

# A comparative study of libido in drakes: from phenotypes to molecules

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**ABSTRACT** Low fertilization rate is the main reason to limit the development of artificial insemination (AI) technology in ducks. However, the libido of male livestock has been confirmed to be related to semen quality and fertilization rate, and we found that the libido of drakes was different. Thus, the research on the libido of drakes may be the key to further develop and apply AI technology. In this research, we established the first scoring standard for libido evaluation in drakes based on the performance of drakes during training period. Phenotypically, the body weight of high libido group was lighter than that of the other groups, while the weight of testis and epididymis in the high libido group was higher than that in the low libido group. Furthermore, we constructed the first expression profile of hypothalamus, pituitary, testis, and epididymis of drakes with high or low libido. There were 2, 1822, 214, and 892

differentially expressed genes (DEGs) in hypothalamus, pituitary, testis, and epididymis. The expression and sequence of *Translocation Associated Membrane Protein 2* (*TRAM2*) were different in high and low libido drakes, indicating that it may be a candidate gene related to drake's libido. The estrogen, prolactin, and oxytocin signaling pathways were all activated in the pituitary of the low libido group. Meanwhile, the metabolic and oxidative phosphorylation pathways were enriched by DEGs in pituitary, testis and epididymis. Our research reveals that the difference in metabolic may cause changes in body weight of drakes, resulting in altered hormone levels and oxidative phosphorylation of gonad, which negatively affects libido and spermatogenesis in drakes. These results provide novel insights into the avian libido and will help better understand the underlying molecular mechanisms.

**Key words:** drakes, libido, obesity, transcriptome

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## INTRODUCTION

Artificial insemination (AI) technology of poultry began in the 1930s, while the development and application of duck AI technology is far less than that of chicken due to its low fertilization rate. With the transformation of duck raising system from ground rearing to caged rearing (Zhang et al., 2019; Abdel-Hamid et al., 2020), the original natural mating mode will be replaced by AI. Studies have shown that the libido of male livestock is significantly related to semen quality and fertilization rate (Singh et al., 2020; Kowalczyk et al., 2021). Furthermore, selection for libido also increased the

testosterone concentration, scrotal circumference, physical and morphological semen traits of the males (Quirino et al., 2004; Ren et al., 2009; Chang et al., 2017). Moreover, in the production practice, there are obvious differences in the libido of drakes, so it is urgent to study it.

To evaluate libido, observing natural reproduction is the most effective method (Chenoweth, 1986). For practical, health and ethical reasons, other methods of evaluating libido are often used. For example, Singh et al. have scored the libido of bulls when they were in presence of teaser (Singh et al., 2020). In the related research of bull, stallion, and boar, scholars usually establish the criteria of libido score from the following aspects: interest in teaser female, mounting, penile erection, and semen collection (Dinger and Noiles, 1986; Kozink et al., 2002; Hoflack et al., 2006). However, there is a lack of scoring criteria for libido in male poultry.

Currently, libido has been reported to be regulated by nutrition (Chen et al., 2018), season (Bonato et al., 2014), age (Ren et al., 2009), environment

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(Muvhali et al., 2020), and genetics (Spitzer et al., 1988; Chang et al., 2017). Hence, it is difficult to study the regulation of libido by traditional methods due to the complex and huge network. With the development of transcriptome sequencing technology, it has been widely used in the study of complex reproductive traits (Słowińska et al., 2020; Ran et al., 2021). Therefore, the objectives of this study were to: 1) establish the criteria for scoring the libido of duck and 2) construction of mRNA expression profile of hypothalamic-pituitary-gonad (testis and epididymis) axis (HPG axis) in drakes with different libido. These data will provide new insights into the genetic mechanisms of libido in poultry.

## MATERIALS AND METHODS

### **Ethics Approval and Consent to Participate**

All drakes were obtained from the Waterfowl Breeding Experimental Farm of Sichuan Agricultural University. All experimental procedures that involved in animal manipulation were approved by the Institutional Animal Care and Use Committee (IACUC) of Sichuan Agricultural University (Chengdu Campus, Sichuan, China).

### **Training of the Drakes**

One hundred and ten drakes were moved to single cages after their maturation on the ground (140 d old). All drakes began massage training at the age of 190 d old. The response of tail upwarping or cloacal intumescencia was recorded during the massage training. When 95% of the individuals showed a response to the massage training, the massage training was stopped and began to use laying female ducks as teaser. During the period of induced stage, the response of drakes was divided into 4 classes: no response (the drakes did not produce any response before the female duck was taken to the drake's cage and the female ducks were put into the drake's cage.), weak (before the female duck was taken the drake's cage, the drake did not have any movement performance. When the female duck was placed in the cage, the drake carried the female duck's head feather, and did not climb and mate.), medium (before the female duck was taken the drake's cage, the drake did not show any movement. When the female duck was placed in the cage, the drake began to take the female duck's head feathers, climb, and mate.), strong (before the female duck was taken to the drake's cage, the drake showed obvious excitement, approaches the female duck, and took the female duck's head feathers. When the female duck was placed in the cage, the drake immediately took the female duck's head feathers, climb, and mate).

### **Sample Collection**

According to the behavior of drakes in artificial sperm collection stage, their libido was scored. Individuals with

a score of 1-2 were classified as low libido group, while those with a score of 5 were classified as high libido group (according to the scoring standard of drake libido). The day after AI training ending, 5 individuals with similar body weight and physiological status were selected in the low and high libido groups. The selected individuals were slaughtered (euthanized by carbon dioxide anesthesia and exsanguination by severing the carotid artery) after female ducks' stimulation, and the indexes of testis, epididymis, and sperm duct were measured. The hypothalamus, pituitary, right testis, and epididymis were collected and placed in liquid nitrogen, and the left testis was placed in 4% formaldehyde fixed solution for subsequent experiments.

### **Histological Observation**

A sliced piece of testicular tissue from the testis was taken and fixed in 10% buffered neutral formalin solution for 24 h. The fixed tissues were dehydrated in alcohol of ascending concentrations, that is, 75%, 85%, 95%, and absolute alcohol, respectively, cleared in xylene, unfiltered, and embedded in molten paraffin wax. A rotary microtome (Leica, Oskar-Barnack, Munich, Germany) was used to cut the specimens into slices of 4  $\mu$ m thickness. Cross-sections were further stained with HE and photographed with a digital trinocular camera microscope BA410 Digital (Motic China Group Co. Ltd., Xiamen, China). The number of sperm was counted by Image-Pro Plus 6.0.

### **RNA-seq and Bioinformatics Analysis**

The Trizol kit (Invitrogen, Massachusetts, CA) was used to extract the total RNA of hypothalamus, pituitary, testis, and epididymis according to the manufacturer's instructions (Pub. No. MAN0000406). The RNA integrity was determined by an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). The RNA samples were used for library construction. The mRNA libraries were sequenced by Novogene Co., Ltd. (Beijing, China) using Nova-PE150 (Illumina, San Diego, CA). The original sequencing data for this study can be found in the Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra>) at NCBI, with the BioProject ID: PRJNA743556. The clean reads were obtained after the filtration of low-quality reads using standard quality control by FastaQC software. Clean reads were mapped to the duck reference genome (GCA\_015476345.1) using the HISAT2 (version 2.2.1) software (Kim et al., 2015). The output SAM (sequencing alignment/mapping) file was converted to a BAM (binary alignment/mapping) file and sorted using SAMtools (version 1.10) (Li et al., 2009). Subsequently, the expression of each transcript was calculated by featureCounts (version 1.6.0) (Liao et al., 2014). DESeq2 was used to identify the different expression genes (DEGs) among different groups, the screening criteria were  $|\log_2 \text{Foldchange}| > 1$ ,  $P_{\text{adjust}} < 0.05$ . KOBAS 3.0 online was

used for functional analysis (<http://kobas.cbi.pku.edu.cn/kobas3/?t=1>) (Xie et al., 2011).

### Quantitative Real-Time PCR Validation

Previously, total RNA extracted from the tissues was reverse transcribed into cDNA using a Prime Script RT Reagent Kit (Takara Biotechnology Co., Ltd., Dalian, China) follow the instruction. Primer 5.0 was used to design the primers (Table 1). A BLAST search against the reference genome was then carried out to confirm primers were specific for the intended target genes. Bio-Rad CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA) was used for RT-PCR, and each sample was assayed 3 times. *β-actin* and *GAPDH* were used as housekeeping genes. The qPCR reaction systems are as follows: TB Green Premix Ex Taq (Takara Biotechnology Co., Ltd., Dalian, China) 12.5  $\mu$ L, PCR Forward Primer (10  $\mu$ M) 1  $\mu$ L, PCR Reverse Primer (10  $\mu$ M) 1  $\mu$ L, cDNA 2  $\mu$ L, ddH<sub>2</sub>O 8.5  $\mu$ L. The qPCR amplification conditions are as follows: 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 30 s. The  $2^{-\Delta\Delta CT}$  method was used for normalization of the qPCR results, after which the normalized data were used for statistical analysis, and  $P < 0.05$  was considered significantly different.

### SNP Detection of TRAM2 in Drakes With Different Libido

According to the sequences of *TRAM2* sequence of Peking duck (GenBank No. NC\_051774.1), Primer premier 5.0 was used to design primers for different exons (Table 1). The prepared whole blood genomic DNA was used as the amplification template to amplify the DNA sequences by PCR specificity. The 25  $\mu$ L reaction system was used for PCR: 1.0  $\mu$ L for upstream and downstream primers, 1.0  $\mu$ L for template, 22.0  $\mu$ L for 1.1  $\times$  T3 Super PCR Mix (Tsingke, Beijing, China). Reaction conditions are as follows: 98°C 2 min; 98°C for 10 s, 60°C 10 s, 72°C for 10 s, 35 cycles; 72°C for 2 min, 4°C. Qualified PCR reaction products detected by 1.5% agarose gel electrophoresis were sent to Tsingke Biotechnology Co., Ltd. (Beijing, China) for sequencing. According to the sequencing results, sequence processing and comparison were conducted by SeqMan software, and SNP sites were identified by combining with the peak graph of sequence.

### Statistical Analysis

Data were analyzed ANOVA with the SPSS.27 software. The Graph Pad Prism 6.0 software was used to generate the graphs. The graphical results were expressed as mean  $\pm$  standard deviation. Results with  $P < 0.05$  were considered statistically significant.

## RESULTS

### Establishment of Libido Scoring Standard for Drakes

After 6 d of massage training, 95% of drakes showed tail upwarping and cloacal enlargement after massage. We finished the data collection when 80% of the drakes showed a stable response to the female ducks for more than 3 d. Based on the behavior of drakes in the training period (massage training stage and using female ducks as teaser stage) of AI, a criterion of libido was constructed for the first time (Table 2). Furthermore, we found differences in body weight among drakes with different libido scores. Drakes with a libido score of 5 had significantly lighter body weight than those with scores of 4 (Figure 1A).

### Phenotypic and Histological Comparison of Drakes With Different Libido

When we slaughtered drakes with high and low libido, we found that there were differences in gonadal index (Supplementary Table S1). Specifically, the size and weight of testis, epididymis and spermaduct in the high libido group were higher than those of low libido group. Of note, the weight of the right epididymis in the high libido group was significantly higher than that of the low libido group (Figure 1B), and the  $P$ -value of the left testicular long diameter, left testicular weight, right testicular short diameter, and left spermaduct stretch length between different groups were nearly to 0.05 (Supplementary Table S1). In terms of histomorphology, the number of sperm in the testis of the low libido group was lower than that of the high libido group (Figure 1C).

### Overview of the mRNA Transcriptome With High and Low Libido

A total of 540,084,472 raw reads were obtained from 24 samples through mRNA sequencing, and 92.75% of the clean reads were aligned to the reference genome (Supplementary Table S2). As shown in Supplementary Figure. 1A, 3 biological replications of each tissue in different groups were well clustered together. Moreover, there were 2, 1822, 214, and 892 DEGs identified in hypothalamus, pituitary, testis, and epididymis between high and low libido group, respectively (Figure 2A). DEGs in pituitary, testis, and epididymis showed consistent expression patterns across 3 biological replicates in each group (Supplementary Figure. 1B-D). Notably, the expression of *TRAM2* in the hypothalamus, epididymis, and testis was higher in low libido drakes than high libido drakes (Figure 2B). Compared with the sequence of *TRAM2* in Peking duck, 12 SNPs were found in this study. As shown in Figure 2C, SNP<sub>412+91</sub>, SNP<sub>412+96</sub>, SNP<sub>412+224</sub>, SNP<sub>412+235</sub>, SNP<sub>412+256</sub>, and SNP<sub>412+265</sub> were homozygous mutations in high libido group, while heterozygous mutations in low libido group. And, SNP<sub>472+116</sub>, SNP<sub>472</sub>

+365, SNP<sub>816</sub>, SNP<sub>887+151</sub>, SNP<sub>887+164</sub>, and SNP<sub>887+189</sub> were homozygous mutations in high libido group, while heterozygous mutations in some low libido individuals.

### **Functional Analysis of DEGs With Different Libido Level Drakes in Hypothalamus, Pituitary, Testis, and Epididymis**

The number of KEGG pathways that significantly enriched by DEGs in hypothalamus, pituitary, testis, and epididymis tissues was 0, 100, 23, and 51 (Supplementary Table S3). Oxidative phosphorylation, ribosome, spliceosome, metabolic, MAPK signaling pathways, and some pathways related to diseased were overlapped in the 3 tissues. Of note, some hormone-related KEGG pathways were enriched only by DEGs in the pituitary, including estrogen, prolactin, and oxytocin signaling pathways. Furthermore, almost all the expression of DEGs in the hormone-related KEGG pathways were up in the pituitary of low libido groups, and consistent results were also obtained in qPCR validation of these DEGs (Figure 3D). In addition, the DEGs related to oxidative phosphorylation and differentially expressed in pituitary, epididymis and testis were also verified in accordance with the RNA-seq expression trend by qPCR (Figures 3D and 3E).

## **DISCUSSION**

It is generally believed that the libido is regulated by the HPG axis. However, our results showed that there were significant differences in gene expression in pituitary, testis, and epididymis of different libido level groups, but this difference was very weak in hypothalamus. Therefore, hypothalamus regulation of libido may be achieved through other ways other than the transcriptional level. We obtained the largest number of DEGs in pituitary between high and low libido groups, confirming the important role of pituitary in regulating the transcriptional level of libido (Lake et al., 2013; Molitch, 2017). Although the epididymal tissue is quite different between avian and mammalian, some studies have also proved the function of epididymis in reabsorption of testicular fluid (Clulow and Jones, 1988). Thus, the epididymal of the male avian reproductive tract is essential for sperm maturation, and dysfunction of this region results in infertility (Janssen et al., 1998; Janssen et al., 2000; Bahr et al., 2006). Furthermore, we found the number of DEGs in epididymis was more than that in testis. Meanwhile, the expression pattern of epididymis is more similar to that of pituitary through the PCA analysis, and epididymis shares more differential genes with pituitary than testis. These results indicate that the epididymis plays a role in regulating libido in drakes.

Notably, the expression of *TRAM2* was different in 4 tissues between high and low libido groups. Previous study has found that *TRAM2* was closely related to reproductive disorders in dairy cows through GWAS analysis

(Liu et al., 2015). Exposing hFOB cells stably transfected with ER $\alpha$  (hFOB-ER $\alpha$ ) to 0.1 nmol/L E2 and found that a 27-fold increase in *TRAM2* mRNA expression, indicating that *TRAM2* was estrogen-dependent (Liu et al., 2014). On the other hand, DEGs in the pituitary are also enriched in the estrogen signaling pathway. Estrogen is biologically more active than testosterone, therefore, a subtle increase in estradiol levels may have more of an inhibitory effect on testicular function compared with an increase in testosterone levels (Hammoud et al., 2006; Schulster et al., 2016). Estradiol not only regulates sexual behavior in adult men, but also appears to organize the early brain to program sexual behavior (McCarthy, 2009). Studies have shown that exogenous estrogen can increase libido in men with low testosterone levels and decrease it in men with normal testosterone levels (Bancroft et al., 1974; Wibowo et al., 2011). Our results showed that all the DEGs (*SRC*, *ADCY6*, *NCOA1*, *SHC1*, *SHC2*, *MMP9*, *FKBP5*, *NOS3*, *PGR*, *PIK3R2*, *CREB3L4*, and *POMC*) enriched in the estrogen signaling pathway were expressed in the pituitary of the high libido group compared with the low libido group. In addition to estrogen, the oxytocin and prolactin signaling pathways are enriched by differentially expressed genes in the pituitary. Caldwell has proposed that oxytocin is a satiety hormone that acts by de-coupling the G-protein and hence reducing sexual arousability (Caldwell, 2002). Chronic elevations of PRL (hyperprolactinemia) produce pronounced reductions in animal sexual activity, and significant reduction of libido and gonadal function in men (Krüger et al., 2002). These results suggest that the overactivation of estrogen, oxytocin, and prolactin signaling pathway in pituitary of drakes may be one of the reasons for the decreased libido.

When we tried to explain these hormonal changes in the drakes, we noticed that the body weight of the high libido drakes was lighter than that of the low libido drakes. Excess production and hyperactivity of the aromatase cytochrome P450 enzyme in adipose tissue lead to increased conversion of testosterone to estradiol (Hammoud et al., 2006; Katib, 2015). Meanwhile, the metabolic and oxidative phosphorylation pathways that may be caused by obesity are enriched by DEGs in pituitary, testis and epididymis. Previous studies have confirmed that the reproductive tract inflammatory (Dulloo and Montani, 2012; Lavranos et al., 2012) response caused by obesity can lead to oxidative stress in testis and epididymis (Bakos et al., 2011; Tunc et al., 2011). And, oxidative stress in the testis and epididymis of mammals has a negative effect on sperm development and maturation (Bakos et al., 2011). The effect of oxidative stress on avian spermatogenesis was also confirmed by the difference in sperm counts in the testicles of drake with different sexual appetites. Given the differences in epididymal function between birds and mammals (above mentioned), we hypothesize that oxidative stress in the epididymis of birds may contribute to reproductive dysfunction.

In conclusion, we established the first scoring standard for libido evaluation in drakes based on the

performance of drakes during the training period. The body weight of high libido group (score = 5) was lighter than that the other groups. While the weight of testis and epididymis in the high libido group was significantly higher than that in the low libido group. Furthermore, we constructed the first expression profile of hypothalamus, pituitary, testis, and epididymis of drakes in high and low libido groups. Notably, the expression and sequence of *TRAM2* were different in high and low libido drakes, indicating that it may be a candidate gene related to drake's libido. The estrogen, prolactin, and oxytocin signaling pathways were all activated in the pituitary of the low libido group. Meanwhile, the metabolic and oxidative phosphorylation pathways were enriched by DEGs in pituitary, testis, and epididymis. We hypothesized that excess body weight may lead to metabolic changes and lead to oxidative phosphorylation of the testis and epididymis, which negatively affects libido and sperm production in drakes (Figure 4).

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## DISCLOSURES

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.psj.2021.101503](https://doi.org/10.1016/j.psj.2021.101503).

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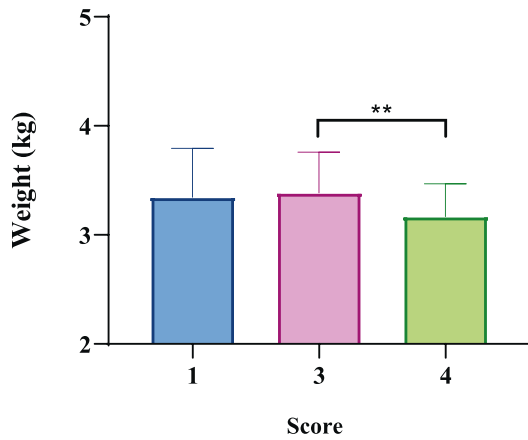
**Table 1.** Primers used in this study.

Primer	Sequence (5'-3')	Usage
TRAM2-1F	GGATGCTGCGGGAGGAAAGA	Amplification of <i>TRAM2</i> exon 1
TRAM2-1R	AGGAGAAGCGAGGTTGGCGA	
TRAM2-2F	GGCACTTTGCGTCTTGTTTCT	Amplification of <i>TRAM2</i> exon 2, 3, 4
TRAM2-2R	GTCCTGTCCACGTTCCCACT	
TRAM2-3F	AGCACTAACCCACCCCACTGT	Amplification of <i>TRAM2</i> exon 5, 6, 7, 8, 9
TRAM2-3R	CTCCGCTCATCCTTACAACTG	
TRAM2-4F	CAGTTGTAAAGGATGAGCGGA	Amplification of <i>TRAM2</i> exon 10, 11
TRAM2-4R	GCTTATTTACGGTGGAAACGAG	
NDUFA13-F	TCAGGTTACAGCCTCTTCGC	qPCR for DEGs
NUDFA13-R	ACACGGATTCCCCGACCT	
NUDFB2-F	ACACGGATTCCCCGACCT	
NUDFB2-R	TCATTCCAGGGATTGCTTCAG	
POMC-F	GGGAAGCGCTCCTACTCCATGG	
POMC-R	CTGACCCTTCTGTAGGCGC	
SHC1-F	CATCCTGGGGAAAAGCAACC	
SHC1-R	TTGGCAATGATCTGCTTGCA	
SRC-F	AGCAGCAAGAGCAAACCCAA	
SRC-R	GTGACGGTGTCCGAGGTGTT	
TRAM2-F	AGGAGTATGCCCTGGACAAA	
TRAM2-R	GACGTACAAGCACCAAATCAAG	

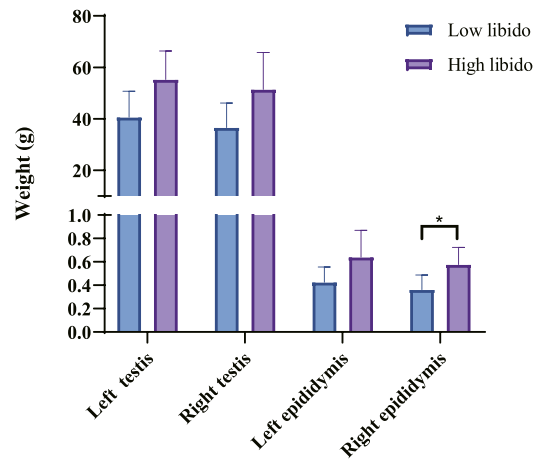
**Table 2.** Scoring standard of drake libido.

Score	Lever of libido		Population	
	Massage training stage	Using female ducks as teaser	Number	Ratio (%)
1	No response	No response	1	0.91
2	Tail upwarping or Cloacal intumescentia	No response	3	2.73
	Tail upwarping and Cloacal intumescentia	No response	6	5.45
3	Tail upwarping or Cloacal intumescentia	Weak	1	0.91
	Tail upwarping and Cloacal intumescentia	Weak	2	1.82
4	Tail upwarping or Cloacal intumescentia	Medium	8	7.27
	Tail upwarping and Cloacal intumescentia	Medium	51	46.36
5	Tail upwarping or Cloacal intumescentia	Strong	2	1.82
	Tail upwarping and Cloacal intumescentia	Strong	36	32.73

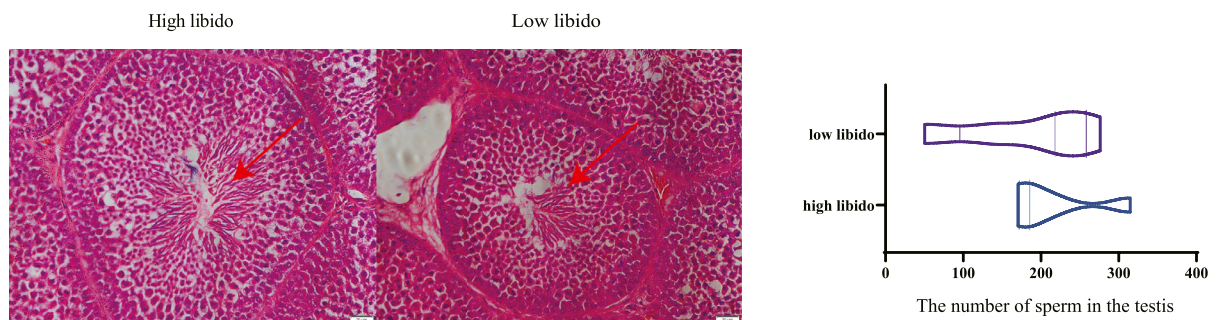
A.



B.



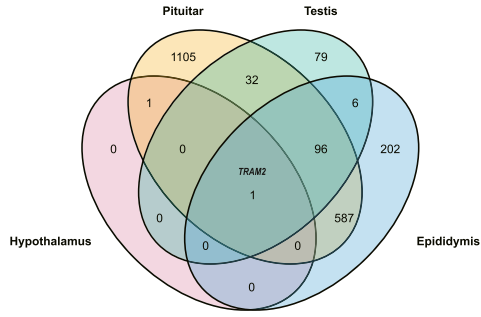
C.



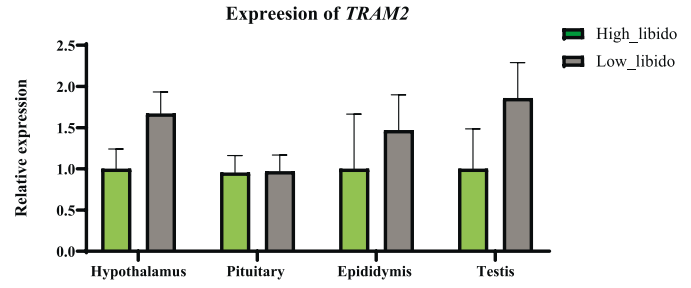
**Figure 1.** Anatomy and histological differences of testis and epididymis in drakes with different libido. (A) Body weight of drakes with different libido level. (B) The weight difference of testis and epididymis between high and low libido drakes. (C) Morphological differences of testis with different libido drakes (400 x). \*Mean the  $P < 0.05$ , \*\* mean the  $P < 0.01$ .



A.



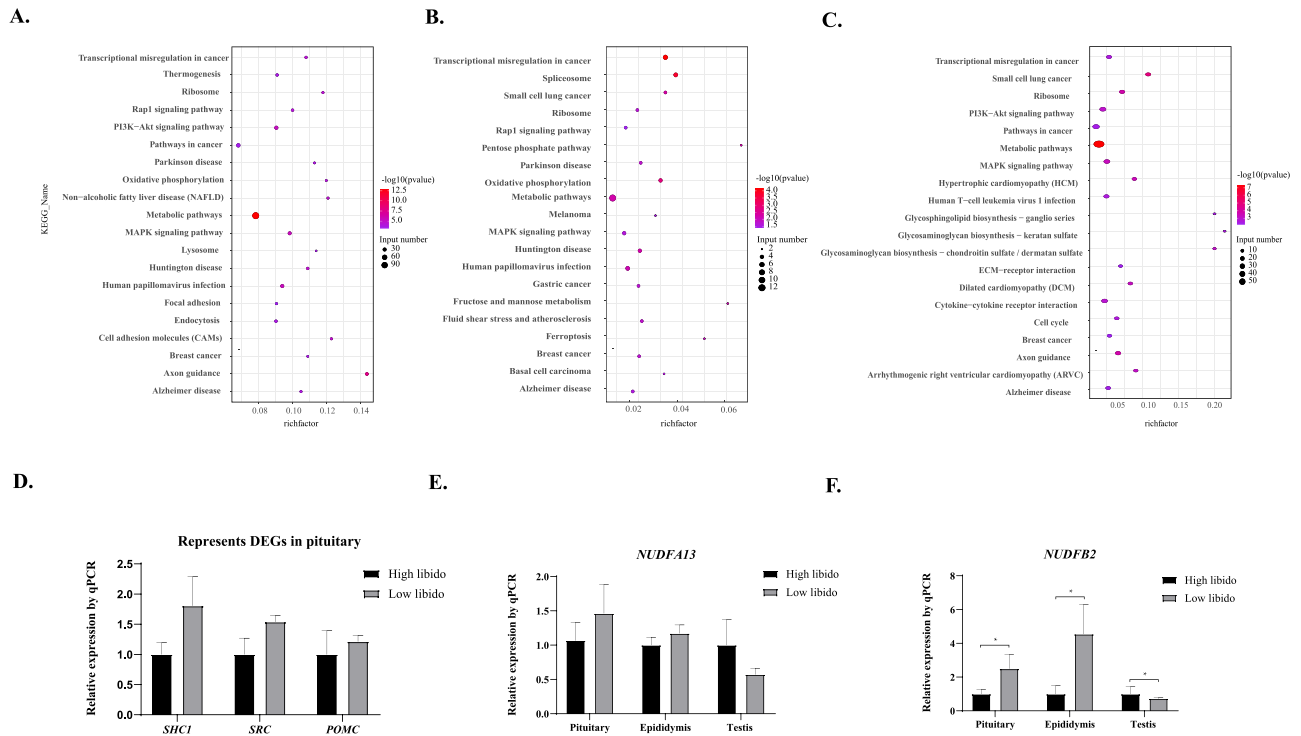
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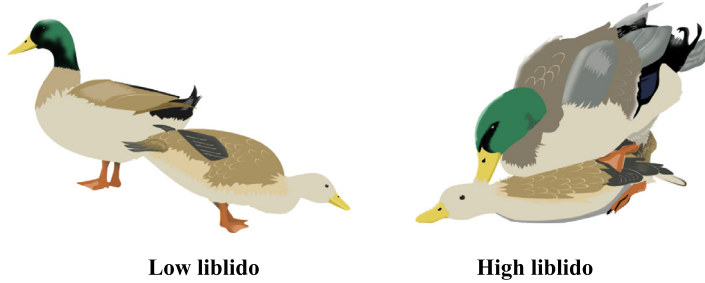
C.

SNP	Intron4						Intron5		Exon9	Intron9		
	SNP <sub>412+91</sub>	SNP <sub>412+96</sub>	SNP <sub>412+224</sub>	SNP <sub>412+235</sub>	SNP <sub>412+256</sub>	SNP <sub>412+265</sub>	SNP <sub>472+116</sub>	SNP <sub>472+385</sub>	SNP <sub>816</sub>	SNP <sub>887+151</sub>	SNP <sub>887+164</sub>	SNP <sub>887+189</sub>
Reference	GG	AA	CC	GG	GG	GG	GG	GG	AA	TT	TT	GG
High_libido-1	AA	GG	TT	AA	AA	TT	AA	AA	GG	AA	CC	AA
High_libido-2	AA	GG	TT	AA	AA	TT	AA	AA	GG	AA	CC	AA
High_libido-3	AA	GG	TT	AA	AA	TT	AA	AA	GG	AA	CC	AA
High_libido-4	AA	GG	TT	AA	AA	TT	AA	AA	GG	AA	CC	AA
High_libido-5	AA	GG	TT	AA	AA	TT	AA	AA	GG	AA	CC	AA
Low_libido-1	AG	AG	CT	AG	AG	TG	AA	AA	GG	AA	CC	AA
Low_libido-2	AG	AG	CT	AG	AG	TG	AA	AA	GG	AA	CC	AA
Low_libido-3	AG	AG	CT	AG	AG	TG	AG	AG	AG	AT	CT	AG
Low_libido-4	AG	AG	CT	AG	AG	TG	AG	AG	AG	AT	CT	AG
Low_libido-5	AG	AG	CT	AG	AG	TG	AG	AG	AG	AT	CT	AG
Homozygous mutation												
Heterozygous mutation												

**Figure 2.** Expression and sequence variation of *TRAM2* in drakes with high and low libido level. (A) Venn diagram of the common DEGs between 4 tissues pairwise comparisons. (B) The expression of *TRAM2* detected by qPCR in hypothalamus, pituitary, epididymis and testis of high and low libido drakes with mean  $\pm$  SD. (C) The differential SNPs between high and low libido level drakes compared with the sequence of Peking duck *TRAM2*.



**Figure 3.** Functional analysis and expression level verification of DEGs. Top 20 KEGG pathways enriched by the DEGs in pituitary (A), testis (B), and epididymis (C) tissues. (D) Expression levels of *SHC1*, *SRC*, and *POMC* detected by qPCR in pituitary of drakes with different libido levels. Expression levels of *NUDFA13* (E) and *NUDFB2* (F) detected by qPCR in pituitary, epididymis, and testis of drakes with different libido levels. \*Mean the  $P < 0.05$ .



**Phenotype**

**Molecule**

**Body weight**

Low libido > High libido

**Metabolism of pituitary and gonad**

Numerous metabolism-related pathways were enriched by the DEGs in pituitary and gonad

**Gonad weight**

Low libido < High libido (testis and epididymis)



**Hormone of pituitary**

Oxytocin/Prolactin/Estrogen signaling pathway was activated in the pituitary of low libido drakes

**Spermatogenesis**

Low libido < High libido (sperm in the testis sections)

**Oxidative phpsphorylation of gonad**

Oxidative phosphorylation pathway was enriched by the DEGs in testis and epididymis

**Figure 4.** The effects of libido differences in drakes (summary of the manuscript).

