Seasonal patterns of prolactin, prolactin receptor, and STAT5 expression in the ovaries of wild ground squirrels (*Citellus dauricus* Brandt)

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ABSTRACT

Prolactin (PRL) is a hormone crucial for normal reproduction, functioning as an autocrine, paracrine, and endocrine factor. This study aimed to examine the immunolocalization and expression patterns of PRL, prolactin receptor (PRLR), and signal transducer and activator of transcription 5 (STAT5) in the ovaries of wild ground squirrels during both breeding and non-breeding periods. Significant seasonal variations were observed in ovarian weights, with higher values during the breeding season and relatively lower values during the non-breeding season. PRL, PRLR, STAT5, and p-STAT5 were immunolocalized in granulosa cells and luteal cells during the breeding season, whereas they were exclusively found in granulosa cells during the non-breeding season. The mRNA expression levels of *Prl, Prlr,* and *Stat5* were increased in ovarian tissues during the breeding season compared to the non-breeding season. Moreover, the mean mRNA levels of *Prl, Prlr,* and *Stat5* exhibited a positive correlation with ovarian weights. Both circulating PRL and ovarian PRL concentrations were significantly elevated during the breeding season. Additionally, transcriptomic analysis of ovarian tissues revealed differentially expressed genes possibly associated with ovarian function and mammary gland development, including ovarian follicle development, steroid synthesis, and regulation of reproductive process. These findings suggest that PRL might play an essential endocrine, autocrine, or paracrine role in the regulation of seasonal changes in the ovarian functions in wild ground squirrels.

Key words: wild ground squirrel; prolactin; prolactin receptor; STAT5; ovary.

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Introduction

Prolactin (PRL) is a peptide hormone mainly synthesized and stored by lactotroph cells in the anterior pituitary gland.[1] PRL mediates its physiologic functions through the engagement of PRL receptor (PRLR), a member of the cytokine receptor superfamily.[2] Its amino acid sequence is similar to that of growth hormone (GH) and placenta lactogen (PL) sharing genomic, structural and biological features and belonging to the same PRL/GH/PL protein family.[3] PRL is highly versatile, with multiple biological functions including effects on water and salt balance, growth and development, endocrinology and metabolism, brain and behavior, reproduction, and immune regulation and protection.[4-6] There are three types of the major membrane-bound PRLR isoforms, and they are the long, intermediate, and short isoforms.[7] The short and long forms differ from each other in the length of their cytoplasmic domain resulting from alternative splicing of a single primary transcript.[1] Generally, the most abundant PRLR is the long isoform, whereas other intermediate and short forms also exist in mammals.[8] All three type isoforms of PRLR are devoid of any intrinsic enzymatic activity, PRL signal transduction begins with hormone-receptor binding, which invokes receptor dimerization and Janus Kinase-2 (JAK2) activation.[9]

Signal transducer and activator of transcription (STAT) proteins, STAT1, STAT3, and STAT5, serve as pivotal transducers in the signal transduction pathways initiated by PRLR activation.[10] Among these proteins, STAT5 is recognized as the most critical transducer for the long and intermediate isoforms of PRLR, while only small amounts of phosphorylated STAT1 and STAT3 are detected.[9-11] Following activation by PRL, STAT5 recruits transcription factors from diverse signaling pathways, influencing cell growth and differentiation.[11] The widespread occurrence of PRLR in multiple extrapituitary sites implicated in PRL production suggests local autocrine and paracrine roles of extrapituitary PRL.[12] PRLR expressions have been observed in the skin, testes, prostate, and reproductive accessory tissues.[13-16] It has been demonstrated that PRL may regulate genes associated with various ovarian events, such as follicular development, as evidenced by PRL gene knockout mice showing reduced follicle size, delayed oocyte release, and impaired oocyte maturation.[17] PRL-null mice are unable to lactate due to a failure of lobuloalveolar differentiation of the mammary epithelium, similar to PRLR-null and STAT5a-conventional knockout mice.[18-20] This underscores the potential role of PRL in promoting reproductive function *via* PRLR. Previous studies have indicated the importance of PRL in the seasonal reproduction of mammals.[21] However, the presence or extent of causative relationships between the seasonal variations in reproduction and the expression patterns of PRL and PRLR in reproductive organs remains unclear. The wild ground squirrel (*Citellus dauricus* Brandt) is a quintessential seasonal breeding mammal, displaying an annual reproductive cycle.[22] During the breeding season (April to May), germ cells maturation and mating, birthing, and pup-rearing activities occur, which are absent during the non-breeding season (June to March).[23] Our previous studies have demonstrated the seasonal expressions of follicle-stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHR), estrogen receptors α (ERα), and estrogen receptors β (ERβ) in the ovaries of wild ground squirrels.[22,24] Moreover, the concentrations of FSH, LH, progesterone, and estrogen were higher during the breeding season in comparison to the non-breeding season.[25-27] These findings indicate the potential roles of gonadotropins and sexual steroid hormones in driving the seasonal changes in ovarian functions of wild ground squirrels. In this study, we investigated the expressions and distribution patterns of PRL, PRLR, and STAT5 in the ovarian tissues of wild ground squirrels during both the breeding and non-breeding seasons. Our objective was to enhance our understanding of the relationship between PRL and the seasonal shifts in the ovarian functions of wild ground squirrels.

Materials and Methods

Animals

The wild adult female ground squirrels (weighing 230-419 g) were captured in Hebei Province, China, during the breeding season (April, n=10) and the non-breeding season (September, n=10). The wild ground squirrels were euthanized with an overdose of sodium pentobarbital (BioDee Co., Beijing, China), and their ovaries were dissected. The ovaries (n=10) were weighed with an electronic scale. One side of ovaries of the wild ground squirrels (n=10) were fixed in 4% paraformaldehyde in 0.05 M PBS (pH=7.4) buffer for histological and immunohistochemical analysis, and the other side of ovaries (n=10) were immediately frozen in liquid nitrogen and stored at -80°C for gene expression analysis. Blood samples were centrifuged at 1500 rpm for 20 min at 4°C and the supernatant was aspirated and stored at -80°C. The plasma samples were analyzed for hormone concentration. All animal testing procedures were approved by the Animal Protection and Utilization Policy of the Beijing Forestry University Ethics Committee (EAWC_BJFU_202008) and approved by the Hebei Provincial Department of Agriculture (JNZF11/2007).

Histology

The ovarian specimens of wild ground squirrels were dehydrated in an alcohol-xylene gradient and paraffin-embedded. The paraffin blocks were sectioned at 5 μm. The tissue sections were mounted on poly-L-lysine coated slides. The deparaffinized tissue sections were stained with hematoxylin and eosin (H&E). The stained sections were gradient-dehydrated and sealed with neutral resin. The prepared tissue sections were observed under the microscope for overall tissue morphology, follicle distribution, and cell types.

Immunohistochemistry

Immunohistochemical experiments were elucidated using KeyGEN One-Step IHC Assay (DAB, compatible for rabbit and mouse). The de-paraffinized ovarian sections were incubated in citrate buffer, washed with PBS, and placed in 3% H_2O_2-methanol solution for 30 min to eliminate endogenous peroxidases. A drop of reagent A (10% goat serum blocking solution) was added to the tissue sections that were incubated for 30 min and then with primary antibody overnight (4°C); 1× PBS was used as a negative control instead of primary antibody. The type of primary antibodies was showed in Table 1. The specificity of PRL, PRLR, STAT5 and

Table 1. Types of immunohistochemistry primary antibodies.

Antibody name	Dilution of primary antibody	Item no.	Company name	Origin
PRL	1:200	bs-0508R	Bioss Biotechnology	Beijing, China
PRLR	1:200	bs-6445R	Bioss Biotechnology	Beijing, China
STAT5	1:200	bs-1142R	Affinity Biosciences	Jiangsu, China
p-STAT5	1:200	AF3304	Bioss Biotechnology	Beijing, China

pSTAT5 antibodies has been described in our previous studies.[28] After washing away the primary antibody, a drop of Reagent B (enhancer) was added and incubated for 30 min, then the slides were washed three times with PBS for 3 min each time. Then, reagent C (HRP polymers with secondary antibodies) was added and incubated for 30 min. The slides were then washed three times with PBS for 3 min each time and DAB chromogenic solution was added for staining that terminated with distilled water. Finally, the nuclei were re-stained with hematoxylin and the tissues were dehydrated and sealed with neutral balsam. We scored the positive signals of DAB staining of ovarian tissues with ImageJ (1.53k, National Institutes of Health, Bethesda, MD, USA) The immunohistochemical staining was determined as positive (+), strong positive (++), very strong positive (+++), and negative (-). Staining that was weak but higher than control was set as positive (+); the highest intensity staining was set as very strong positive (+++); staining intensity between + and +++ was set as strong positive (++).

Total RNA extraction

Total RNA was extracted from the ovarian tissues of wild ground squirrels using TRIzolTM Kit (Invitrogen, Carlsbad, CA, USA); 0.1 g of ovarian tissues were thawed, lysed, and ground, and 1 mL of TRIzol reagent was added and allowed to stand for 5 min at room temperature to allow sufficient lysis. After adding 0.2 mL of chloroform, the tissues were shaken vigorously for 30 s at room temperature and left to stand for 10 min. The samples were centrifuged at 12,000 g for 20 min at 4°*C*, then the aqueous phase was transferred to a new tube, 500 µL of isopropanol was added, and the sample was kept at room temperature for 10 min. The ribonucleic acid pellet was washed twice with 70% cold ethanol and dissolved in 30 µL of ultrapure water. The integrity of ribonucleic acid was detected by gel electrophoresis, and the ribonucleic acid was diluted to 250 ng/µL after measuring the concentration by spectrophotometer.

qPCR

Synthesis of first strand cDNA for total RNA using Hiffair® III 1st Strand cDNA Synthesis (Yeasen Biotechnology, Shanghai, China) for qPCR. Add 8 µL of RNase-free ddH_2O, 3 µL of 5×g DNA digester Mix, and 4 µL of total RNA to an RNase-free centrifuge tube, mixed well with a pipette, and incubated at 42°C for 2 min. 5 µL of 4× Hiffair® SuperMix plus was added to the reaction solution and set at 25°C for 5 min, 55°C for 15 min, and 85°C for 5 min. After the reaction, 80 µL of RNase-free ddH_2O was added and diluted. The Hieff UNICON® Universal Blue qPCR SYBR Green Master Mix Kit (Yeasen Biotechnology, Shanghai, China) was utilized to configure the 10 µL system. Table 2 displayed the designation of primers. Data were measured by the ABI PRISM 7500 fast real-time polymerase chain reaction system (Applied Biosystems, Foster City, CA, USA). 95°C preheat for 10 min was followed by 40 cycles (95°C preheat for 30 s, 60°C preheat for 30 s, and 72°C preheat for 30 s), and finally, 60-95°C melt curve steps. The relative expression of target genes was analyzed according to the expression of the internal reference Actb (β-actin).

Hormone assay

The hormone concentrations were measured in plasma samples from the female wild ground squirrels during the breeding season (April) and in the non-breeding season (September).The plasma samples of both seasons were centrifuged at 3,000 rpm for 15 min at 4°C. The frozen portion of both seasons of the ovary tissues of wild ground squirrels was lysed in a homogenizer containing PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged at 5,000 g for 5 min at 4°C. Aspirate the supernatant and assayed immediately by ELISA Kits (Rat prolactin ELISA Kit, CSB-E06881r Cus-bio Biotech Co., Ltd., Wuhan, China), which were designed for the detection of PLR concentrations. All samples in the same experiment, the intra-assay coefficient of variation was less than 15%. The inter-assay coefficients of variations for PRL were less than 15%.

Transcriptome data analysis

The ovarian tissues (n=3) of wild ground squirrels from both seasons were sent to Yuanquan Eko Biotechnology (Beijing, China) for sequencing *via* the Illumina GAIIx platform. Raw reads from sequencing were filtered for high-quality clean reads by removing reads containing connectors, unknown nucleotides (> 10%), and low-quality (Q≤20) bases (>50%). Differentially expressed genes (DEGs) were identified using the DESeq2 package in R (v4.1.2).[29] Genes with adjusted $p \leq 0.05$ and $|Log2FoldChange| \geq 1$ were designated as differentially expressed. Based on the Gene Ontology (GO) database, functional annotation and enrichment analysis of DEGs were performed with GOseq software to determine the interrelationship of GO terms with genes and analyze the GO molecular function.[30] Based on the specific genes in the GO terms, the expression levels in the breeding season and non-breeding season were analyzed.

Statistical analysis

Data were analyzed using the *t*-test with GraphPad Prism 9 software. All data were presented as the means ± SEM, and a $p<0.05$ was considered statistically significant.

Results

Histological and morphological characteristics in the ovaries of wild ground squirrels

Significant seasonal variations were observed in the ovarian weights of wild ground squirrels, with higher values during the breeding season and relatively lower values during the non-breeding season (Figure 1). It was found that ovary morphology was rounder and more enlarged during the breeding season compared to the non-breeding season (Figure 1a), and ovarian weight was significantly higher during the breeding season than that of the non-breeding season (Figure 1b). Primordial follicles, primary follicles, secondary follicles, tertiary follicles and corpus luteum were observed in ovaries during the breeding season (Figure 1c); whereas ovaries composed of primary and secondary follicles, and only a few tertiary follicles were observed in the non-breeding season (Figure 1d).

Table 2. Oligonucleotide sequences for real-time quantitative PCR.

Gene	Primers (5'-3')	Size(bp)
Prl	F:GGTCATCAATGACTGCCCCA R:CTCGAGGACTGCACCAAACT	150
Prlr	F:CATCTGCTGGAGAAGGGCAA R:CATTAGCCGCTCGTCCTCAT	135
Stat5	F:AAAGAATCAAGCGTGCTGACAGA R:GGACAGGGTCTTCACCTGGAA	125
Actb	F:GACTCGTCGTACTCCTGCTT R:AAGACCTCTATGCCAACACC	223

Immunohistochemical results of PRL, PRLR, STAT5, and p-STAT5 in the ovaries of wild ground squirrels

Immunohistochemical results of PRL, PRLR, STAT5 and p-STAT5 were showed in the ovaries of wild ground squirrels (Figure 2). PRL (Figure 2 a,b), PRLR (Figure 2 d,e), STAT5 (Figure 2 g,h) and p-STAT5 (Figure 2 j,k) were expressed in ovarian granulosa cells and luteal cells of the wild ground squirrels during the breeding season. The positive staining of PRL, PRLR, STAT5 and p-STAT5 in the ovarian granulosa cells were stronger in the breeding season than those of the non-breeding season (Figure 2 a-l, Table 3).

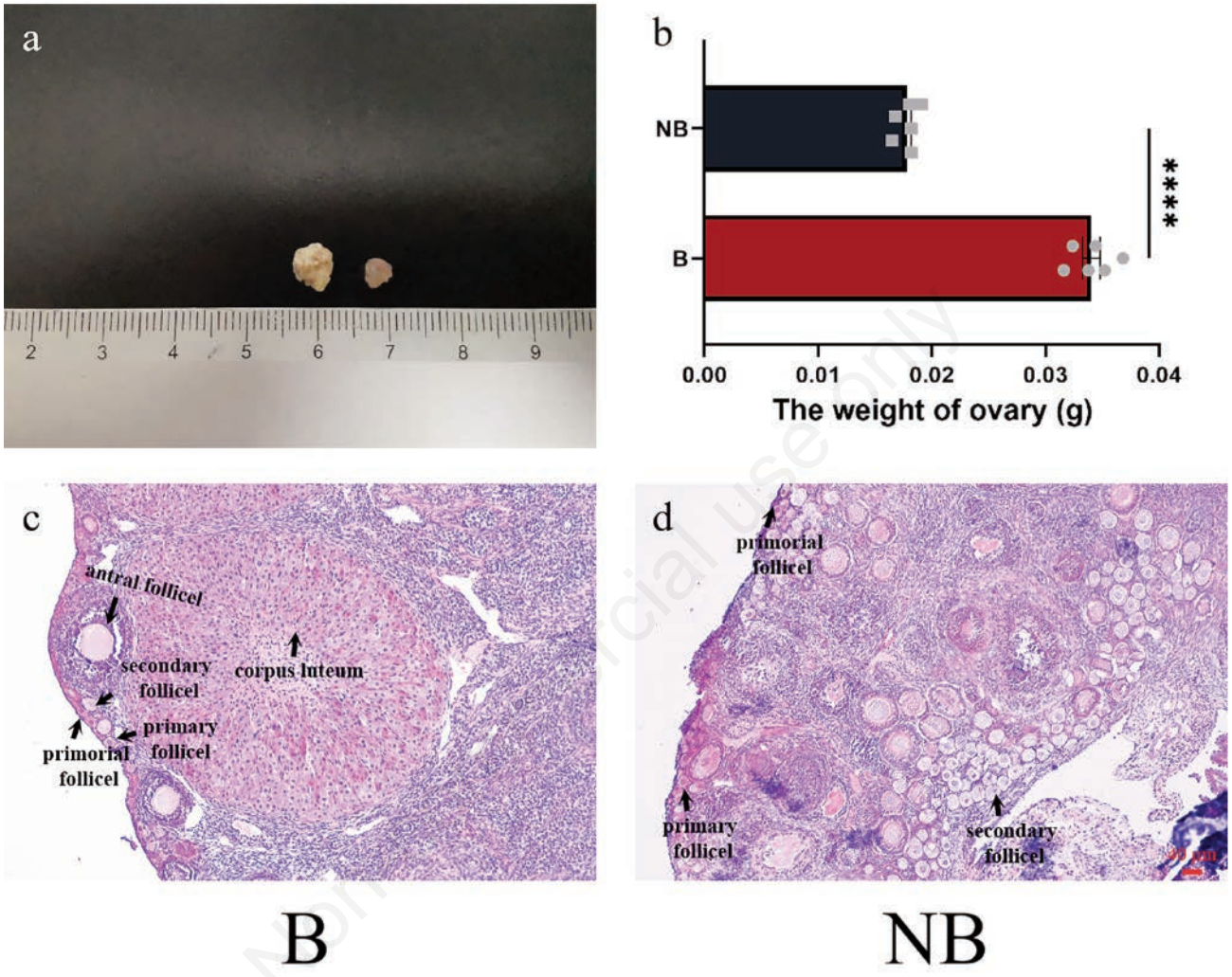

Figure 1. Seasonal changes in the morphology and histology of the ovaries of wild ground squirrels. Morphological observation of the wild ground squirrels during both breeding and non-breeding seasons (**a**). The mean weight (**b**) of the ovaries during the breeding and non-breeding seasons. H&E staining of the ovaries during both two seasons (**c,d**). Data are shown as the mean ± SEM. B, the breeding season; NB, the non-breeding season; scale bars: 40 μm (c,d); ****p<0.0001.

Table 3. Relative abundance of PRL, PRLR, STAT5, p-STAT5 in the ovaries of wild ground squirrels during both breeding and non-breeding seasons.

Antibodies	Breeding season				Non-breeding season		
	GC	IC	LC	TC	GC	IC	TC
PRL	++	++	+++	+++	+	+	++
PRLR	+++	++	+++	+++	+	+	+
STAT5	+++	+++	+++	+++	++	+	+
P-STAT5	++	++	+++	++	+	++	+
NC	-	-	-	-	-	-	-

B, the breeding season; NB, the non-breeding season; GC, granulosa cells; TC, theca cells; IC, interstitial cells; LC, luteal cells; –, negative staining; +, positive staining; ++, strong positive staining; +++, very strong positive staining.

The mRNA expression levels of *Prl, Prlr, Stat5* in the ovaries of wild ground squirrels

The mRNA expression levels of *Prl*, *Prlr,* and *Stat5* were increased in the ovarian tissues of wild ground squirrels during the breeding season compared to the non-breeding season (Figure 3 a-c). Moreover, the mean mRNA levels of *Prl*, *Prlr,* and *Stat5* exhibited a positive correlation with ovarian weights (Figure 3 d-e).

Concentrations of PRL in the plasma and the ovaries of wild ground squirrels

The plasma and ovarian tissues concentrations of PRL were measured in the wild ground squirrels during the breeding and the non-breeding seasons (Figure 4). The results showed that the plasma PRL concentration was 52.364±11.394 ng/mL in the breeding season and 16.595±2.724 ng/mL in the non-breeding season (Figure 4a). The ovarian PRL concentration was 0.948±0.035 ng/mg in the breeding season while 0.617±0.110 ng/mg in the non-breeding season (Figure 4b).

Transcriptome data analysis in the ovaries of wild ground squirrels

The ovarian tissues of wild ground squirrels (n=3) from both periods for transcriptome data analysis (Figure 5). By chord diagram analysis, the five GO terms specified for steroid-related pathways, including steroid biosynthetic process, ovarian follicle development, regulation of reproductive process, primary ovarian follicle growth, and cellular response to estrogen stimulus, and DEGs were mainly expressed in *Igf1*, *hcgr*, *Fshr*, *3b-hsd*, and *Hsd17b7* genes (Figure 5a). In GO functional analysis, biological processes (BP) related to reproduction were upregulated in the pathways of development of primary sexual characteristics, sex differentiation, and Sertoli cell differentiation (Figure 5b). The screening of genes related to ovarian function and mammary gland development for differential analysis, and the results showed that the expression of *hcgr*, *Fshr*, *Stat6*, and other genes were significantly higher in the breeding season than those of the non-breeding season (Figure 5c).

Discussion

This study presents, for the first time, the seasonal patterns of PRL, PRLR, and STAT5 expression in the ovarian tissues of wild ground squirrels during both the breeding and non-breeding seasons, revealing the occurrence of local PRL synthesis within ovarian tissues. The expression levels of PRL, PRLR, and STAT5 showed a positive correlation with changes in ovarian weight between the two seasons. In the breeding season, both circulating PRL and ovarian PRL concentrations were significantly elevated in comparison to the non-breeding season. Furthermore, transcriptomic analysis of ovaries from both seasons identified DEGs associated with ovarian follicle development, steroid synthesis, regulation of reproductive process, and steroid hormone receptor activity. These findings indicate that the ovarian tissues of wild ground squirrels possess the capability to locally synthesize PRL, which may critically regulates ovarian function through endocrine, autocrine or paracrine mechanisms. The morphology and histology of reproductive organs in seasonal breeders can be tightly regulated by the combined effect of reproductive hormones and local intrinsic factors.[28,31,32] Experimental evidence suggests a functional role for seasonal changes in PRL in controlling gonadotrophin secretion, gonadal activity, and sexual behavior.[33-35] Studies on muskrats have demonstrated that both circulating PRL and scented glandular PRL can directly regulate scented glandular function.[10,28] In our current study, mRNA expression levels of *Prl*, *Prlr,* and *Stat5* increased in tandem with elevated circulating PRL and ovarian PRL concentrations during the breeding season, which correlated with changes in ovarian weights. Additionally, histological examination of the ovaries revealed the presence of primordial fol-

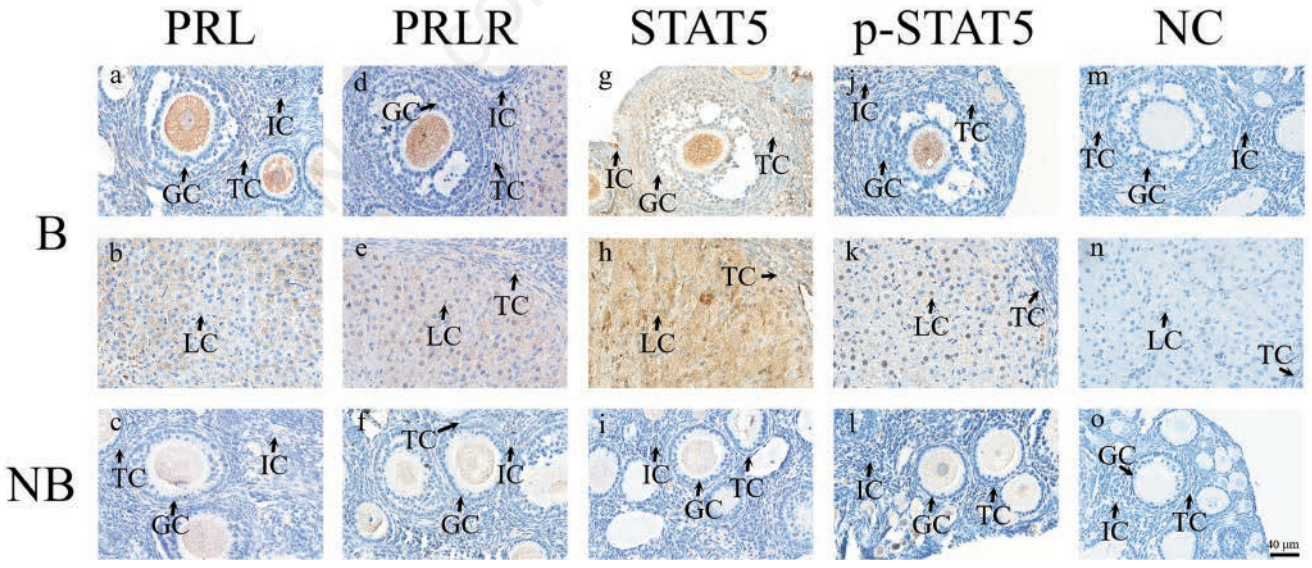

Figure 2. Immunohistochemical staining of PRL, PRLR, STAT, and p-STAT5 in the ovaries of wild ground squirrels during the breeding and non-breeding seasons. Positive staining of PRL (**a,b**), PRLR (**d,e**), STAT5 (**g,h**) and p-STAT5 (**j,k**) were detected in the GC, LC and IC of ovaries during the breeding season. The staining of PRL (**c**), PRLR (f), STAT5 (**i**) and p-STAT5 (**l**) were weak during the non-breeding season. No immunostaining was detected in the negative control sections (**m-o**). B, breeding season; NB, non-breeding season; GC, granulosa cells; TC, theca cells; IC, interstitial cells; LC, luteal cells; NC, negative control.

licles, primary follicles, secondary follicles, tertiary follicles, and corpus luteum during the breeding season. These findings align with previous reports indicating that PRL is integral to both cellular proliferation and differentiation during ovarian development,[28] suggesting a heightened cell proliferative rate during the breeding season as compared to the non-breeding season.[36] These results further support the notion that the ovaries of seasonally breeding mammals undergo morphological changes, including increased ovarian mass, during the breeding season, with PRL playing a critical role in ovarian growth and development.[21,37]

The presence of PRLR within ovary suggests the involvement of PRL in ovarian physiology.[38] PRL is locally produced in the

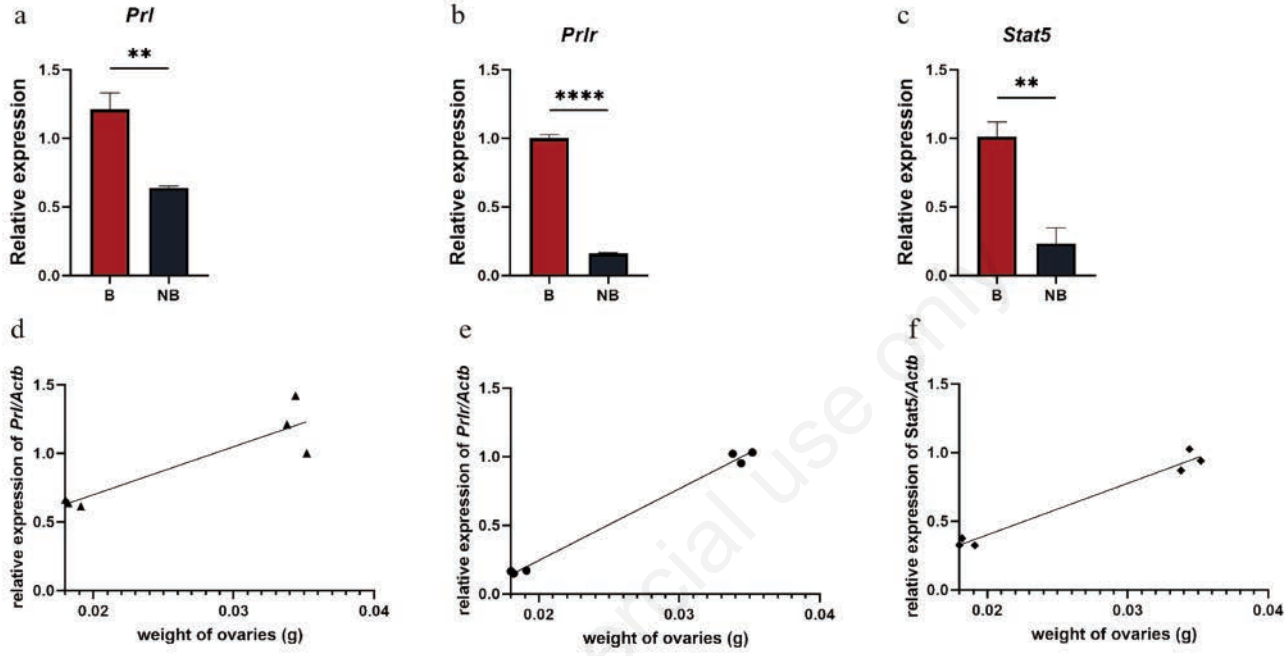

Figure 3. Relative expression levels of *Prl* (**a**), *Prlr* (**b**) and *Stat5* (**c**) in the ovaries of wild ground squirrels during both breeding and non-breeding seasons. The linear correlation and scatter diagrams of *Prl* (**d**), *Prlr* (**e**), and *Stat5* (**f**) and the weight of the ovaries of different individual wild ground squirrels in the breeding season and non-breeding season. Bars represent means ± SEM for three independent experiments. B, breeding season; NB, non-breeding season; **p<0.01; ****p<0.0001.

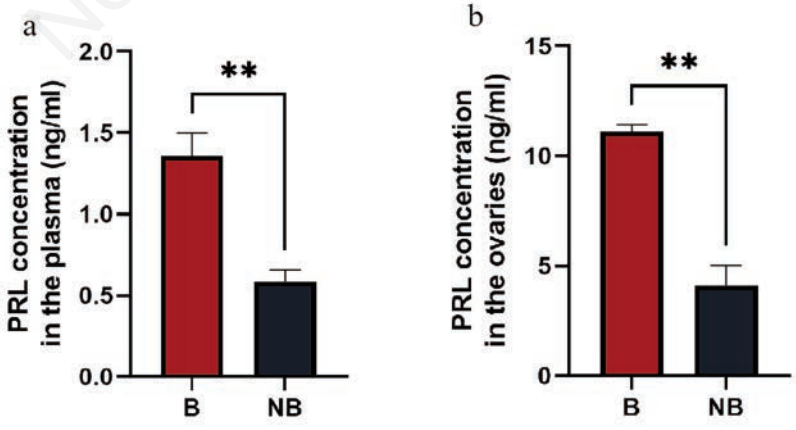

Figure 4. The concentration of related hormones in the plasma and the ovaries of wild ground squirrels in the breeding and non-breeding seasons. There was a significant increase of PRL (**a**) concentration in the plasma of wild ground squirrels in the breeding season compared with the non-breeding season. There was a significant increase of PRL (**b**) concentration in the ovaries of wild squirrels in the breeding season compared with the non-breeding season. Bars represent means ± SEM for three independent experiments. B, breeding season; NB, non-breeding season; **p<0.01.

ovary and functions as a potent survival factor against C2-ceramide-induced apoptosis in human granulosa cells. In sheep ovaries, PRL has been identified in theca cells of preantral to ovulatory follicles and in granulosa cells of antral follicles up to the gonadotropin-dependent stage, indicating that maximum PRL responsiveness may occur during the preovulatory phase of the estrous cycle.[39] In hamsters, PRL synergized with FSH to stimulate granulosa cell proliferation and foster ovarian follicular development.[40] Furthermore, PRLR has been detected in the granulosa cells of chicken ovaries, suggesting that PRL can bind to PRLR on granulosa cells and directly affect their function.[41] Although PRL's actions in the ovary may differ among species, these observations collectively indicated the indispensability of PRL in folliculogenesis and ovarian development. In our study, higher concentrations of PRL and mRNA levels were detected in ovarian tissues during the breeding season, suggesting the ovaries of wild ground squirrels can locally synthesize PRL, influencing ovarian growth and follicular development *via* endocrine and autocrine/paracrine pathways.

PRL is considered the most important component of the luteotropic complex in rodents and pigs, with varied functions.[42,43] PRLR knockout mice exhibited abnormal luteal function and reproductive activity, including reduced ovulation rate, aberrant oogenesis, and implantation failure.[6] In bovines, *Prl* and *Prlr* mRNA were expressed in the corpus luteum throughout the luteal phase, suggesting the bovine corpus luteum as an extrapituitary PRL production site.[44] The transcription factor STAT5 was acknowledges as a pivotal mediator of PRLR signaling, governing diverse PRL-related biological activities.[28,45] In our study, PRL, PRLR, and STAT5/pSTAT5 were localized in luteal cells of wild ground squirrels during the breeding season, implying that the PRLR/STAT5 pathway, activated by PRL, might play a role in maintaining and regulating ovarian luteal function. The ovarian transcriptome data of DEGs in both seasons were mainly related to ovarian function and mammary gland development, including ovarian follicle development, steroid synthesis, and regulation of reproductive process. Moreover, differential expressions of *Fshr*, *Lhr*, *3β-hsd*, and *17β-hsd* were highly significant in both seasons. Notably, *Wnt5a*, *Hoxd9*, and *Areg* were important factors in mammary gland development regulation, displaying upregulation during the breeding season. Further, GO annotation of these DEGs

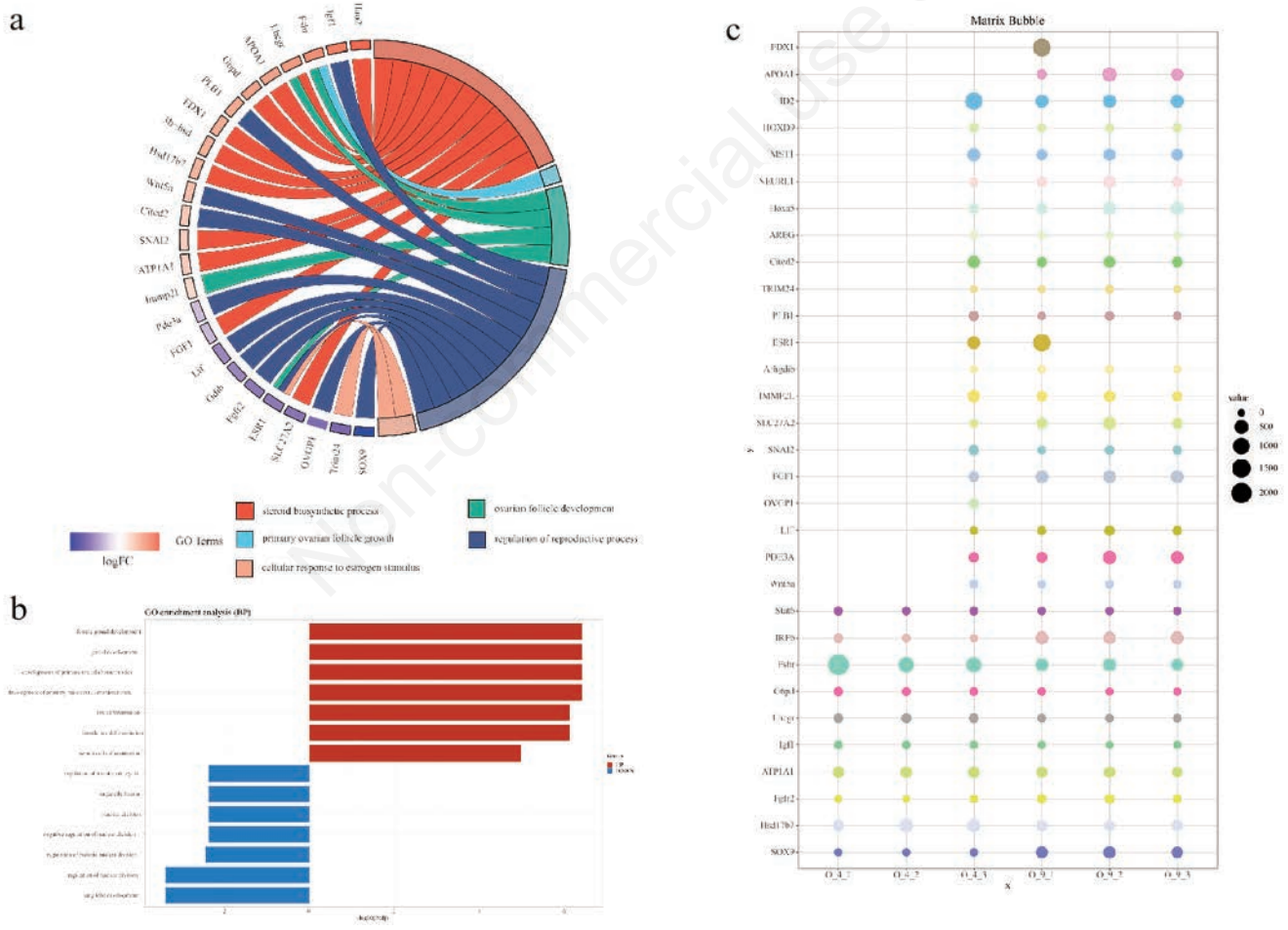

Figure 5. Transcriptomic analysis revealed significant changes in the pathways related to prolactin metabolic process and steroid metabolic process in the ovaries of wild ground squirrels during different seasons. Circle chord diagram of GO analysis enriched by differentially regulated genes during breeding and non-breeding seasons (**a**). Two-way bar chart of GO MF analysis from differentially regulated genes during breeding and non-breeding seasons (**b**). Matrix bubble chart analysis enriched by differentially regulated genes during breeding and non-breeding seasons (**c**). B, breeding season; NB, non-breeding season.

indicated a significant enrichment in female gonad development. In addition, STAT6, a member of the STAT family associated with glandular follicle development in mammary glands, exhibited higher expression during the breeding season compared to the non-breeding season. Therefore, these results suggest the vital role of PRL in corpus luteum maintenance, the production of ovarian steroid hormones, and mammary gland development.

In conclusion, this study has elucidated PRL, PRLR, and STAT5 expression patterns in the ovarian tissues of wild ground squirrels, demonstrating their seasonal fluctuations aligned with changes in circulating PRL and ovarian PRL concentrations. Remarkably, these levels were notably higher during the breeding season compared to the non-breeding season. Collectively, these findings suggest PRL's potential influence on the ovarian functions of wild ground squirrels through endocrine, autocrine or paracrine mechanisms. The insights provided by this study enhance our understanding of the effect and mechanism of the PRLR/STAT5 signaling pathway in the seasonal reproduction of animals. Future studies will investigate the molecular mechanism of the PRLR/STAT5 signaling pathway *in vitro* on the ovarian function of wild ground squirrels.

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