson *et al.* 1994, O'Reilly *et al.* 2014). It was also reported that sex hormone imbalance was causally related with visceral adipose tissue (AT) dysfunction and visceral obesity (Zhang & Sairam 2014). Besides, it was found that the vertebral bone marrow fat fraction increased during the follicular phase and showed a non-significant decrease during the luteal phase. They demonstrated that 17-β estradiol can rapidly reduce the marrow fat fraction in women (Limonard *et al.* 2015).

In our study, we found that compared to the control group the estrogen and FSH levels in ovary fat padremoved mice decreased overall especially at proestrus of estrus cycle (Fig. 3A). As we all know, in the follicular phase, gonadotropins especially FSH can enhance the production of estrogen in granulosa cells (Erickson & Hsueh 1978, Jamnongjit & Hammes 2006). Besides, with the follicle development, the estrogen secretion increases and then activates the FSH receptor expression (Chakraborty & Roy 2015). We observed that, after ovary fat pad removal, the estrogen level decreased and Fshr expression was downregulated (Fig. 4E). In addition, as shown in Fig. 2A and B, compared to the control group, more follicles were blocked at the secondary follicle stage and fewer antral follicles were observed in the ovary of ovary fat pad-removed mice. During the transition from preantral to antral follicle stage (also named gonadotropinresponsive phase), FSH plays important roles (McGee & Hsueh 2000, Fortune 2003). Therefore, we believed that the low level of FSH could not support this transition from preantral to antral follicle stage and a large portion of follicles were blocked at the secondary follicle stage in ovary fat pad-removed mice. Thus, at the gonadotropindependent phase, only a few follicles developed to antral stage because of the low level of estrogen and FSH.

Interestingly, the LH level in the experimental group was higher than that of control group and the relative LH receptor expression was also higher (Figs 3A and 4E). It was reported that the pulsatile GnRH/LH release is negatively regulated by circulating estrogen from the

ovary to fine-tune gonadotropin secretion for follicular maturation (Akema et al. 1984, Karsch 1987). It was reported that dynorphin-kappa opioid receptor signaling can partly mediate estrogen negative feedback effect on GnRH/LH pulses by reducing the kisspeptin release in female rats (Mostari et al. 2013). Besides, LH can decrease FSH sensitivity during preantral-early antral transition (Orisaka et al. 2013). Taking this factor into consideration, the oversecreted LH in ovary fat pad-removed mice may lead to FSH sensitivity downregulation and less matured follicles formation. Evolutionarily, in order for species continuation, mice are more naturally inclined to generate a large amount of offspring, with the minority strong individual standing up to natural selection. But in our studies, because of the less matured follicles in ovary fat pad-removed mice, the high level of LH and Lhr still cannot afford to generate the enough MII oocytes and offsprings eventually.

The abnormal estrus cyclicity in ovary fat padremoved mice was mainly due to disordered sex hormone secretion. However, in primiparous Charolais cows, subcutaneous body lipids can affect cyclicity and estrus behavior, and estrus duration was longer in low adipose group at calving than high adipose group (Recoules *et al.* 2013). Therefore, as a part of subcutaneous fat, the loss of fat around ovary may also lead to the irregular estrus cycle (Fig. 3B).

For the low level of FSH, two possible reasons are suggested. It was reported that estrogen receptor  $\beta$  could regulate estradiol-mediated stimulation of granulosa cell growth and modulation of FSH action (Krege et al. 1998), and there may be a positive feedback pathway between estrogen and FSH. Thus, one possibility is that decreased estrogen can downregulate FSH secretion. As we all know, both underweight and overweight women find it hard to become pregnant. In fact, adolescent girls show a significant decrease in waist-to-hip ratio and large amount of fat accumulates at lower abdomen (Roemmich & Rogol 1999). With adipose tissue distributing to the lower abdomen, an appropriate environment is ready for not only fetal gestation but also ovary development. In mammary gland development, mammary ducts are surrounded by fat pad, which can supply necessary nutrients and cell factors to promote mammary ducts growth (Howard & Gusterson 2000). Similarly, fat around ovary may play important roles for folliculogenesis. It is believed that adipose tissue can secrete many cytokines, and some adipokines can not only simulate the cell proliferation but also regulate gonadotropin secretion

in HPG axis (Mitchell *et al.* 2005, Maleszka *et al.* 2014). Thus, the other possibility for decreased FSH level is that the level of adipokines and some growth factors that are secreted by ovary fat pad decrease after this adipose tissue is removed, which may affect the follicular development through a paracrine-like pathway. As it was reported in bovine ovary and buffalo, locally produced leptin and its receptor play important roles in promoting angiogenesis, steroidogenesis and the development of the corpus luteum through an autocrine/paracrine effect (Sarkar *et al.* 2010, Kumar *et al.* 2012).

However, as Fig. 2B showed, after 2-month recovery, the fat pad-removed mice showed a tendency of recovery in fertility (even there was no statistical difference). But little fat adjacent to ovary was observed even after 2-month recovery (Fig. 1A), which also made us puzzled. Maybe the ovary fat pad can affect fertility through short-time effects (such as within one month), after which the body can modify these defects through some ways, like the compensatory mechanism. Of course, the specific mechanism about ovary fat pad functions still needs to be properly studied in the future.

In conclusion, our study indicates that ovary fat pad is important for female fertility in mice. It can influence folliculogenesis of ovary by the regulation of estrogen secretion and the release of gonadotropins.

## **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.

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### Author contribution statement

Qing-Yuan Sun, Jun-Yu Ma and Hong-Hui Wang helped with conception and design. Qian Cui, Teng Zhang, Lei Guo, Ming-Zhe Dong, Yi Hou, Zhen-Bo Wang and Wei Shen contributed to acquisition of data. Qing-Yuan Sun, Jun-Yu Ma and Hong-Hui Wang contributed to the analysis and interpretation of data, and drafting the article. All authors approved the final version of the manuscript.

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