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Metabolomics and transcriptomics analyses reveal the complex molecular mechanisms by which the hypothalamus regulates sexual development in female goats

Qing Li[1,2], Tianle Chao[1,2*], Yanyan Wang[1,2], Peipei He[1,2], Lu Zhang[1,2] and Jianmin Wang[1,2*]

Abstract

Background The hypothalamus is a critical organ that regulates sexual development in animals. However, current research on the hypothalamic regulation of sexual maturation in female goats remains limited. In this study, we conducted metabolomic and transcriptomic analyses on the hypothalamic tissues of female Jining grey goats at different stages of sexual development (1 day old (neonatal, D1, $n=5$), 2 months old (prepuberty, M2, $n=5$), 4 months old (sexual maturity, M4, $n=5$), and 6 months old (breeding period, M6, $n=5$)).

Results A total of 418 differential metabolites (DAMs) were identified in this study, among which the abundance of metabolites such as anserine, L-histidine, carnosine, taurine, and 4-aminobutyric gradually increased with the progression of sexual development. These metabolites may regulate neuronal development and hormone secretion processes by influencing the metabolism of histidine and phenylalanine. Through combined transcriptomic and metabolomic analyses, we identified that differentially expressed genes such as mitogen-activated protein kinase kinase kinase 9 (*MAP3K9*), prune homolog 2 with BCH domain (*PRUNE2*), and potassium voltage-gated channel interacting protein 4(*KCNIP4*) may jointly regulate the development and energy metabolism of hypothalamic Gonadotropin-releasing hormone neurons in conjunction with DAMs, including LPC22:5, 2-Arachidonyl Glycerol ether, LPE22:5, and Lysops22:5. Additionally, we elucidated the molecular mechanism through which glutathione metabolism regulates sexual maturation in goats.

Conclusions In summary, this study illustrates the dynamic changes in metabolites and mRNA within hypothalamic tissue during postnatal sexual maturation in female Jining grey goats. This research may provide significant scientific insights for future animal breeding.

Keywords Precocious goat, Multi-omics analysis, Hormone secretion, Glutathione metabolism

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Introduction

Sexual maturation is a dynamic developmental process that is regulated by a variety of factors such as genetics, endocrine, and nutrition, and is primarily achieved through the hypothalamic-pituitary-gonadal (HPG) axis [1, 2]. Gonadotropin-releasing hormone (GnRH) stimulates the pituitary gland to synthesize and secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [3, 4], activates the hypothalamic-pituitary signaling cascade, promotes ovarian development, and promotes gonadotropin-induced gene expression [5, 6, 7], while steroids secreted by the gonads provide feedback regulation of GnRH secretion [8, 9]. This complex transcriptional network that regulates reproduction has been extensively studied, with the highest levels of network regulation controlled by key genes and metabolic pathways, such as genes and effectors associated with Kisspeptin, GABAergic, and glutamatergic neurons and glial cells, which act as upstream regulators of GnRH neurosecretion [10]. In cases of negative energy balance, such as energy/nutrient deficiencies due to fasting, the onset of puberty and the acquisition of fertility are often delayed or inhibited [11]. In addition, Kisspeptin neurons play a major role in energy expenditure, which may be mediated by Kisspeptin and/or its cotransmitters such as glutamate [12].

Mutations in the kisspeptin (*KISS1*) or its receptor (*KISS1R*) have been shown to lead to decreased hypothalamic GnRH secretion and inability to enter puberty [13, 14]. GABAA receptor activation, on the other hand, produces excitatory responses primarily to embryonic and prepubertal GnRH neurons [15]. In addition to the regulation of gene expression, the process of sexual development is also tightly regulated by metabolic factors [16], such as lipid metabolism [17], amino acid metabolism [18, 19], and others. Compared with young mice, there were significant reductions in multiple metabolites and neurotransmitters in the hypothalamus of older mice, and there was an increase in the levels of genes associated with the production and utilization of monocarboxylates [20]. 3-Hydroxybenzoic acid and proline glutamine have been reported to be biomarkers of precocious puberty in women [21], while ceramides affect ovarian steroid hormone production and the synthesis of central nervousamides promotes precocious puberty in women [22]. Metabolomics and transcriptomics techniques have been widely used to study the regulatory mechanisms of mammalian reproduction [23].

As the main source of meat, milk, and fur, goats play a vital role in animal husbandry [24]. However, the low fecundity of goats poses a major challenge to the development of the goat industry. Precocious puberty can accelerate the intergenerational succession of livestock, reduce the cost of feeding, and effectively improve the fertility of goats [25]. Understanding the physiological and molecular pathways involved in the sexual maturation of goat is crucial for genetic improvement and breeding practice. Compared with other goat breeds, The Jining grey goat is a nationally recognized indigenous breed in China, renowned for its exceptionally early sexual maturity and high reproductive efficiency. This breed initiates puberty as early as 2–3 months postpartum and achieves full sexual maturity by 4 months of age [26], which is an ideal model for studying the regulation mechanism of reproduction [27, 28].

Although the study of hypothalamic tissue has been abundant, the specific mechanism of its initiation of complex neuroendocrine signaling cascade, regulation of neuronal development and GnRH secretion to promote sexual development has not been fully elucidated. This study hypothesizes that during the development of goats after birth, the transcriptomic and metabolomic expression profiles of the hypothalamus undergo significant changes, which drive sexual maturation by regulating GnRH secretion and related neuroendocrine signals. In this study, metabolomics and transcriptomics were combined to analyze the dynamic changes of hypothalamic metabolites and mRNA at different stages after birth (1 day old (neonatal stage, D1, $n=5$), 2 months old (prepuberty, M2, $n=5$), 4 months old (sexual maturity stage, M4, $n=5$) and 6 months old (reproductive stage, $n=5$). This study revealed the transcriptional regulation mechanism and metabolic characteristics of the hypothalamus during goat sexual development, and provided important insights for genetic improvement and breed selection.

Materials and methods

Animals

In this study, the hypothalamic tissues of 20 female Jining grey goats were collected from the original breeding farm of Jining grey goats (Jiaxiang County, Shandong Province, China). The goats were categorized into the following age groups:1 day old (neonatal stage, D1, $n=5$), 2 months old (prepuberty, M2, $n=5$),4 months old (sexual maturity, M4, $n=5$), and 6 months old (breeding period, M6, $n=5$). All goats were raised under the same environmental conditions. 1-day-old and 2-month-old kid goats lived with their mother goats; 4-month-old and 6-month-old goats were separated from their mother goats and housed in the same shed but in different pens. The conditions of water source, lighting, temperature, and feed in the shed were completely consistent. All selected goats are healthy, disease-free, and are able to eat and drink freely. Starting from 4 weeks of age, the goats were weaned from milk to solid feed, and fully weaned at 10 weeks of age. All experimental goats were slaughtered at the same time, with the D1 group goats having already ingested colostrum. The selected goats were either in anestrus or in the diestrus

and had similar weights in the same group. Experimental goats were slaughtered on the same day after electroshock anesthesia. According to the method of Sesti and Britt [29], the hypothalamus was divided into the preoptic area (POA), mediobasal hypothalamus (MBH), and stalk median eminence (SME). The POA spanned ~ 5 mm anterior to the optic chiasm, dorsally bounded by the MBH's anterior edge. The MBH extended from the optic chiasm's posterior edge to the mammillary bodies' anterior edge, laterally limited by the hypothalamic sulcus and dorsally by a 5 mm posterior depth. The SME was ventrally dissected from the MBH along the third ventricle base midline. After sampling, the tissues were pooled, flash-frozen in liquid nitrogen, and stored at -80 °C for metabolomic and transcriptome analysis. To improve the accuracy of metabolome analysis, reduce pretreatment and instrument errors, and better perform subsequent combined analysis of transcriptome and metabolome data, we divided each biological replicate into two technical replicates [30].

Extraction, detection and quantification of hypothalamic metabolites

One hundred milligrams of hypothalamic tissue were ground in liquid nitrogen and transferred to an Eppendorf tube, where it was combined with 500 µL of an 80% methanol aqueous solution. The mixture was vortexed and then kept in an ice bath for 5 min before being centrifuged at 15,000 g for 20 min at 4 °C. The supernatant was collected and diluted with mass spectrometry-grade water to achieve a methanol concentration of 53%. The prepared supernatant was centrifuged again under the same conditions [31].

A UHPLC-MS/MS analysis was performed using a Vanquish UHPLC system coupled with an Orbitrap Q Exactive™ HF mass spectrometer (Thermo Fisher, Germany) [32]. Sample separation occurred on a Hypersil Gold column (100 × 2.1 mm, 1.9 µm), applying a linear gradient for 17 min at a flow rate of 0.2 ml/min. In positive polarity mode, solvent A was 0.1% formic acid in water and solvent B was methanol; in negative polarity mode, solvent A consisted of 5 mM ammonium acetate (pH 9.0) with methanol as B. The gradient protocol included holding at 2% B for 1.5 min, increasing to 85% B over 3 min, ramping to 100% B in 10 min, then returning to 2% B at 10.1 min for an additional 12 min. The mass spectrometer was set at a spray voltage of 3.5 kV and a capillary temperature of 320 °C. For quality control, all samples were mixed in equal volumes to create mixed QC samples.

Data processing and metabolite quantification

The UHPLC-MS/MS raw data files were processed using Compound Discoverer 3.1 (CD3.1, ThermoFisher) for peak alignment, extraction, and metabolite quantification. Parameters included a retention time tolerance of 0.2 min, mass tolerance of 5 ppm, 30% signal strength tolerance, a signal-to-noise ratio of 3, and a minimum intensity threshold [33]. Peak intensities were normalized to the total spectral intensity, enabling the prediction of molecular formulas from adducts, molecular ion peaks, and fragment ions. Finally, identified peaks were cross-referenced with mzCloud (https://www.mzcloud.org/), mzVault, and MassList databases for accurate qualitative and relative quantitative analyses [34].

Metabolome data analysis

We conducted metabolite annotation for the samples and utilized the KEGG (https://www.genome.jp/kegg/pathway.html), HMDB (https://hmdb.ca/metabolites), and LIPI DMaps (http://www.lipidmaps.org/) databases to gather further metabolic information. To examine the overall metabolic differences among the groups, we employed principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) [35]. Statistical significance was determined using univariate analysis (t-test), and p-values were adjusted using the Benjamini-Hochberg correction method [36]. The thresholds for differentially expressed metabolites (DAMs) were set as VIP > 1, false discovery rate (FDR) < 0.05, and fold change (FC) ≥ 1.5 (upregulated) or VIP > 1, FDR < 0.05, and FC ≤ 0.67 (downregulated). The identified differential metabolites were then clustered and visualized using a heatmap generated by the Pheatmap package in R (version 3.3.2). To investigate the expression patterns of metabolites, we conducted k-means clustering analysis using the mfuzz package [37]. Metabolite annotations were performed using a dual matching strategy based on metabolite names and molecular formulas against the KEGG database (version 110.0, released on April 1, 2024). Data visualization was carried out using the R package ClusterGVis (V.0.1.1).

Transcriptome data analysis

According to the manufacturer's protocol, total RNA was extracted from hypothalamic tissues at four developmental stages using TRIzol reagent (Takara Bio, Dalian, China). The RNA integrity number (RIN) of the sequencing RNA was assessed using the Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). Total RNA concentration was measured with the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the quality of total RNA was evaluated through agarose gel electrophoresis. RNA sequencing was conducted on the Illumina NovaSeq 6000 platform, with raw data filtered through FastQC (v0.11.8) to ensure high-quality reads. A reference genome index was created using HISAT2 (v2.0.5)

[38], and clean reads were aligned to the goat reference genome (GCF_001704415.2_ARS1.2). The data was assembled with StringTie (1.3.3b) [39], and gene expression was normalized to Fragments Per Kilobase of transcript per Million mapped reads (FPKM) using featureCounts (v1.6.2). Differentially expressed genes (DEGs) were identified with DESeq2 (v1.22.1) using |log2(Fold Change)| ≥ 1 and p.adj < 0.05. K-means clustering analysis was performed with the mfuzz package [37] in R, and KEGG enrichment analysis was conducted using clusterProfiler for DEGs exhibiting different expression patterns [40].

Metabolome and transcriptome WGCNA analysis

The co-expression network modules for all DEGs and DAMs were created using the WGCNA package (version 1.70-3) in R. The transcriptome and metabolome used soft thresholds of $\beta = 7$ and $\beta = 6$, respectively, with parameters minModuleSize = 30 and mergeCutHeight = 0.25 to build the topological network. Combining this data with the serum hormone indexes of the same batch of goats published in our laboratory in the past [41], we evaluated the module-trait relationship. A significant correlation between modules and features was defined as $p \leq 0.05$. KEGG enrichment analysis of DEGs and DAMs in key modules was performed using clusterProfiler. We filtered hub DEGs and hub DAMs in the module based on the top 20 of Module Eigenvector connectivity (KME). The correlation coefficient was then calculated using Spearman correlation analysis.

Integrative analysis of metabolomic and transcriptomic data

Perform Spearman correlation analysis using R packages to analyze the hub DEGs and hub DAMs identified by WGCNA. Additionally, based on the expression pattern analysis and KEGG pathway analysis results in this study, compare those with similar or opposite expression patterns and their corresponding KEGG outcomes to identify shared KEGG pathways. This will determine the key signaling pathways jointly regulated by DAMs and DEGs.

Result

Metabolic profiling of hypothalamic tissue

In order to better understand the dynamics of hypothalamic metabolites during the sexual maturation of goats, we performed a non-targeted metabolomic analysis of goat hypothalamic tissue using UPLC-MS/MS. Metabolomic data under the combination of positive and negative modes were pooled and analyzed. A total of 925 metabolites were identified from the hypothalamus (D1, M2, M4, M6) at different stages of sexual development after birth in goats (Figure S1). Excluding unannotated metabolites, lipids and lipid-like molecules (292) and organic acids and derivatives (159) represented the most abundant metabolite categories. PCA analysis showed that the D1, M2, M4, and M6 groups had an obvious tendency for separation, and the M4 and M6 groups were close to each other. PC1 and PC2 together accounted for 41.32% of the total variation (Fig. 1a). The results of PLS-DA are shown in Figure S2. The $R^2Y \geq 0.97$ and $Q2 \geq 0.80$ among the different comparison groups indicated that the model had good predictive power and a low risk of overfitting.

Identification and analysis of differential metabolites in the hypothalamus

A total of 418 DAMs were identified according to the screening criteria (VIP > 1, FDR < 0.05, FC ≥ 1.5, or FC ≤ 0.667) (Table S1-6). Specifically, pairwise comparisons identified 174 DAMs (80 upregulated, 94 downregulated) between M2 and D1, 70 DAMs (24 upregulated, 46 downregulated) between M4 and M2, 71 DAMs (28 upregulated, 43 downregulated) between M6 and M4, 201 DAMs (70 upregulated, 131 downregulated) between M4 and D1, 247 DAMs (76 upregulated, 171 downregulated) between M6 and D1, and 128 DAMs (32 upregulated, 96 downregulated) between M6 and M2 (Fig. 1b). The highest number of DAMs was identified in M6 vs. D1, suggesting significant differences in the physiological status of goats after birth and sexual maturity. In addition, the Venn diagram (Fig. 1c) illustrates common and unique DAMs across comparative groups. A sequential temporal analysis (M2 vs. D1, M4 vs. M2, M6 vs. M4) identified one common DAM, 17-AAG, which exhibited significantly higher abundance during the M2 stage. Comparative analysis against the D1 stage revealed 87 common DAMs in M2, M4, and M6, displaying two distinct expression patterns: higher abundance at postnatal D1 and increased abundance during sexual development (Fig. 1c).

Further, the identified DAMs were classified (Fig. 1d), and after removing unannotated metabolites, lipids and lipid-like molecules accounted for the highest proportion (33.49%), followed by organic acids and derivatives (18.42%). The results of cluster analysis showed that lipids and lipid-like molecules were mainly composed of glycerophospholipids, fatty acids, steroids and their derivatives (Figure S3). Among the glycerol phospholipid metabolites, the abundance of Lyso-phosphatidylcholine (LPC), Lyso-phosphatidylethanolamine (LPE), and Phosphatidylcholine (PC) was significantly higher at D1 stage than at other stages. The abundance of most fatty Acyls metabolites such as FAHFA was highly expressed at the M2 stage. At the same time, the abundance of several organic acid metabolites, such as L-Ornithine, betaine, L-serine, threonine, and valine, was also higher at the D1 stage (Figure S4). In addition, the expression abundance

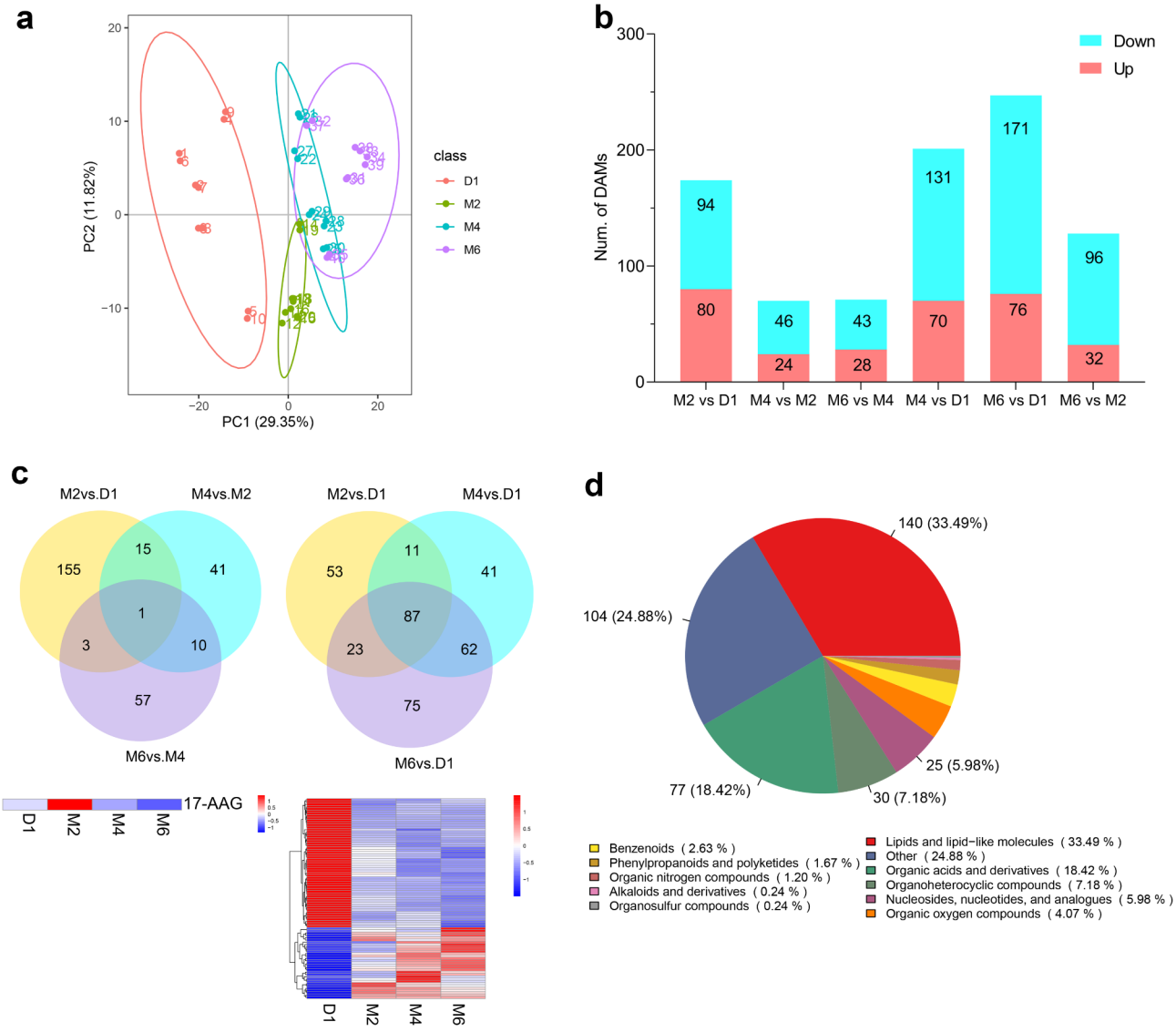

Fig. 1 Metabolomic analysis of the hypothalamus during postnatal sexual development in goats. (**a**). PCA analysis of metabolomics data in D1, M2, M4 and M6 periods. (**b**). Histogram of up- and down-regulated differentially expressed metabolites (DAMs). (**c**). Venn diagram and cluster heatmap of DEGs identified in hypothalamic tissue. (**d**). Classification pie chart of DAMs

of metabolites such as anserine, L-Histidine, carnosine, and gamma-glutamylmethionine was low in D1 stage, but remained high in the subsequent stages of sexual development. These results suggest that different metabolites may play different biological functions during sexual maturation in goats.

Dynamic changes and functional analysis of differential metabolites in hypothalamic tissue during sexual development

To investigate the temporal dynamics of hypothalamic metabolite profiles, we conducted K-means clustering and KEGG pathway enrichment analysis on the identified DAMs. The results showed that the 418 DAMs could be divided into 6 clusters according to their expression patterns (Fig. 2, Table S7).

Cluster 1 comprised 80 DAMs exhibiting a progressive decline in abundance with advancing sexual development. These metabolites were enriched in pathways associated with HIF-1 signaling pathway, neuroactive ligand-receptor interactions, circadian entrainment, tryptophan metabolism, and glutathione metabolism. In contrast, Cluster 4 contained 58 DAMs that demonstrated an initial increase followed by a decline, peaking at the M2 stage. This cluster showed functional enrichment in histidine metabolism, beta-Alanine metabolism, and AMPK signaling pathway. Cluster 6 (76 DAMs) exhibited an inverse expression pattern relative to Cluster 1, with metabolite levels progressively increasing during

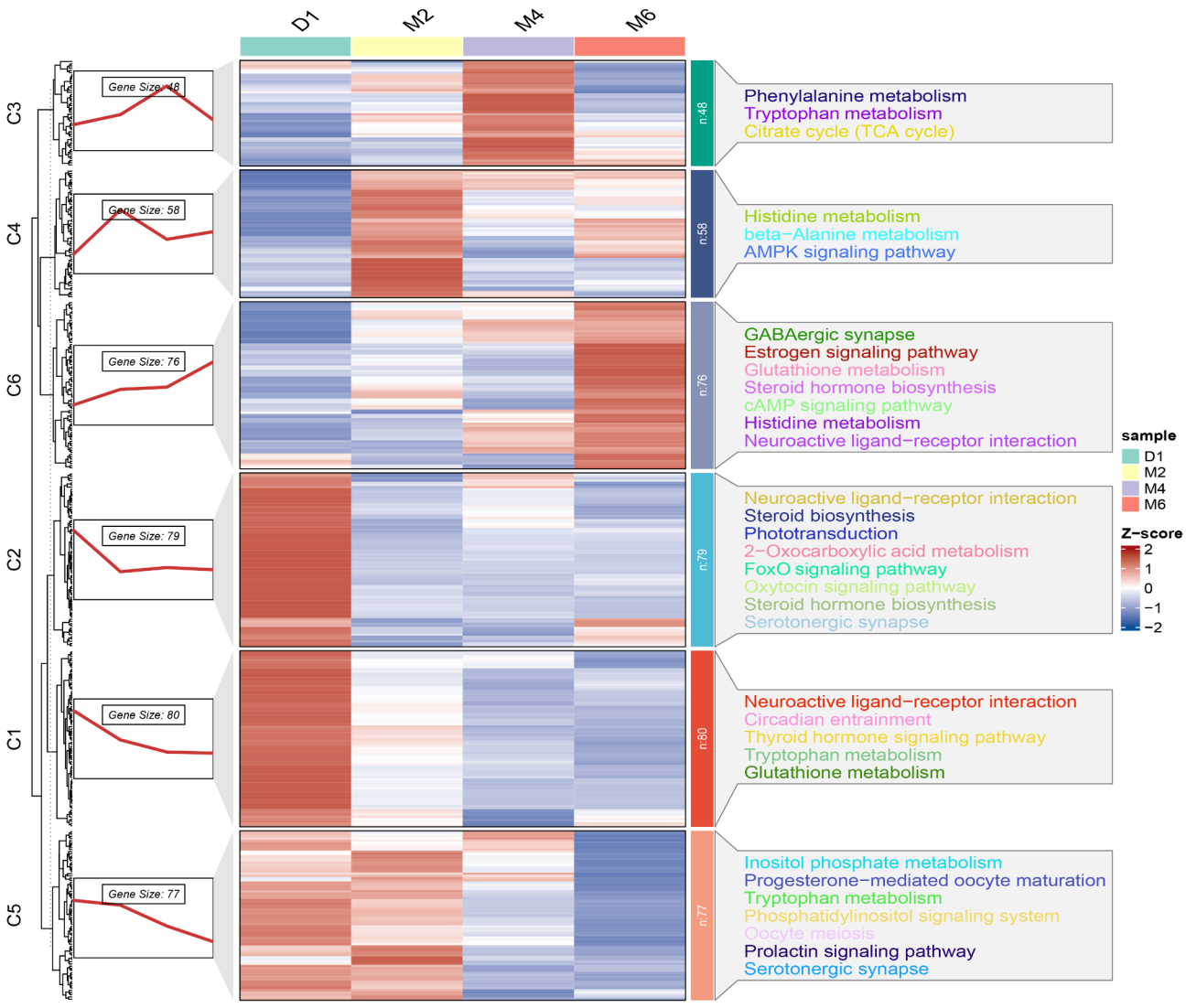

Fig. 2 Heatmap and hierarchical clustering KEGG annotation diagram of DAMs at four stages of postnatal sexual development in Jining grey goats. The color indicates the expression value of the normalized adjusted DAMs. Red and blue indicate upward and downward adjustments, respectively

sexual maturation. These DAMs were predominantly associated with GABAergic synapse, estrogen signaling pathway, glutathione metabolism, steroid hormone biosynthesis, cAMP signaling pathway, and histidine metabolism. These results suggest that these pathways may be involved in the sexual maturation of goats by regulating amino acid synthesis and metabolism, hormone secretion, and signaling.

WGCNA analysis identified key metabolites during postnatal sexual development in goats

To reduce data dimensionality and minimize interference from background noise, thereby facilitating the identification of key DAMs associated with phenotypic traits, we integrated previously acquired goat serum hormone data with the identified DAMs and conducted a WGCNA (Table S8) [41]. Using a soft threshold of $\beta = 7$, we identified a total of five modules (Figs. 3a and S5a). Among these, the brown module exhibited significant positive correlations with GnRH ($R = 0.56$, $P < 0.01$), FSH ($R = 0.33$, $P = 0.04$), progesterone (PROG) ($R = 0.44$, $P < 0.01$), and estradiol (E2) ($R = 0.37$, $P = 0.02$) (Fig. 3b). In contrast, the turquoise module revealed significant negative correlations with GnRH (R = -0.45, $P < 0.01$), FSH (R = -0.58, $P < 0.01$), luteinizing hormone (LH) (R = -0.63, $P < 0.01$), PROG (R = -0.37, $P = 0.02$), and E2 (R = -0.57, $P < 0.01$).

Based on the gene significance and module membership (GS-MM) results (Fig. 3c and f), we selected the turquoise and brown modules for further analysis. KEGG pathway enrichment analysis indicated that the DAMs in the brown module were primarily associated with arginine biosynthesis, phenylalanine metabolism, and glutathione metabolism pathways (Fig. 3d), whereas the

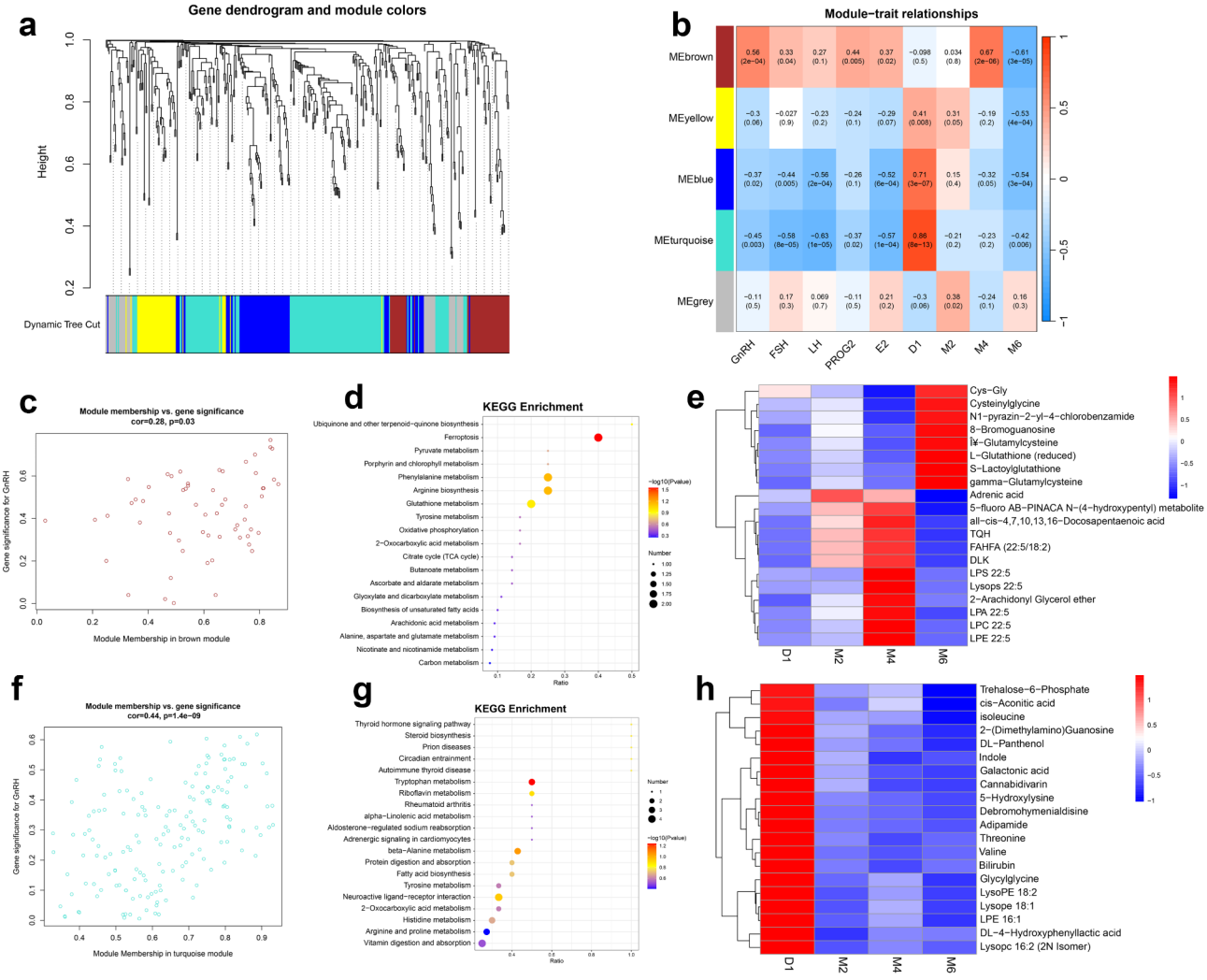

Fig. 3 Weighted gene co-expression network analysis (WGCNA) of DEGs identified at different developmental stages of goat hypothalamic tissue. (**a**). Hierarchical cluster tree of co-expression modules identified. (**b**). Heatmap of the correlation between module characteristic metabolites and serum hormone content (GnRH, FSH, LH, PROG2, E2) and developmental stage (D1, M2, M4, and M6). Red indicates a positive correlation between DAMs and serum indices, and blue indicates a negative correlation. (**c**). GS-MM scatterplot of the brown module with GnRH. (**d**). GS-MM scatterplot of the turquoise module with GnRH. (**e**). KEGG enrichment analysis scatterplot of DAMs in the brown module. (**f**). KEGG enrichment analysis scatterplot of DAMs in the urquoise module. (**g**). Expression heatmap of the top 20 DAMs of the brown module. (**h**). Expression heatmap of the top 20 DAMs of the turquoise module

DAMs in the turquoise module were mostly enriched in tryptophan metabolism, β-alanine metabolism, and neuroactive ligand-receptor interaction pathways (Fig. 3g).

Utilizing |KME| for filtering, we identified the top 20 hub DAMs to elucidate the key metabolites regulating hormone secretion during goat sexual development. Notably, DAMs in the brown module demonstrated the highest abundance during the M4 and M6 stages (Fig. 3e), whereas the DAMs in the turquoise module peaked in expression at the D1 stage (Fig. 3h). Interestingly, certain DAMs in the brown module, such as adrenic acid, DLK, TQH, and FAHFA (22:5/18:2), displayed elevated expression levels during both M2 and M4.

Hypothalamic transcriptome WGCNA analysis

WGCNA analysis was performed to further identify key genes during postnatal sexual development in goats using 506 previously identified DEGs (Table S9) [42]. Based on a soft threshold of β = 6 (Figure S5b), a co-expression network modeling was constructed. Combining the phenotypic data of serum hormones after birth in Jining grey goats (Table S8) [41], 506 genes were further divided into 6 modules (Fig. 4a). Among them, the blue and green modules were significantly positively correlated with serum GnRH and FSH levels ($p < 0.05$) (Fig. 4b). The GS-MM correlation results showed that there was a significant positive correlation between DEGs and GnRH content in the blue and green module (Cor = 0.44, $P < 0.01$; Cor = 0.41, $P < 0.01$) (Fig. 4c and f). The KEGG

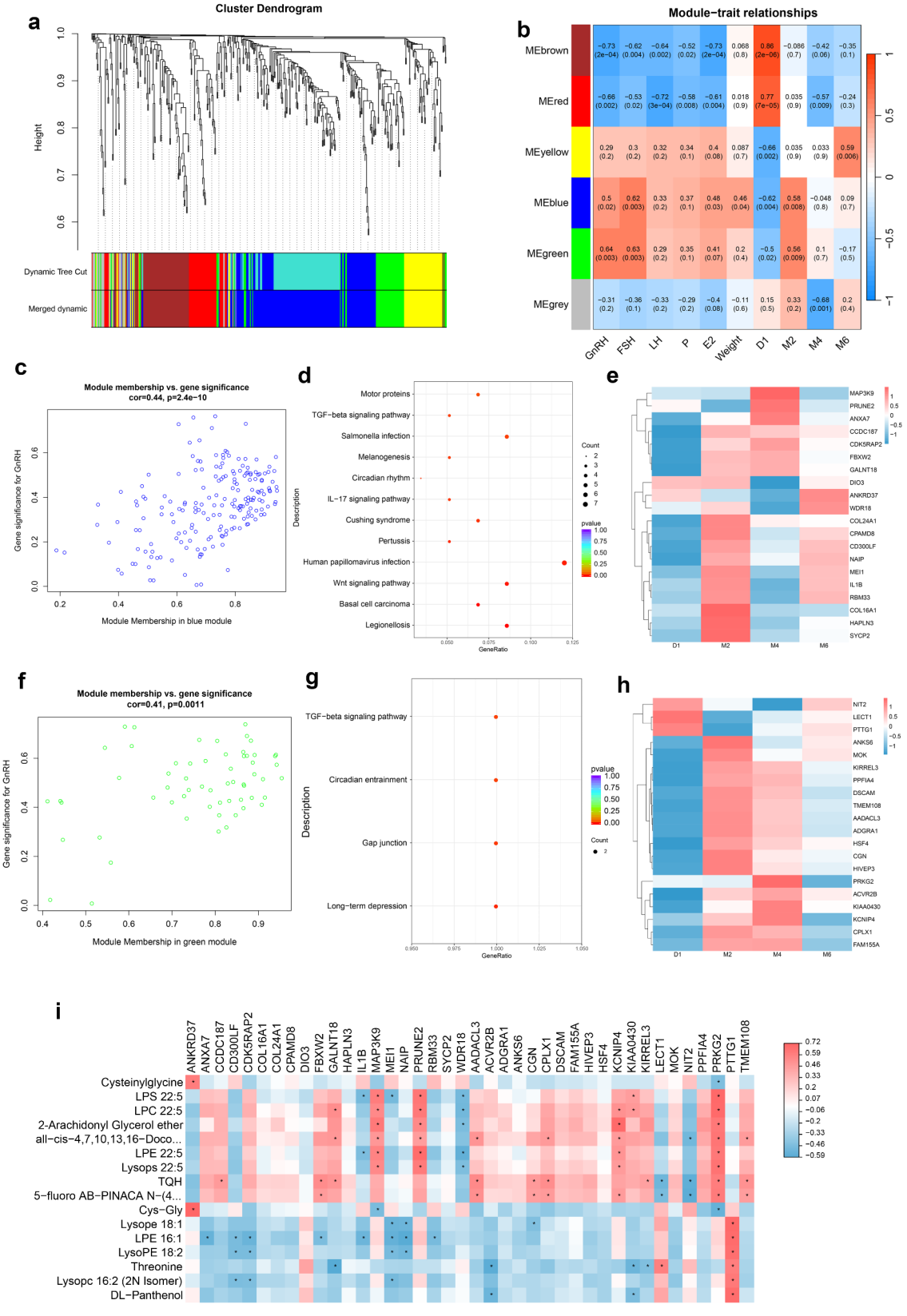

Fig. 4 (See legend on next page.)

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Fig. 4 WGCNA of DEGs identified at different stages of sexual development in goat hypothalamic tissue. (**a**). Hierarchical clustering tree of hypothalamic DEGs. (**b**). Correlation heat map between gene co-expression network modules and phenotypic indicators such as serum hormones. The red and blue modules indicate positive and negative correlations, respectively. (**c**). GS-MM scatterplot between the blue module and GnRH. (**d**). KEGG enrichment analysis scatterplot of DAMs in the blue module. (**e**). The heatmap of DEGs expression in the top 20 of the blue module. (**f**). GS-MM scatterplot between the green module and GnRH. (**g**). KEGG enrichment analysis scatterplot of differential DAMs in the green module. (**h**). he heatmap of DEGs expression in the top 20 of the green module. (**i**). Correlation heat map between Hub DEGs and Hub DAMs. Red signifies a positive correlation, while blue represents a negative correlation. Asterisks (*) denote significance levels of $p < 0.05$

enrichment analysis results indicated that the DEGs within the blue module were significantly enriched in several pathways, including the Wnt signaling pathway, TGFβ signaling pathway, and IL-17 signaling pathway ($p < 0.05$) (Fig. 4d). Interestingly, DEGs in the green module were also significantly enriched in the TGFβ signaling pathway ($p < 0.05$) (Fig. 4g). The top 20 DEGs in each module were defined as hub genes, and the clustered heat map showed that most of the hub genes in the blue module were highly expressed during the M2 period (Fig. 4e), while most hub genes in the green module were highly expressed during the M2 and M4 periods (Fig. 4h).

Additionally, this study computed the Spearman correlation coefficients between the hub DAMs identified through metabolomic WGCNA analysis and the hub DEGs in the green and blue modules. The results indicated that prune homolog 2 with BCH domain (*PRUNE2*), mitogen-activated protein kinase kinase kinase 9 (*MAP3K9*), potassium voltage-gated channel interacting protein 4(*KCNIP4*), protein kinase cGMP-dependent 2 (*PRKG2*), and PTTG1 regulator of sister chromatid separation, securing (*PTTG1*) were significantly positively correlated with LPC22:5, 2-Arachidonyl Glycerol ether, LPE22:5, and Lysops22:5 ($P < 0.05$). In contrast, WD repeat domain 18 (*WDR18*) exhibited a significant negative correlation with these DAMs ($P < 0.05$). Furthermore, *PTTG1* demonstrated a significant positive correlation with various metabolites, including threonine ($P < 0.05$). Furthermore, our previous research has shown that the transcriptomic sequencing data is consistent with the qRT-PCR data (Figure S7), indicating the reliability of our sequencing results [42].

Association analysis of metabolome and transcriptome data

Integrating transcriptomic and metabolomic analyses provides a more comprehensive perspective on the regulatory mechanisms underlying complex biological processes. Initially, K-means clustering analysis and KEGG enrichment analysis were utilized to further explore the key genes and pathways involved in goat sexual development (Figure S6). Combining these results with metabolomic data, we found that the DEGs in cluster 3 of the transcriptomic analysis exhibited a similar expression pattern to the DAMs in cluster 1 of the metabolomic analysis, while demonstrating an opposing expression pattern compared to the DAMs in cluster 6. Both clusters were significantly enriched in the glutathione metabolism pathway ($p < 0.05$) (Figs. 2 and S6). By integrating these results, we further elucidated the characteristics of glutathione metabolism in hypothalamic tissues during goat puberty (Fig. 5), reflecting the precise regulatory patterns of gene expression in the hypothalamus.

Discussion

The hypothalamus is the most upstream tissue of the gonadal axis and plays an important role in the sexual development of goats after birth. Therefore, this study employed non-targeted metabolomics and transcriptomics techniques to analyze the important regulatory mechanisms in female goat hypothalamic tissue during sexual maturation.

In this study, the abundance of lipids and lipid-like molecules, as well as organic acids and their derivatives, in hypothalamic tissue changed significantly during goat sexual maturation. Among them, most lysophosphatidylcholine (LPCs, including LPC14:0, LPC22:3, LPC18:2, LPC20:3, LPC16:2, LPC18:3, LPC16:1), lysophosphatidylethanolamine (LPE, including LPE16:0, LPE22:3, LPE18:0, LPE20:3, LPE20:2, LPE18:1, LPE18:2, LPE14:0), and other metabolites were highly expressed in goats after birth. Their expression abundance decreased significantly before the first puberty period and then remained stable. Lipids in the hypothalamus play a key role in maintaining biological processes such as cellular homeostasis, signal transduction, and energy storage [43, 44, 45]. LPCs exert biological functions by activating ion channels, promoting the release of inflammatory cytokines, and inducing apoptosis and oxidative stress [46]. In addition, LPC activates the MAPK signaling pathway, enhances nerve growth factor (NGF) production [47], and is also involved in the synthesis of acetylcholine in the brain, thereby promoting nerve conduction [48, 49, 50]. However, LPE is structurally similar to LPC and is involved in cell signaling and enzyme activation [51]. It can also induce neuronal differentiation in PC12 cells [52, 53]. The highest levels of LPE and LPC were found in the D1 stage of Jining goats, possibly related to the regulation of lipid metabolism in the hypothalamus after birth, supporting thermoregulation and nervous system development [54]. FAHFAs are a new class of endogenous lipids that have been shown to promote glucose metabolism and have anti-inflammatory and antioxidant effects [55]. Unlike most FAHFAs in the serum during

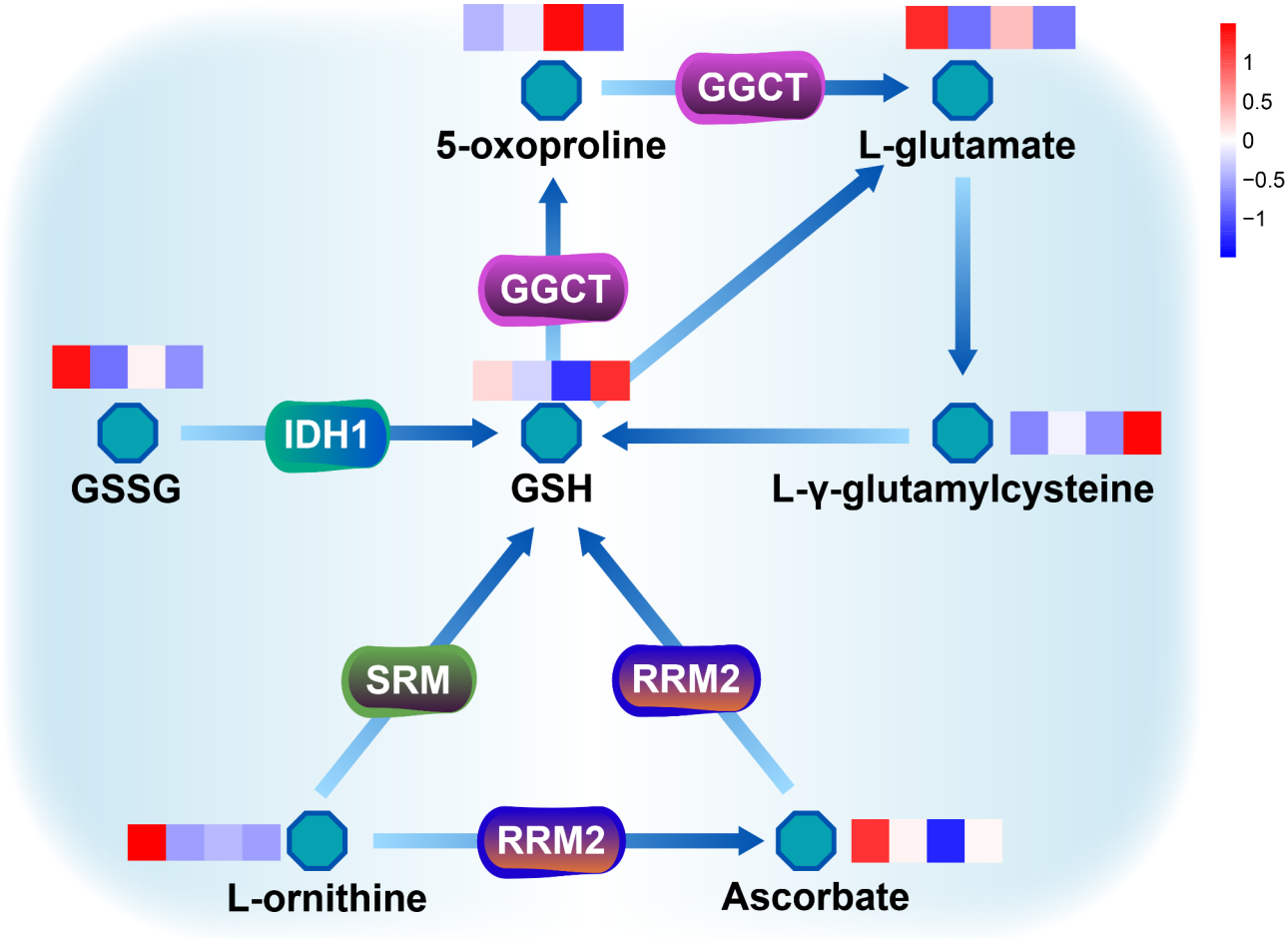

Fig. 5 Schematic diagram of the involvement of DEGs and DAMs in hypothalamic tissue in the synthesis of glutathione during postnatal sexual development of goats

goat sexual development, which are highly expressed at the M4 stage [56], hypothalamic FAHFA-like metabolites exhibit higher expression levels at the D1 and M2 stages. This may be related to the role of FAHFAs in regulating glucose homeostasis, helping neurons in the hypothalamus perceive energy status in vivo, which in turn affects their growth and development [57, 58, 59]. In addition, ACar is produced when L-carnitine combines with fatty acids, promoting thermogenesis in brown adipose tissue and participating in multiple energy metabolism pathways [60, 61]. It was found that most ACar metabolites were lowest after birth and increased with the gradual maturation of sexual development. This suggests that ACar may be involved in regulating energy homeostasis during the development of goats after birth. As the body develops and body weight reaches a specific threshold, signals are transmitted to the hypothalamus, promoting the secretion of GnRH and triggering sexual maturation in animals [62, 63, 64]. Overall, these studies indicate that lipids play a key role in goat sexual maturation and nervous system development.

Metabolites with different expression patterns may play a similar role during sexual development in goats. In this study, six different expression patterns of metabolites were identified, which were mainly enriched in histidine metabolism, β-alanine metabolism, neuroactive ligand-receptor interaction, glutathione metabolism and other pathways. All of these, including Anserine, L-histidine, and Carnosine, were enriched in the histidine metabolism and β-alanine metabolism pathways [65]. In mammals, histaminergic neurons are predominantly found in the hypothalamus, and L-Histidine is converted to histamine to regulate feeding behavior and energy metabolism, and is involved in the synthesis and release of growth hormone and thyroid-stimulating hormone (TRH) [66]. Carnosine is a dipeptide composed of alanine and L-histidine, and its analogue Anserine is present in skeletal muscle and nervous system, and has been identified in the rat hypothalamus [67]. Carnosine and Anserine have antioxidant activity and also modulate nerve signaling [68, 69]. Moreover, neuroactive ligand-receptor interactions have been confirmed to play an

important role in hypothalamic reproductive regulation [70, 71]. Metabolites identified in this study, such as taurine and 4-aminobutyric acid (GABA), are enriched in this pathway. Taurine is one of the most abundant free amino acids in the hypothalamus and pituitary tissue of animals [72], with the highest levels found in the fetal brain, which decrease with age [73]. This finding aligns with the age-dependent reduction of taurine observed in the sexual maturation process of goats in this study. Research has shown that taurine can inhibit the release of pituitary LH via the hypothalamus [74]. Additionally, taurine-mediated GnRH release may activate the GABAergic system, significantly suppressing HPG axis activity and leading to delayed sexual development [75]. GABA stimulates GnRH release in immature male rats while inhibiting GnRH secretion in adult rats [76]. In this study, GABA levels increased from D1 to M2, potentially related to the rise in GnRH secretion from the hypothalamus. Our study reveals dynamic changes in hypothalamic metabolites during sexual maturation in goats. During this process, changes in neuron development and hormone secretion are accompanied by changes in the expression levels of signaling-related metabolites (Anserine, L-histidine, Carnosine, taurine, and 4-aminobutyric).

Through WGCNA analysis by transcriptomics and metabolomics, we identified a number of DEGs and DAMs associated with hypothalamic GnRH secretion and performed spearman correlation analysis on them. This opens up the possibility of further research into the association between these DEGs and DEMs. *MAP3K9* regulates signal transduction through mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) pathways [77]. The MAPK signaling pathway is critical to neurodevelopment and GnRH hormone secretion [78, 79, 80]. Studies have shown that PRUNE2 may be involved in neurodevelopmental processes in sheep [81]. KCNIP4 is a small calcium-binding biomolecule that regulates neuronal excitability in response to changes in intracellular calcium levels [82, 83], and has also been identified as a candidate gene for reproductive traits in buffalo [84], poultry [85, 86], and sheep [87]. 2-Arachidonoylglycerol (2-AG) is a key regulator of neurotransmitter release [88] and plays an important role in food intake and metabolism [89]. In our study, the expression levels of *MAP3K9*, *PRUNE2* and *KCNIP4* gradually increased with sexual development after birth, reaching a peak at M4 stage, and all of them were significantly positively correlated with 2-Arachidonyl Glycerol Ether. These results suggest that *MAP3K9*, *PRUNE2*, *KCNIP4*, and 2-Arachidonyl Glycerol Ether may be involved in the sexual development of goats after birth by regulating hypothalamic GnRH neuronal development and energy metabolism.

The results of joint analysis showed that the glutathione metabolism pathway was enriched in both transcriptome and metabolome. Glutathione (*GSH*) is a tripeptide composed of glutamate, cysteine, and glycine that is involved in biological processes such as redox, apoptosis, and neuromodulation [90, 91, 92]. Glutamate and its receptors are present in all key hypothalamic regions closely related to reproduction [93] and have been shown to regulate mammalian GnRH neuronal development and neuroendocrine function [94, 95]. In this study, the elevated L-glutamate content in the hypothalamus at the D1 and M4 stages may relate to its regulation of GnRH neuronal development and secretion associated with sexual maturity. GSH is mainly converted from Glutathione disulfide (*GSSG*). *IHD1*, on the other hand, is mainly involved in the conversion of *GSSG* to *GSH* via *NADPH* from the pentose phosphate pathway [96]. Goats have higher levels of hypothalamic *GSSH* and *IDH1* after birth, and the ability of *GSSG* to convert to GSH increases, ultimately leading to higher postnatal hypothalamic *GSH* content, consequently, this may be related to postnatal hypothalamic neuronal development [97]. Therefore, homeostasis of glutathione metabolism may play an important role in the sexual maturation of goats.

However, this study has some limitations. Firstly, molecular biology experiments have not yet been conducted on the identified key genes and metabolites. Additionally, since this research focuses on Jining grey goats, further validation is needed to determine whether these findings are applicable to other early-maturing goat breeds.

Conclusion

In this study, metabolites such as Anserine, L-histidine, Carnosine, taurine, and 4-aminobutyric may affect neuron development and hormone secretion processes during sexual maturation in goats via histidine and phenylalanine metabolic pathways. Furthermore, *MAP3K9*, *PRUNE2*, and *KCNIP4* may jointly regulate the development and energy metabolism of hypothalamic GnRH neurons in conjunction with DAMs such as 2-Arachidonyl Glycerol Ether. Moreover, the homeostasis of glutathione metabolism may be involved in the regulation of GnRH hormone secretion during sexual maturation. This study offers a new perspective for understanding the regulatory mechanisms of the hypothalamus in goat sexual development, which may yield important insights for enhancing reproductive traits in future animal breeding efforts.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11492-2.

Supplementary Material 1

Supplementary Material 2

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Author contributions
J. W. and Q.L. designed the research; Q.L., Y. W., P. H. and L.Z. performed sampling; Q. L. analyzed the data and wrote the manuscript. T. C. and J. W. edited the manuscript. All authors reviewed the manuscript.

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Data availability
Data is available in the article and supplementary information. The dataset supporting the conclusions of this article is available in the Gene Expression Omnibus (GEO) repository, GSE244004 in https://www.ncbi.nlm.nih.gov.

Declarations

Ethics approval and consent to participate
The study was approved by the Animal Care and Use Committee of Shandong Agricultural University ethics committee (SDAUA-2023-157), and all methods are reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Supplementary Information
The datasets supporting the conclusions of this article are included within the article (and its additional files).

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