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Short-term exposure of sleep deprivation male model rats to dutasteride promotes the discovery of gene profiles related to fertility impairments

Shengxiao Zhang[1,2,3], Wei Li[2,4*] and Guirong Zhang[1,2,3*]

Abstract

Background Fertility was impaired in sleep deprivation (SD) male rats. Dutasteride (DUT), a 5-α reductase inhibitor, also had negative effects on fertility, however, its definite mechanisms were still unknown. To this end, SD male model rats, exposed to DUT for a short period of time, were used to observe whether DUT would exacerbate fertility damage and further to explore the possible mechanism of its fertility damage effects.

Methods Male rats were randomly divided into 3 groups: Model control (MC) group and the two model groups (i.e. SD group and DUT group). For model groups, the modified multi-platform method was used to model SD for 42 consecutive days. The rats in DUT group were administered by gavage at DUT doses of 20 mg-kg^{-1} for 21 consecutive days. Whether the short-term administration of DUT would bring the aforesaid rats about fertility damage was confirmed by observing rats' behavioral changes, detecting serum testosterone levels, testing sperm-related parameters, and pathological analysis of testicular and epididymal tissue changes. RNA sequencing (RNA-seq) was applied to explore the overall transcriptional profile of genes related to DUT-induced exacerbation of fertility impairment in SD model male rats. By analyzing the differentially expressed genes (DEGs) profiles among those groups, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were used to explore the gene profile associated with DUT promoting fertility impairments and further to find the mechanism of common fertility damages between SD and DUT. Finally, the transcription of key genes was verified by quantitative reverse transcription polymerase chain reaction (qRT-PCR). This experimental work was carried out in 2023–2024.

Results After exposure to DUT, the rats had significantly changed sperm-related parameters and showed further aggravated gonadal tissue damage. Compared with the model control, the SD rats had 445 DEGs. And compared with the SD group, the DUT group had 477 DEGs. Ibsp, Runx1, Fgf18, Areg, Stat6, Mapk3, and Gng4 were screened as the hub genes by Cytohubba. 142 cross-DEGs were identified. Notably, Abca5 expression was continuously decreased

*Correspondence:
Wei Li
leewei318@163.com
Guirong Zhang
guirong_zhang@126.com

Full list of author information is available at the end of the article

in this exposure process. In the cross-DEGs, ABCA5 interacted indirectly with LOR, SLC22A2, PCARE and RCVRN by its family proteins.

Conclusions Short-term exposure to DUT aggravated the fertility impairment of SD rats and unique actions on the aforesaid hub genes might be one of the possible reasons. The 142 common fertility-impairing DEGs involved in immunological, neurological and metabolic aspects. The abnormal expression of Abca5 and its indirectly associated other cross-DEGs might be the important gene targets for fertility disorders in male rats.

Keywords Dutasteride, Sleep deprivation, Fertility, RNA-seq

Background

Dutasteride (DUT) is a dual inhibitor of SRD5A types 1 and 2, which prevents the conversion of testosterone to dihydrotestosterone (DHT) and affects multiple parts of the endocrine system [1]. It is widely used in the treatment of androgenetic alopecia and benign prostatic hyperplasia [2].

Studies have reported that DUT was associated with a series of adverse events such as gynecomastia [3], erectile dysfunction (ED) [4]. In addition, a case was reported of male external genital abnormalities at birth after maternal exposure to spironolactone and DUT at 8 weeks of gestation [5]. Moreover, even fewer studies have reported that DUT had little effect on men with normal semen quality but had a negative effect on sperm count in men with oligozoospermia [6]. Whereas the results of long-term DUT interference, such as gender identity, sexual function, hormonal maturation throughout puberty and fertility remain unknown [5]. Limited evidence suggested that DUT was significantly inhibited male fertility in rats by regulating the expression of the Ca influx related gene CatSper [7], reducing serum DHT levels, inhibiting Claudin1 and β-catenin expression, and disrupting epididymal epithelial cell junctions [8]. Furthermore, exposure to DUT significantly reduced fish fecundity and impacted various aspects of reproductive endocrine function in males and females [9]. These findings indicated that the drug adversely affects sperm maturation and fertility.

DUT has been shown to reduce fertility, yet the underlying mechanisms of its fertility toxicity remain unclear. Therefore, further research is essential to identify the specific fertility targets affected by DUT. However, due to the limitations of conventional methodologies, fully elucidating the extent of DUT-induced fertility impairment and pinpointing its exact targets remains a significant challenge.

Previous studies showed that sleep deprivation (SD) could adversely affect male rats' fertility, which was reflected in abnormal gene expression in the testis [10, 11], defective spermatogenesis, reduced sperm quality and damage to the integrity of the acrosome [12]. Moreover, Chronic sleep deprivation disrupted the permeability of the reproductive tract barriers (blood-testicular and blood-epididymal barriers), leading to a decrease in ejaculatory function and a reduced likelihood of pregnancy [13]. Obstructive sleep apnea was shown to compromise organ function and led to erectile dysfunction [14]. Sleep loss caused by rapid eye movement (REM) sleep deprivation affected spermatic cord function and impaired reproduction [15]. Following SD and a 7-day recovery period, sperm parameters, particularly sperm motility and testosterone levels, did not return to baseline levels [16]. In addition, SD led to impaired fertility in female animals [17]. Seventy-two hours of total SD induced transcriptomic changes in oocytes of female mice, resulting in oocyte defects and impaired early embryonic development [18]. Nocturnal sleep disturbances were associated with a slight reduction in fertility, and shorter sleep duration had a weaker inverse association with fertility [19]. Pathological sleep patterns adversely affected women's health, is closely associated with sub-infertility and early pregnancy loss, and reduced the success rate of assisted reproductive technology [20, 21]. In conclusion, SD can lead to impaired fertility in humans and experimental animals.

Given the increasing incidence of SD, a key unanswered question is whether exposure to additional reproductive stressors, such as pharmacological agents, could lead to a significantly greater impairment in fertility in sleep-compromised individuals. Therefore, we proposed to use the established SD model as the background to investigate the subsequent changes in fertility following short-term administration of DUT. The SD model of male rats was established and then exposed to DUT. High-throughput RNA sequencing (RNA-seq) was performed by next generation sequencing (NGS). By comparing the transcriptomes of SD model rats and DUT-exposed rats, we aimed to address the following two key questions: (A) Did short-term exposure to DUT aggravate fertility impairment in SD model rats? If so, what was the possible mechanism? (B) Was there a common mechanism of fertility impairments in SD model rats and DUT-treated rats? Were there any overlapping genes involved in fertility damage, and how were they expressed? What functions were they involved?

Methods

Experimental grouping and drug administrations

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals of Hebei North University and was approved by its Animal Ethics Committee (HBNU202307012104). The number of animals used and their suffering were minimized. In-bred 7-week-old male Wistar rats (24 total, 220 ± 20 g, SPF (Beijing) Biotechnology Co., Ltd.) were maintained in plastic cages in the Animal Barrier Center of the Department of Pharmacy, Hebei North University, with the license number: SYXK (Ji) 2024-006 (25 ± 2 °C, 50–60% humidity, natural light-dark cycle, < 55dB noise) with ad libitum access to standard diet and water. The sample size was set at $n = 8$ per group, consistent with common practice in comparable rodent studies. Rats were randomly assigned to three groups: Model control (MC), Sleep deprivation (SD), and Dutasteride (DUT), with the individual animal defined as the experimental unit. The random allocation sequence was generated using the RAND function in Microsoft Excel. To minimize confounding biases, the order of behavioral tests and tissue collection was randomized. All animals that successfully underwent the modeling procedure were included in the analyses. No pre-established exclusion criteria were defined, and no animals or data points were excluded, resulting in a final sample size of $n = 8$ for all groups.

Treatment

SD and DUT groups prepared SD model by the modified multi-platform method (MMPM) with reference to our previous research [10]. After 21 days of SD, animals in the DUT group were given DUT (Macklin, Shanghai, China) (20 mg·kg^{-1}, dissolved in 0.5% carboxymethyl cellulose (CMC)) by gavage for 21 days, while the SD group was received 0.5% CMC for the same duration. The rats were placed in a 120 × 80 × 40 cm size tank containing eight circular platforms (each 6.5 cm in diameter) with water levels approximately 1 cm below the platform. The rats could move by jumping between platforms, and when they entered REM sleep, muscle relaxation caused their head to touch the water and wake them up. They were deprived of sleep for 18 h per day (16:00- next day 10:00) for 6 weeks. Rats in the MC group, which served as the negative control were placed in a tank equipped with a partition (18 h per day, 6 weeks), and 1 cm below the partition was filled with water to exclude the influence of external water environment on male fertility. From the fourth week, they were given 0.5% CMC until the experiment ended (Fig. 1). All groups were given normal diet and drinking water except for the corresponding treatment factors. The study was conducted between 2023 and 2024.

Open field tests

According to the method of Talaee et al. [22], the open-field behavior test was performed after modeling. The total distance of activity, movement speed and center residence time of each rat during the 5-minute session were recorded by animal behavior analysis software (Montech, Chengdu, China) to evaluate their exploration ability and anxiety.

Sperm-related parameters

According to the method of Chen et al. [10], after the rats were euthanized (3% sodium pentobarbital, 200 mg/kg), the left epididymis cauda was cut out and placed into a preheated 37 °C TYH Medium (EasyCheck, Nanjing, China) to prepare sperm suspension.

The assessment of sperm parameters was conducted according to established methodologies. Briefly, sperm concentration was measured using a hemocytometer (Neubauer chamber). To facilitate accurate counting, the seminal fluid was first diluted at a ratio of 1:100 with pre-warmed TYH medium [10].

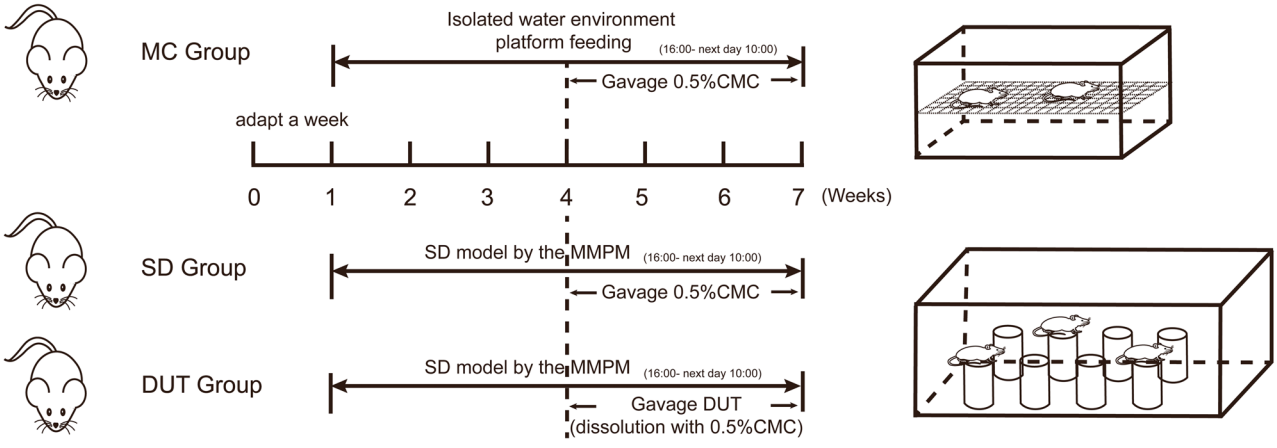

Fig. 1 Experimental grouping and experimental timeline

For sperm motility analysis, the seminal fluid was diluted with pre-warmed (37 °C) TYH medium at a ratio of 1:100. The diluted sample was examined under a phase-contrast microscope at 400× magnification. A minimum of 300 spermatozoa per sample were evaluated and categorized as fast progressive, slow progressive, or non-motile. Sperm vitality was represented by the proportion of motile sperm to all sperm counted.

The abnormal sperm morphology rate was determined by evaluating 200 sperm per sample under 400× magnification. Sperm were categorized as either normal or exhibiting specific abnormalities, including defects in the head only, tail only, or the presence of a double head [10].

Hormonal analysis in serum

Blood samples were collected from aorta abdominalis and stored at 4℃ overnight. After that, the blood samples were centrifuged (3500 × g) at 4℃ for 10 min. The serum testosterone levels were determined according to the manufacturer's instructions (Cusabio, Wuhan, China).

Histopathological studies

Three rats were randomly selected from each group. According to the description in Cheng report [23], the right testis and epididymis were collected and fixed in 4% paraformaldehyde solution for 48 h. After dehydration and embedding, the tissue (testis and epididymis) was sliced at 5 μm thickness and stained using the hematoxylin-eosin (H&E) kit (Solarbio, Beijing, China), then observed under a 40× optical microscope (Leica, Germany).

RNA-sequencing

Transcriptome sequencing was performed on three groups of rat testis tissues, based on Illumina sequencing platform. Some of the routine steps and analytical methods of RNA sequencing included RNA extraction and detection, library construction, sequencing, quality control, reference genome alignment and quantitative analysis. The expression difference multiple | log2Fold Change | > 0.5 and p-value < 0.05 were used to screen the differentially expressed genes (DEGs). DEGs were identified for the comparisons of SD vs. MC and DUT vs. SD. The above-mentioned DEGs were submitted to the David database (https://David.ncifcrf.gov/) for Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of genes and genomes (KEGG) pathway enrichment analysis to obtain relevant biological functions and signaling pathways. Protein-protein interactions (PPI) networks of DEGs-encoded proteins were constructed using Cytoscape software and STRING databases (https://cn.STRING-db.org/).

qRT-PCR

According to Vahabi Barzi [24], total RNA was extracted from testis using Trizol reagent. The mRNA expression of Abca5, Dohh, Mybbp1a and Tff2 genes were detected by qRT-PCR (Bio-rad, USA). All steps followed the manufacturer's instructions (Takara, Beijing, China). The relative mRNA expression was calculated using the $2^{-\triangle\triangle Ct}$ method.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9, normality of the data was analyzed using the Kolmogorov-smirnov test, and homogeneity of variance was analyzed using the Bartlett test. Data that met the assumptions of normality and equal variance were analyzed using analysis of variance (ANOVA). For data that violated these assumptions, non-parametric tests were employed. $p < 0.05$, the difference was statistically significant. All data were expressed as mean ± standard deviation.

Results

DUT changed the general physical signs of SD model rats

Compared with MC group, the rate of weight gain in SD group was lower, and there were more bite marks in SD group. The SD group rats showed impatience in the early stage and listlessness in the later stage. There was a continuing trend of weight loss after DUT exposure. Compared with SD group, the testis mass of DUT group decreased significantly ($p < 0.01$). Compared with MC group, both SD and DUT groups showed a significant decrease in testosterone content ($p < 0.001$, $p < 0.01$). No significant change in testosterone levels was observed between the DUT and SD groups, with levels being almost identical (Fig. 2).

DUT changed the behavior of SD model rats

Compared with MC group, both SD and DUT groups showed significant differences in total distance, speed and time of central region ($p < 0.001$, $p < 0.001$). The DUT group showed a significant increase in both speed and central residence time compared with MC group ($p < 0.001$, $p < 0.001$). Compared with SD, the time in the central region of DUT group was significantly increased ($p < 0.001$) and there was no significant difference in total movement distance and speed (Fig. 2).

DUT can change sperm parameters of SD model rats

Compared with MC group, the total sperm count, sperm motility and sperm viability of SD group were significantly decreased, and the abnormal rate was significantly increased ($p < 0.001$). The DUT group showed significantly lower sperm concentration, motility rate, and vitality, but a higher abnormal rate than the MC group

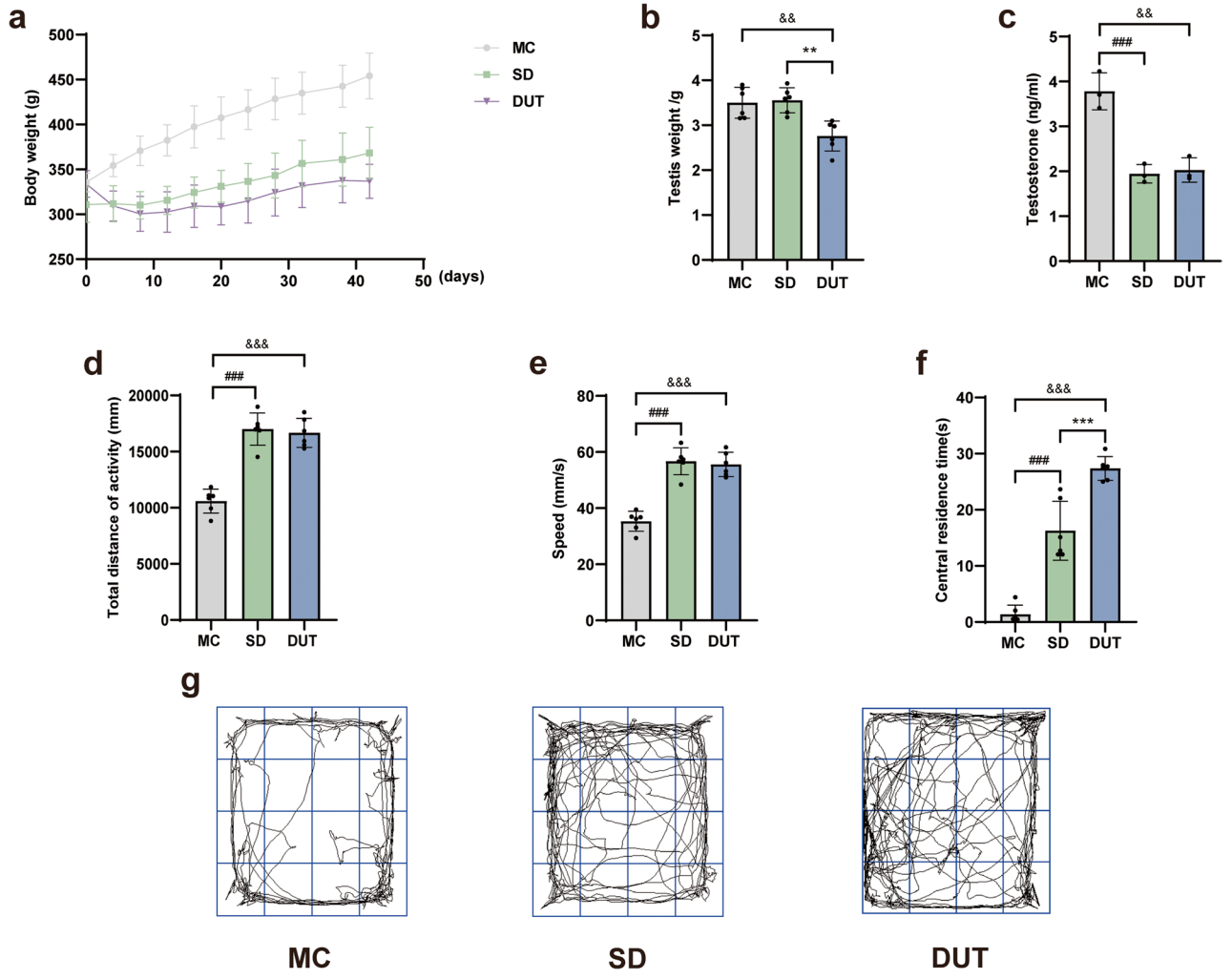

Fig. 2 Changes in general signs of rats in each group. Changes in body weight **a**, testicular weight **b** and testosterone content **c** of rats in each group. Changes in total distance of activity **d**, speed **e**, central residence time **f** and representative route of movement **g** of rats in each group $n=6$ in indicators a-b and d-f, $n=3$ in indicators c $^{\#\#\#}p<0.001$ SD vs. MC group. $^{**}p<0.01$, $^{***}p<0.001$ DUT vs. SD group. $^{\&\&}p<0.01$, $^{\&\&\&}p<0.001$ DUT vs. MC group

(all $p<0.001$). Compared with SD group, the total sperm count and sperm motility rate of DUT group were significantly decreased ($p<0.01$, $p<0.001$), the sperm viability was decreased and the deformity rate was significantly increased ($p<0.001$) (Fig. 3).

DUT changed testis and epididymis pathological manifestations in SD model rats

Histopathological examination of the testis showed that the testicular cytoarchitecture was preserved in the MC group rats. However, the SD group was obviously abnormal, which showed that the seminiferous tubules were arranged loosely and the spermatozoa in the lumen were decreased greatly. Compared with the SD group, the DUT group was severely damaged, the seminiferous tubules were arranged disorderly, the layers of spermatogenic cells at all levels were basically disappeared (Fig. 4a-f).

Histopathological examination of the epididymis showed that the epididymal cytoarchitecture was preserved in the MC group rats with normal sperm density within the lumen. Nevertheless, the SD group showed reduced sperm density within the lumen. The DUT group had more severe damage, a substantial decline in the number of sperm, and most of the lumen was vacuolar (Fig. 4g-l).

Gonadal histomorphometry indicated significant alterations in both the SD and DUT groups relative to MC group. Specifically, the diameter of seminiferous tubules ($P<0.01$, $P<0.001$), the average number of spermatogonia ($P<0.01$, $P<0.01$), and the height of seminiferous epithelium ($P<0.05$, $P<0.01$), were significantly reduced in both the SD and DUT groups, whereas the height of epididymal epithelium was increased ($P<0.01$, $P<0.01$). When comparing DUT group to SD group, exposure to DUT was associated with a further significant reduction

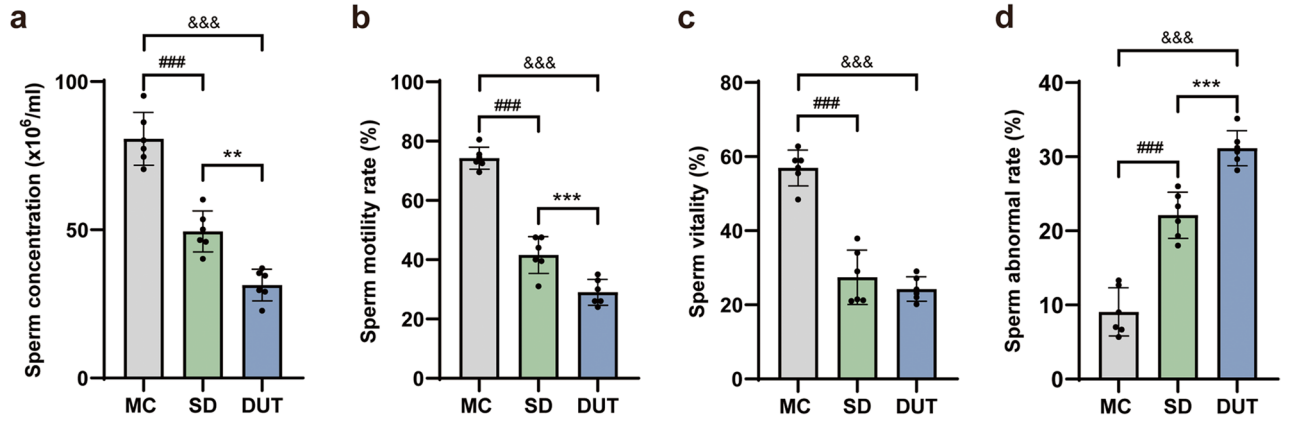

Fig. 3 Changes of sperm-related parameters, Sperm concentration **a**, Sperm motility rate **b**, Sperm vitality **c**, Sperm abnormal rate **d** in each group. $n = 6$ (All analysis) for each group $^{\#\#\#}p < 0.001$ SD vs. MC group, $^{**}p < 0.01$, $^{***}p < 0.001$ DUT vs. SD group, $^{\&\&\&}p < 0.001$ DUT vs. MC group

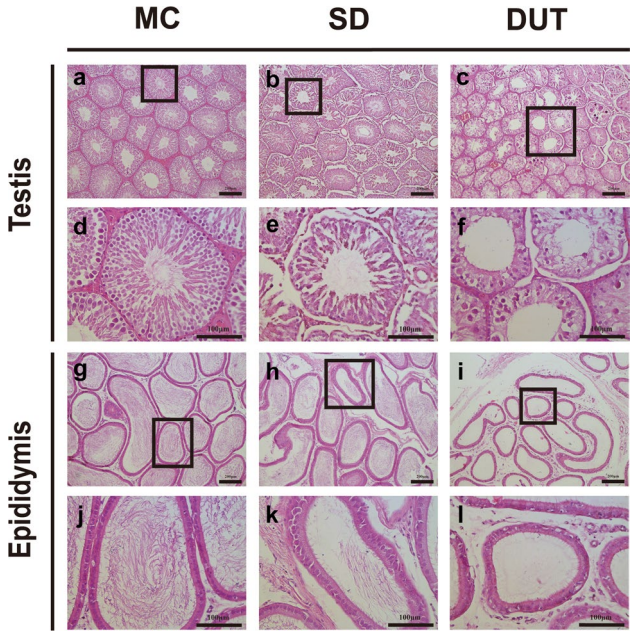

Fig. 4 Histopathological changes in the testis and epididymis (magnification, 100X, 400X; scale bar represents 200 μm, 100 μm.)

in seminiferous tubule diameter ($P < 0.001$). A decreasing trend in the average number of spermatogonia was also noted, but no significant differences were found in the other parameters (Fig. 5).

Effects of DUT on the transcriptome of rat testis

Expression of DEGs in SD vs. MC

The gene expression results of MC, SD and DUT groups were compared in testis. Compared with MC group, there were 445 DEGs in SD group, including 223 up-regulated genes and 222 down-regulated genes. GO was a functional classification system to describe genes and gene products in organisms, which was mainly reflected in cellular component (CC), molecular function (MF) and biological process (BP). GO enrichment for SD vs. MC revealed that these DEGs were mainly involved in circadian rhythm, hormone secretion, negative regulation of immune system processes, and other BP, including steroid binding, hormone activity and other MF. KEGG of SD vs. MC enrichment analysis indicated that DEGs were mainly involved GnRH secretion, steroid hormone biosynthesis and retinol metabolism etc. The interacting

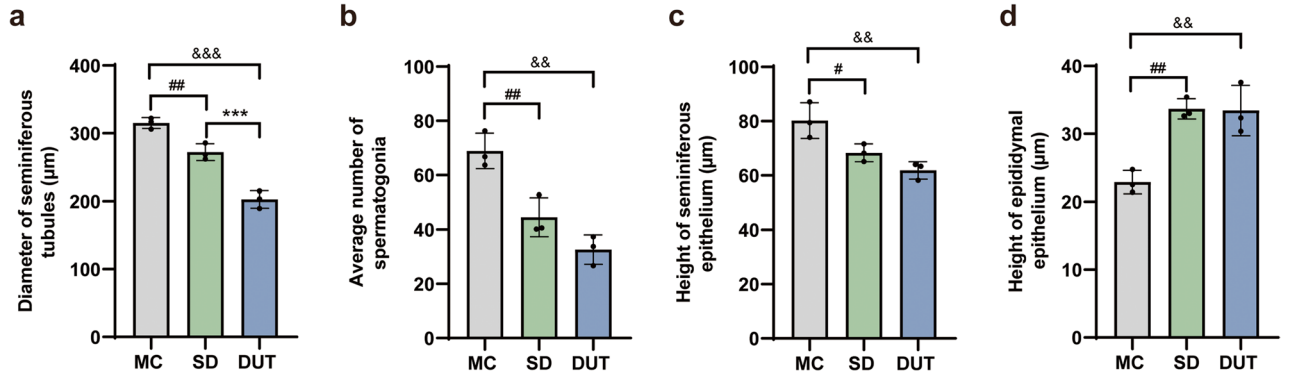

Fig. 5 Gonadal morphometric analysis of male rats in various groups, Diameter of seminiferous tubules **a**, Average number of spermatogonia **b**, Height of seminiferous epithelium **c**, Height of epididymal epithelium **d** in each group. $n = 3$ (All analysis) for each group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ SD vs. MC group, $^{***}p < 0.001$ DUT vs. SD group, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ DUT vs. MC group

DEGs encoding proteins in the PPI network were identified by Cytoscape. They were arranged in descending order from red to orange to yellow according to degree values. Maximal clique centrality (MCC) performed better in the accuracy of predicting essential proteins from PPI networks. Cytohubba was used to screen SPP1, IBSP, TNFSF11, SP7, IHH, POSTN, NPW, NMS, GPSM3, and OPRM1 as the core targets of DEGs in SD vs. MC according to the MCC algorithm. Among them, the expression of Spp1, Ihh, Postn, Npw, Gpsm3, Oprm1 were up-regulated, and the expression of Ibsp, Tnfsf11, Sp7, and Nms were down-regulated. These genes identified above were found to be potential targets for SD induced fertility impairment (Fig. 6).

Expression of DEGs in DUT vs. SD

Compared with the SD group, 477 DEGs were screened out in the DUT group, of which 267 were up-regulated and 210 were down-regulated. GO enrichment for DUT vs. SD revealed that the aforesaid DEGs were mainly involved in BP such as response to glucocorticoid, response to corticosteroid, arginine metabolism, and regulation of somatostatin secretion. They were involved in CC such as lipid transporter activity, arginine binding, and MF such as extracellular matrix and dopaminergic

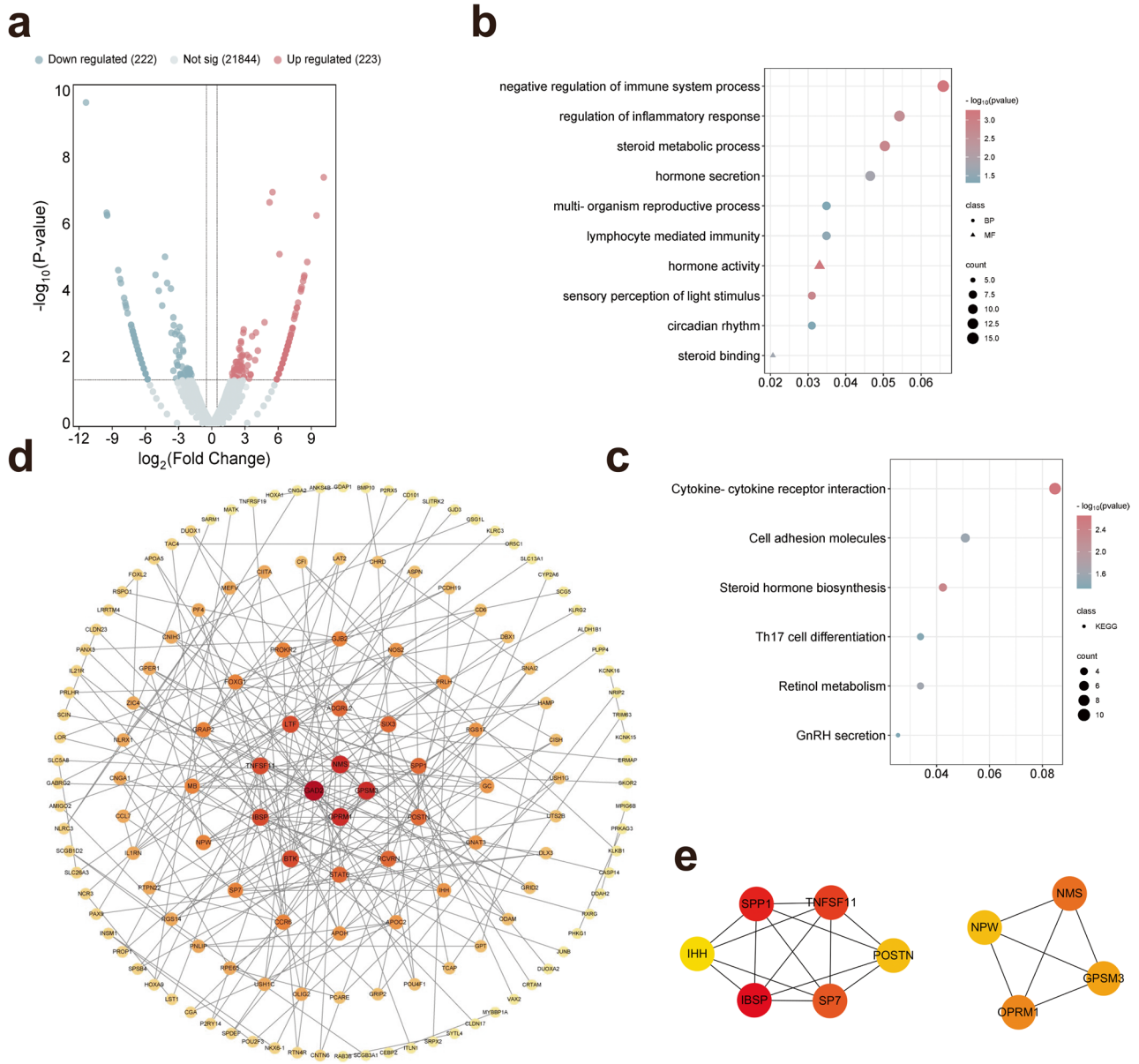

Fig. 6 Analysis of DEGs in SD vs. MC. Volcano plot of SD vs. MC **a**, GO enrichment analysis of SD vs. MC **b**, KEGG enrichment analysis of SD vs. MC **c**. Cytoscape was used to screen the protein interactions encoded by DEGs **d**. Hub genes of DEGs were screened by Cytohubba **e**

synapse. KEGG of DUT vs. SD enrichment analysis indicated that the DEGs were mainly concentrated neuroactive ligand-receptor interaction and calcium signaling pathway. IBSP, RUNX1, FGF18, AREG, STAT6, MAPK3, GNG4 were screened as hub targets of DEGs in DUT vs. SD using Cytohubba. Among them, the expression of Ibsp, Runx1, Areg, Mapk3 and Gng4 were up-regulated, and the expression of Fgf18 and Stat6 were down-regulated. The above genes might be the potential targets of impaired fertility in DUT exacerbated SD model rats (Fig. 7).

Expression interactions of common DEGs among MC, SD, and DUT groups

The overlapping DEGs between SD vs. MC and DUT vs. SD were analyzed, with a total of 142 cross-DEGs. These 142 cross-DEGs were mainly involved in the following five functional categories. Tnfrsf19, Nos2 and Clec2g are related to the immune system. Tff2, Scgb3a1 and Gnat3 are related to nerve development and conduction. Rhox11, Tmem154 and Six3 are related to embryonic development and organ formation. Dohh, Abca5 and Phkg1 are related to metabolism and transport. Prl7d1, Prkag3 and Junb are related to cell growth and proliferation. GO and KEGG enrichment analyses showed that the cross-DEGs were involved in BP such as response

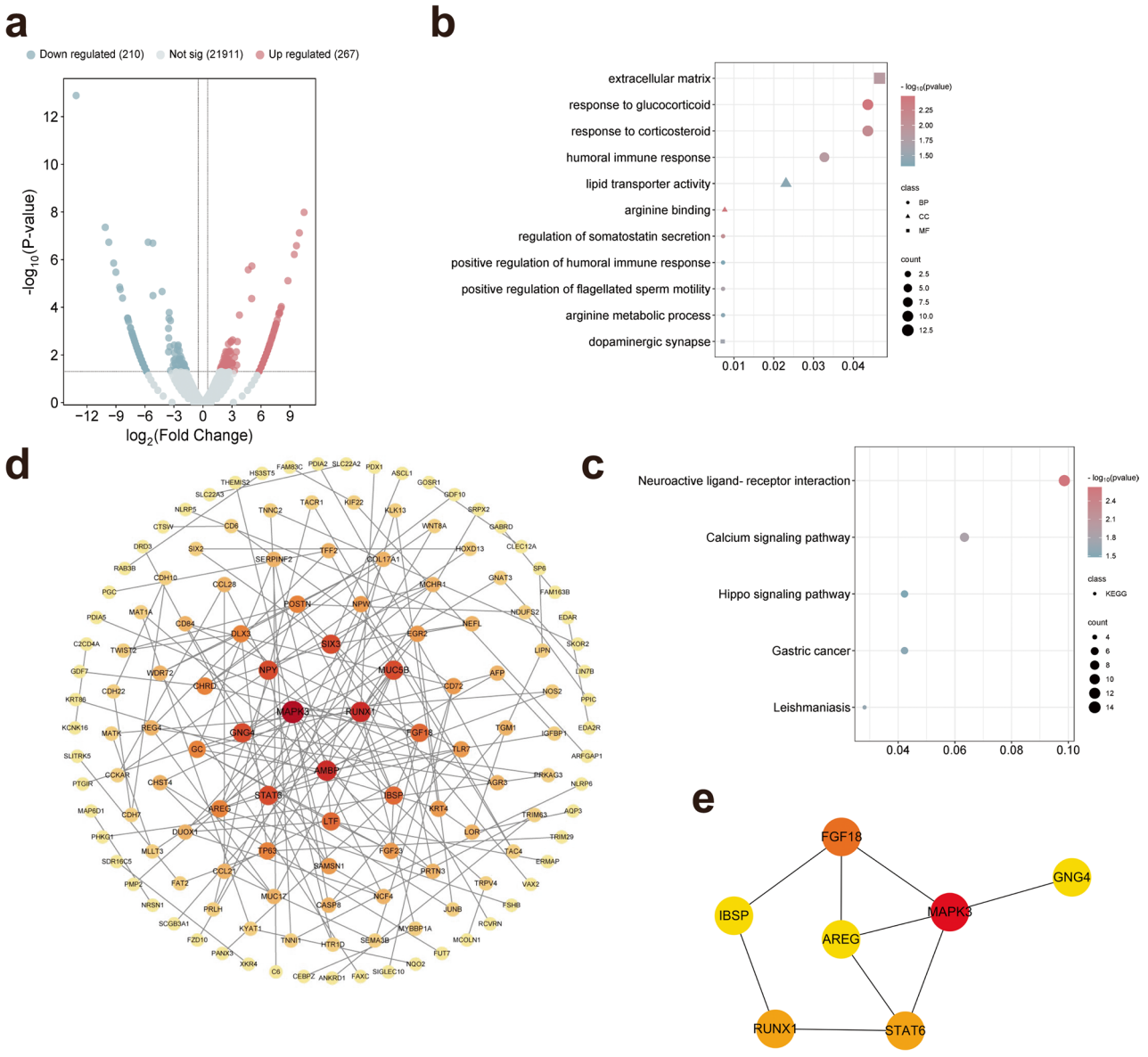

Fig. 7 Analysis of DEGs in DUT vs. SD. Volcano plot of DUT vs. SD **a**, GO enrichment analysis of DUT vs. SD **b**, KEGG enrichment analysis of DUT vs. SD **c**. Cytoscape was used to screen the protein interactions encoded by DEGs **d**. Hub genes of DEGs were screened by Cytohubba **e**

to estradiol and regulation of neuroblastoma proliferation, in MF such as hormone activity and calcium channel activity, and in cell adhesion molecules signal pathways. PPI results showed that there were 87 DEGs encoding proteins. A few proteins, such as NOS2/TAT6/JUNB、DLX3/IBSP/PANX3、RCVRN/SIX3/CHRD/VAX2, interact with each other, but most of them were not directly related. The interactions of proteins encoded by the cross-DEGs were demonstrated using Cytoscape. Notably, the Abca5 gene was found to be impaired after SD, and the impairment was exacerbated by exposure to DUT (Fig. 8).

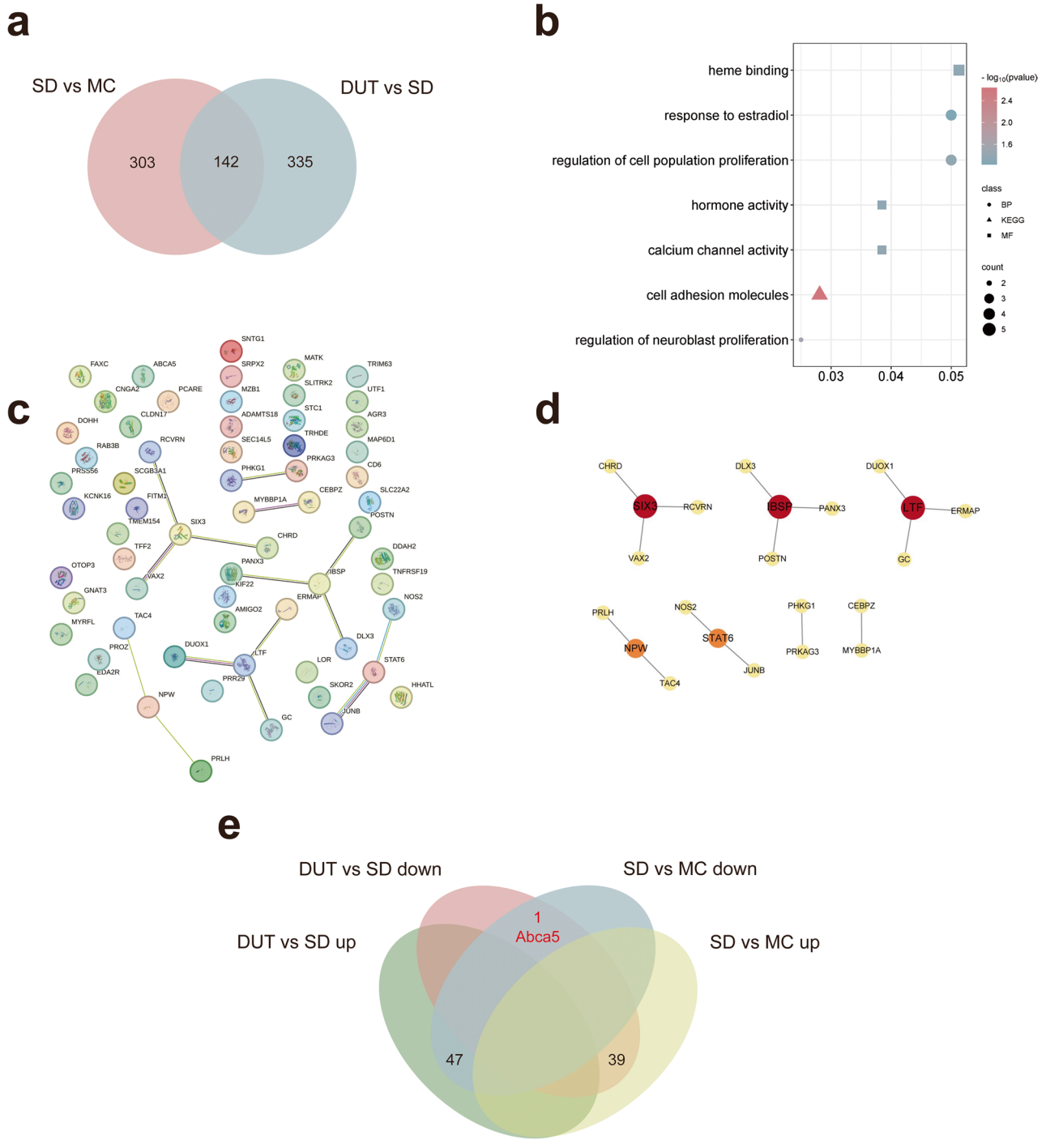

Fig. 8 Cross-DEGs analysis of SD vs. MC and DUT vs. SD. Veen plots of SD vs. MC and DUT vs. SD **a**, GO and KEGG enrichment analysis of the cross-DEGs **b**, PPI of the cross-DEGs **c**. Cytoscape was used to screen the interaction of proteins encoded by the cross-DEGs **d**, up-regulated and down-regulated Veen plots of the cross-DEGs **e**

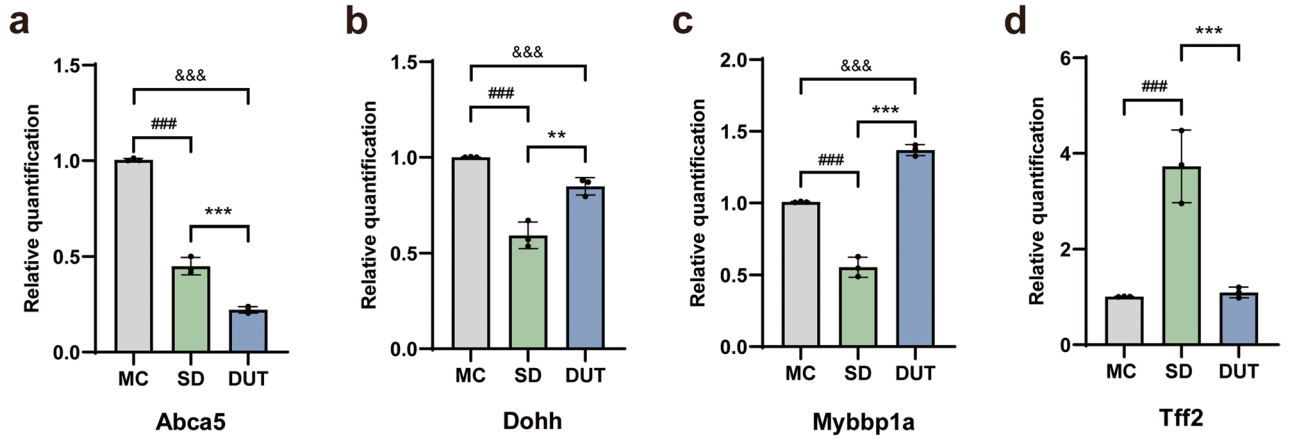

Fig. 9 mRNA expression of Abca5 **a**, Dohh **b**, Mybbp1a **c** and Tff2 **d**. $n = 3$ for each group $^{\#\#\#}p < 0.001$ SD vs. MC group, $^{**}p < 0.01$, $^{***}p < 0.001$ DUT vs. SD group, $^{\&\&\&}p < 0.001$ DUT vs. MC group

Table 1 Primer sequences of genes

Gene Name	Forward Primers 5′→3′	Reverse Primers 5′→3′
Abca5	TGGAGTACCCCGTTCATTCAG	TGGCAAAGTAAGGTGGCATTG
Mybbp1a	CGGGAGCTGAGAGTGTGTTT	CAGGGTCTGGGTGGTCTTTC
Tff2	CCACTTCCAAACCAAGCGTC	AGAAACACCAGGGCACTTCG
Dohh	CCCGAAAGTTCTAGGCCTCC	CACTCTAGACGCCTAACGGC
β-actin	GATGACGATATCGCTGCGCTC	CTGACCCATACCCACCATCAC

DUT changed the mRNA expression of Dohh, Abca5, Tff2 and Mybbp1a

The mRNA expression levels of Abca5, Dohh, Mybbp1a and Tff2 were analyzed by qRT-PCR (Fig. 9). Compared with MC group, Abca5, Dohh, Mybbp1a and Tff2 in SD group were marked changed, and the differences were statistically significant ($p < 0.001$). Relative to MC group, gene expression in DUT group was significantly down-regulated for Abca5 and Dohh ($p < 0.001$, $p < 0.001$), significantly up-regulated for Mybbp1a ($p < 0.001$), but remained unchanged for Tff2. Compared with SD group, the expression of Abca5 in the DUT groups was down-regulated ($p < 0.001$), while Dohh, Mybbp1a and Tff2 were up-regulated ($p < 0.01$, $p < 0.001$, $p < 0.001$). The results were consistent with the RNA-seq results, and the primer sequences were shown in Table 1.

ABCA5 interaction with the cross-DEGs

We further investigated the direct or indirect links between Abca5 and the overlapping DEGs, in response to the observation that Abca5 expression was exacerbated by damage. The Cytoscape results revealed that ABCA5 interacted with ABCA12, and ABCA12 interacted with LOR in the overlapping DEGs. Additionally, ABCA5 was found to interact with ABCB1, which interacted with SLC22A2 in the cross-DEGs. Furthermore, ABCB1 interacted with ABCA4, which was associated with PCARE, RCVRN, SIX3, CHRD, and VAX2 in the common DEGs (Fig. 10).

Discussion

This is the first study to confirm that short-term DUT administration aggravates fertility impairment in the SD model rats. It provides the first overview of the gene profile associated with DUT exacerbating fertility impairment. By comparing the overlapping genes of SD model animals before and after the short-term exposure to DUT, the possible mechanism of common fertility damage in male rats is preliminarily discussed.

Our results showed that short-term exposure to DUT in SD male model rats exacerbated their reproductive dysfunction, which was reflected in multiple representations. The findings of weight loss in SD model rats were consistent with previous studies [17, 25]. It was the first time to have been observed the continued weight loss in SD model rats after short-term exposure to DUT. This phenomenon was not consistent with previous findings that DUT intervention alone had no effect or gain on body weight [26, 27]. It was speculated that short-term exposure to DUT exacerbated SD-induced stress in the body, thereby promoting lipolysis. As a key hormone for male reproductive health, testosterone is critical for sperm production and maturation [28], and low testosterone levels are often the main cause of male infertility [29, 30]. The decrease in testosterone induced by SD was the same as previously reported [31], but the intervention of DUT did not cause further decrease of testosterone in SD model rats. For some people with low testosterone, the level of testosterone will be increased after DUT intervention [32]. This suggested that the DUT intervention can induce a compensatory increase in the level of testosterone in SD model rats, thereby regulating testosterone levels. SD causes anxiety [33] and anxious patients often exhibit lower fertility [34]. The results of open-field

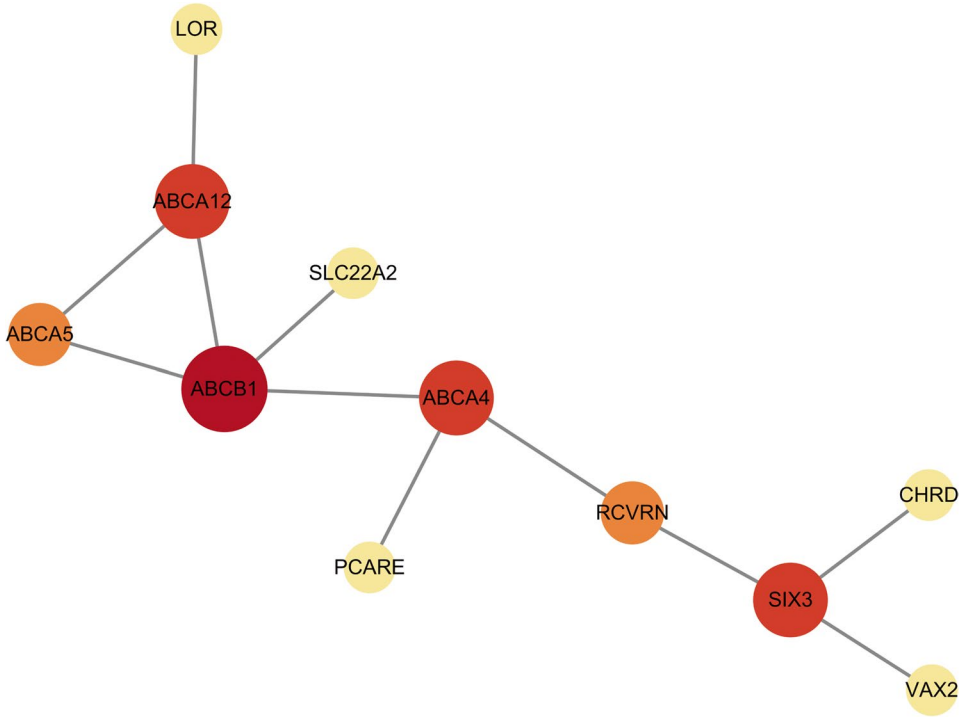

Fig. 10 ABCA5 interacted indirectly with the proteins encoded by the cross-DEGs

behavior suggested that the anxiety state of SD model rats was not aggravated after short-term exposure to DUT, and the reason of aggravation of fertility damage might not be caused by depression and anxiety. Sperm parameters are the gold standard for evaluating male fertility. After short-term exposure to DUT, the rate of sperm abnormality rate reached 30%, and the damage of interstitial cells was aggravated. DUT reported on sperm quality and gonadal tissue vary [7, 35–37]. This experiment may be due to the widespread adverse effects of SD on various systems of the body to exacerbate DUT damage to fertility.

By RNA-seq, we found a gene profile associated with fertility impairment promoted by DUT. In SD vs. DUT, Cytoscape screened Ibsp, Runx1, Fgf18, Areg, Stat6, Mapk3 and Gng4 as hub genes. Among them, Stat6 plays an important role in allergic reaction, inflammatory reaction and cytokine regulation of immune system [38]. Fgf18 [39] is a pleiotropic growth factor that stimulates major signaling pathways involved in cell proliferation and growth and is involved in the development and homeostasis of many tissues. Mapk3 is a crucial signaling molecule during cell proliferation and differentiation, and also play an important role in chronic inflammatory [40], immune-mediated [41] diseases. It was suggested that the aberrant changed in our above genes affect cell proliferation and growth, induce inflammation response. This might have been a key factor in the exacerbation of fertility damage by DUT.

The results from this study showed that there were 142 cross-DEGs within the DEGs of SD vs. MC and DUT vs. SD. It was highly likely that there were new targets related to injury to fertility among these genes. Crossed DEGs are broadly involved in 5 functional classes: immune system, nerve development and conduction, embryonic development and organ formation, metabolism and transport, cell growth and proliferation. It was suggested that these functions might be associated with injury to fertility. In this study, we selected Mybbp1a, Dohh, and Tff2 to perform qRT-PCR experiments with Abca5, and the results were consistent with RNA-seq. Down-regulation of Mybbp1a affects the regulation of DNA replication and cell division [42]. Decreased Dohh expression may cause impaired cell proliferation [43]. These were required for the normal development of some germ cells. We hypothesized that Mybbp1a and Dohh knockdown can affect germ cell proliferation and differentiation. It is reported that Tff2 is highly expressed in ovarian cancer [44]. Ovarian cancer patients often have abnormal expression of steroid synthetase and receptor [45]. This suggested that the up-regulation of Tff2 may be highly related to gonadal damage. The reason for the elevation of these genes after DUT intervention, which may be compensatory, but cannot be ignored for its important role in fertility effects.

Notably, the expression of Abca5 was downregulated after SD, and continued to decrease following DUT intervention in this study. The Abca5 gene encodes an

ATP transporter protein family binding cassette protein involved in a variety of biological processes. It is reported that the protein level of ABCA5 is significantly lower in osteoarthritic cartilage samples than in normal samples [46]. Furthermore, ABCA2 and ABCA5 play an important role in the development of Alzheimer's disease [47]. Cholesterol turnover in nerve cells and testis suggests that Abca5 is involved in intracellular lipid transport and maintains the rate of cholesterol efflux [48], and knockdown of Abca5 shows dysregulation of cholesterol homeostasis [49]. At the same time, Abca5 is also involved in intracellular sterol/steroid transport [50]. In summary, Abca5 played important roles in neurodevelopment, inflammation, sterol transport, and lipid metabolism, and downregulation of Abca5 may affect the above functions. The downregulation of Abca5 expression in this study was speculated to potentially affect the efflux and transport of steroids in the testes. Furthermore, as most sex hormones belong to the steroid class, it suggested that Abca5 could impact fertility by influencing the transport of sex hormones. Mining the interaction of ABCA5 with the cross-DEGs revealed that indirect interactions with LOR, SLC22A2, PCARE, and RCVRN/SIX3/CHRD/VA-X2 among the proteins encoded by the cross-DEGs could be produced through the ABCA5 family proteins ABCA12, ABCB1, and ABCA4. This suggested that Abca5 was able to associate with the cross-DEGs, the interactions emphasized the importance of Abca5, and the network opened up new directions in the search for reproductive targets of injury.

Admittedly, we still have many questions to discuss further in this experiment. We lack specific experiments on the effects of SD and DUT on mating fertility, and the effects of some genes on fertility rate need to be further explored. One limitation of our morphometric analysis is the reliance on lower-magnification micrographs. While sufficient for the primary measurements reported, the lack of higher-resolution imagery may limit the observation of finer ultrastructural details. Compared with 35 days of SD in our previous study [10], the DEGs induced by 42 days of SD were enriched in immune regulation, nervous system development, and had a tendency to affect steroid metabolism. In the future, we will explore the changes of DEGs with the extension of SD time and find the related targets of fertility by affecting sleep impairment.

Conclusions

This study provided a genetic profile of DUT-promoted reproductive impairment and showed that short-term exposure to DUT exacerbated the reproductive impairment in SD rats. The screened Hub genes Is, Runx1, Fgf18, Areg, Stat6, Mapk3, and Gng4 were found to be important targets for DUT-exacerbated fertility injury in SD model rats. By comparing the overlapping genes of SD and DUT groups, 142 cross-DEGs were found to be mainly involved in immunity, nerve and metabolism etc. Among them, the aberrant expression of Abca5 and the cross-DEGs associated with it were identified as important causes of impaired fertility in male rats.

Abbreviations

DUT	Dutasteride
DHT	Dihydrotestosterone
SD	Sleep deprivation
MC	Model control
RNA-seq	RNA sequencing
NGS	next generation sequencing
qPT-PCR	quantitative reverse transcription polymerase chain reaction
ED	Erectile dysfunction
REM	Rapid eye movement
MMPM	Modified multi-platform method
CMC	Carboxymethyl cellulose
H&E	Hematoxylin-eosin
DEGs	Differentially expressed genes
GO	Gene ontology
KEGG	Kyoto encyclopedia of genes and genomes
PPI	Protein-protein interactions
ANOVA	analyzed using analysis of variance
CC	cellular component
MF	molecular function
BP	biological process
MCC	Maximal Clique Centrality

Supplementary Information

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Supplementary Material 1

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Authors' contributions

ZSX performed the experiments, analyzed the results and wrote the paper. ZGR and LW supervised the study and edited and reviewed the paper. All authors read and approved the final manuscript.

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Data availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA024417) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals of Hebei North University. Ethical approval for this study was obtained from the Animal Ethics Committee of Hebei North University (HBNU202307012104). Approval date: 2023.7.26.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Shandong Xiehe University, Jinan 250100, China
[2]College of pharmacy, Hebei North University, Zhangjiakou 075000, China
[3]Bioscience Today (Beijing) Biotechnology Research Institute, Beijing 102629, China
[4]Hebei Provincial Key Laboratory of neuropharmacology, Zhangjiakou 075000, China

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