Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

A Proteomics Study of Tianzhu White Yak Ovary during Estrus and Pregnancy

Guo Shun Zhao, Jin Zhong Tao[1], Shan Shan Liu[2], Xiao Hu Wu, Jun Jie Hu, Yong Zhang and Xing Xu Zhao*

College of Veterinary Medicine, Gansu Agricultural University, Lanzhou, 730070, Republic of China; [1]Agricultural College, Ningxia University, Yinchuan, 750021, Republic of China; [2]College of Life Sciences and Technology, Gansu Agricultural University, Lanzhou, 730070, Republic of China
*Corresponding author: zhaoxx@gsau.edu.cn

ARTICLE HISTORY

Received: May 13, 2013
Revised: July 17, 2013
Accepted: September 30, 2013

Key words:
Estrus
Ovarian proteomics
Pregnancy
Tianzhu white yak

ABSTRACT

Estrus refers to the phase when the female is sexually receptive and ovarian follicles are maturing under regulation by gonadotropic hormones. Pregnancy is the process of fertilization and development of offspring. To study the ovarian proteome changes of Tianzhu white yak between estrus and pregnancy, 12 ovaries from 6 pregnant and 12 ovaries from 6 estrous yak cows were used and ovarian proteins were separated by 2-DE and analyzed by PDQuest 8.0 software. 20 differentially expressed spots were identified with MALDI-TOF-TOF/MS. Totally 15 different proteins were detected. COMMD1, PMK, TM, CG, VIM, ALDH1A1, SNX7, MRPs, MDH were related to steroid hormone synthesis and regulation, energy metabolism, pregnancy maintenance and early embryo development. These differentially expressed proteins might be useful to detect protein candidates of ovarian function, clarify the mechanisms of ovarian development and understand the reproductive physiological mechanism of the Tianzhu white yak.

To Cite This Article: Zhao GS, JZ Tao, SS Liu, XH Wu, JJ Hu, Y Zhang and XX Zhao, 2014. A proteomics study of Tianzhu white yak ovary during estrus and pregnancy. Pak Vet J, 34(1): 87-91.

INTRODUCTION

Tianzhu white yak (*Poephagus grunniens*) lives on the cold prairie at elevation above 3000m (Tianzhu Tibetan Autonomous County, China). Its reproductive patterns resemble most bovine species, but the fertility is rather lower under the natural grazing and rearing conditions (Yu *et al.*, 2007). Female white yak do not show estrus until more than two years old and yak cows have a higher silent estrus and lower pregnancy rate than cattle (Wiener *et al.*, 2003). They bear a calf every two years on average, and give birth to four to five calves over their lifetimes (Yu and Chen, 1993). Although these reproductive characteristics may be influenced by human and environmental factors (Ma, 2009), their concrete physiological mechanism remains unknown.

Ovary serves as both reproductive and endocrine organs. So far, many interested genes related to ovarian function have already been identified by transcriptomic technique (Satoh *et al.*, 2009). Proteomics study provided a better understanding of posttranscriptional characteristics of bioprocess (Rho *et al.*, 2008), and served as an effective tool for detecting differential proteins and explaining mammalian reproduction physiological function (Miarelli and Signorelli, 2010). Proteomics was used in ovarian research of mice and 52 proteins had been identified (Qian *et al.*, 2004). Wang *et al.* (2005) identified 138 individual proteins from human ovary, and 7 of which were differentially expressed between follicular and luteal phases. Satoh *et al.* (2009) treated gonadotropin-primed immature female mice with PMSG and hCG and compared ovarian proteome of preovulation, ovulation and postovulation, and found 253 differentially expressed proteins, 99 of which had related to ovary function. Since differences exist between species and proteome research on white yak ovaries may help to develop and understand reproduction physiology of the specie, a study on Tianzhu white yak ovarian proteomics during estrus and pregnancy was carried out.

MATERIALS AND METHODS

Ovarian tissue collection and preparation: Ovary of Tianzhu white yak were obtained from a local slaughter house and kept in 37°C physiological saline before laboratory processing. 6 ovaries were collected from 3 cows experiencing pregnancy and 6 ovaries from the other 3 cows in estrus. Proteins were extracted as Wang *et al.* (2005) described with slight modification. Ovaries were removed follicular fluid before preparing ovarian tissues.

Then ovary tissues were physically homogenized on ice with 1 mol/L EDTA, 4% (3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate), 8 mol/L urea and 100 mmol/L DL-Dithiothreitol (DTT) incubated for 1 hour at 4°C, vortexed for 30 s every 20 min. Then centrifuged at 12,000 g for 20 min at 4°C and discarded the supernatant prior to a repeated centrifugation.

Two-dimensional gel electrophoresis: Two-dimensional gel electrophoresis (2-DE) was carried out according to BioRad (Hercules, CA, USA) protocols. Briefly, protein concentration was determined by modified Bradford assay. Samples were then diluted with hydration solution and centrifuged at 10,000 g for 5 min. Carefully applied the supernatant to a 17 cm gel strips (pH 3-10, NL, Bio-Rad) in a protean IEF cell (Bio-Rad) for isoelectric focusing (IEF) following the program: 50 V for 14 h; 1 h at 250 V; 1 h at 1000 V; 6 h at 9000 V; 80,000 Vh at 9000 V.

After IEF, gels were equilibrated in 6 ml equilibration buffer [20% glycerol, 130 mM DTT, 50 mM Tris-HCl (pH 8.8), 2% SDS, 6 M urea] for 15 min. The equilibration was repeated with DTT replaced by 2.5% iodoacetamide for another 15 min. Gels were applied to a 1 mm 12% SDS-PAGE gel after rinsed. The second dimension electrophoresis was performed in Protean II xi cell (BioRad) following the program: 50V for 1h, at 200V until finished.

Staining and image analysis: Gels were stained with coomassie brilliant blue G-250 and then scanned (UMAX, PowerLook 2100XL, Taiwan). The maps were analyzed with PDQuest 8.0 software (Bio-Rad).

Spot identification: Interested protein and polypeptide spots were excised for sequencing analysis by Beijing Protein Innovation (Beijing, China) and Shanghai Applied Protein Technology (Shanghai, China). Namely, spots were manually excised from the gel and analyzed by matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI-TOF/TOF-MS). Peptides were matched using MASCOT software with NCBI non-redundant protein database. Proteins were validated if the scores were above 60 or score C.I. was above 95%.

RESULTS AND DISCUSSION

In this study, triplicate gels of each sample were subjected to PDQuest 8.0 for difference analysis. Totally 33 spots were differentially expressed in a staining density of three-fold or more. The 20 obviously differential spots were characterized by mass spectrometry. Figure 1 shows the 2-DE map of Tianzhu white yak ovaries during estrus and pregnancy and 20 differentially expressed protein spots.

The result of mass spectrum shows that these 20 spots represented 15 individual proteins. Table 1a and 1b show the differentially expressed spots represented proteins. Among them, spots 4, 5 and 7 are albumin, spots 2 and 9 are 60 kDa heat shock protein, spots 6, 15 and 16 are retinal dehydrogenase 1, and the others are identified as unique proteins. Albumin and an unnamed protein are specifically expressed during estrus. Zinc finger protein 48, mitochondrial ribosome protein and a heat shock protein 60 are specifically expressed after pregnancy. phosphomevalonate kinase, tropomyosin and two albumin are down-regulated after pregnancy. The other proteins are up-regulated after pregnancy. The intimate change in the content of every protein between estrus and pregnancy are shown in Fig. 2.

Specifically expressed proteins during estrus: In this study, one albumin was specifically expressed during estrus and the other two albumins were up-regulated during estrus. Elzanaty *et al*.(2007) found that seminal plasma albumin was associated with sperm morphology. However, no report indicates whether albumin is related to follicular development or oocyte maturation. There are many developmental follicles during estrus, and albumin is one main protein of follicular fluid (Angelucci *et al.*, 2006). Although the antral follicle have been punctured to remove follicular fluid before preparing the ovary tissues, it's difficult to exclude the possibility that there are still a few residual. So, specifically expressed/increased albumin may also be related to follicular fluid.

Specifically expressed proteins after pregnancy: Mitochondrial ribosome protein (MRPs), located in the mitochondrial matrix, is responsible for the synthesis of 13 proteins related to oxidative phosphorylation in eukaryotic cells (Sharma *et al.*, 2003; Mears *et al.*, 2006). But in this study, MRPs was specifically expressed after pregnancy. In the germplasm of *Drosophila* embryos, mitochondria-type ribosomes were detected outside the mitochondria and it is important for germ-line formation (Amikura *et al.*, 2001). Whether MRPs function in Tianzhu white yak and *Drosophila* are similar still needs further study.

Down-regulated proteins after pregnancy: In this study, the content of phosphomevalonate kinase (PMK) during estrus is nearly 100 times more than during pregnancy. PMK catalyzes the rate-limiting step in isoprenoid/sterol biosynthesis from mevalonate, and it can provide a variety of products, such as sterols, vitamin K, retinol, carotenoids, ubiquinone (Chang *et al.*, 2008). Cholesterol is the precursor of adrenal cortical hormone, estrogens and androgen (Boonsri *et al.*, 2012). So, PMK may be related to steroid hormone biosynthesis. In human lymphoblast subcultured, PMK is regulated by sterol. The consumption of steroid hormones is increasing after pregnancy, but the level of estrogens and androgen precursor is decreasing. Thus, PMK may be regulated by sterol through feedback inhibition in ovary of yak cows, which means, PMK will inhibit sterol's biosynthesis when the level of PMK rise to a certain amount.

Tropomyosin (TM), an actin-binding protein, was down-regulated after pregnancy in this study. Ben-Ze'ev *et al.* (1989) also found that the mRNA level of TM decreased during ovarian luteinization (luteal, preantral and preovulatory stage) in rat. It has been reported that TM is indispensable for kinesin binding to the ribonucleoprotein complexes in *Drosophila* oocyte. In the study of FSH induced the differentiation of an immature undifferentiated rat ovary granulosa (ROG) cell line, tropomyosin-4 was found has a key role in intracellular

Table 1a: Identification of differential proteins in white yak ovary during estrus and pregnancy by MALDI-TOF/TOF-MS

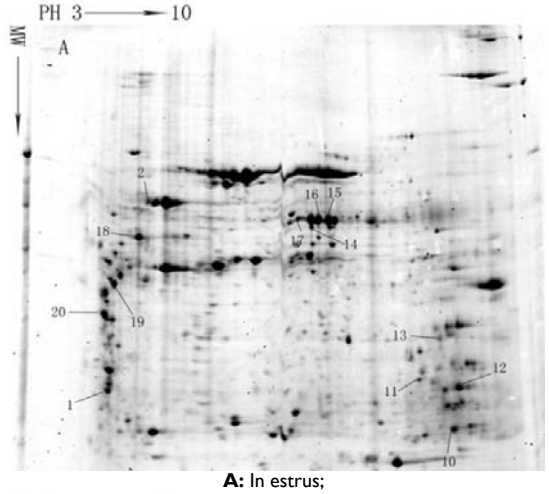
Spot No	Protein name	Accession	pI/MW	Coverage (%)	Score	Phase
1	Zinc finger protein 48 [Pan paniscus]	397472014	9.83 (137168)	20	83	E
2	60 kDa heat shock protein, mitochondrial	296439571	4.73 (29133)	45	136	E
3	Human Phosphomevalonate Kinase	194709154	6.49 (23360)	47	52	E&P
6	retinal dehydrogenase 1 [Bos taurus]	27806321	6.24 (55426)	45	144	E&P
7	ALB protein [Bos taurus]	154425704	5.95 (71244)	28	84	P
8	unnamed protein product [Homo sapiens]	221045918	4.83 (46936)	33	125	P
10	COMM domain-containing protein 1-like [Papio anubis]	402891016	10.09 (10138)	52	65	E&P
11	SNX7 protein [Bos taurus]	158455095	4.99 (45315)	29	75	E&P
12	The Mammalian Mitochondrial Ribosome	99032321	10.41 (7555)	44	41	E
13	mitochondrial malate dehydrogenase 2, NAD [Bos taurus]	89574145	7.7 (30388)	36	76	E&P
14	aldehyde dehydrogenase, mitochondrial precursor [Bos	115496214	7.55 (57073)	43	134	E&P
15	retinal dehydrogenase 1 [Bos taurus]	27806321	6.24 (55426)	39	88	E&P
16	retinal dehydrogenase 1 [Bos taurus]	27806321	6.24 (55427)	57	138	E&P
17	mCG148265 [Mus musculus]	148704773	12.18 (7303)	62	46	E&P

Spot No. is number of protein spot in Figure 2; Accession is accession number from the NCBInr database; Score is based on NCBInr database using the MASCOT searching program (>60 score was considered statistically significant). Phase is the phase of protein detected, E represents the estrus, P represents the pregnancy.

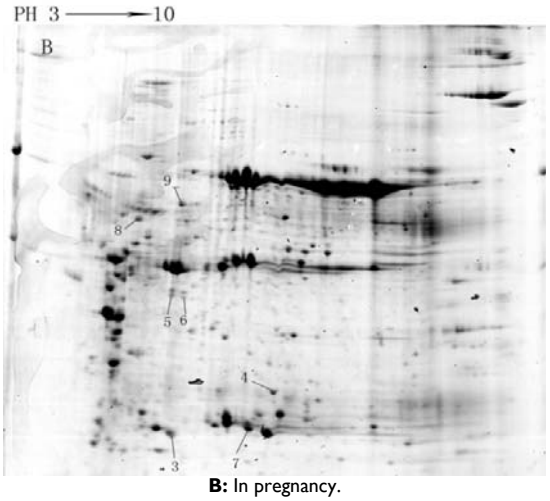
Table 1b: Identification of differential proteins in white yak ovary during estrus and pregnancy by MALDI-TOF/TOF-MS.

Spot No	Protein name	Accession	pI/MW	Score C.I.%	Score	Phase
4	Serum Albumin	367460260	5.6 (68415.6)	100	812	E&P
5	Serum Albumin	76445989	6.09 (55486.7)	100	681	E&P
9	60 kDa heat shock protein, mitochondrial	262205483	5.71 (61110.4)	100	1390	E&P
18	Bovine F1-Atpase Covalently Inhibited With 4-Chloro-7-Nitrobenzofurazan	3660252	4.97 (51396.9)	100	1040	E&P
19	vimentin [Bos taurus]	110347570	5.06 (53752.1)	100	1060	E&P
20	tropomyosin beta chain-like, partial [Cricetulus griseus]	354485729	4.58 (29966.1)	100	611	E&P

Score C.I.% is based on NCBInr database using the MASCOT searching program (>95% score was considered statistically significant).



A: In estrus;



B: In pregnancy.

Fig. 1: 2-DE map of Tianzhu white yak ovaries. Ovarian proteins were separated by IPG strip (pI 3-10) and a 12% SDS-PAGE gel. The numbers (1-20) indicate the identified proteins (Table 1a, 1b).

morphogenesis, transportation and differentiation (Grieshaber *et al.*, 2003). And there is a 2.0 kb maternal cytoskeletal TM mRNA were transported into the oocyte during *Drosophila* oogenesis after it was synthetized in the nuese cells (Hales *et al.*, 1994). TM was expressed in both estrus and pregnancy of Tianzhu white yak. So, a high TM level during estrus may be related to the development of oocyte and granulosa and a low TM level during pregnancy may be related to oogenesis.

Up-regulated proteins after pregnancy: Heat shock protein 60 (HSP60) plays an important role in mitochondria protein folding processes. In rat, HSP60 was detected in developing fetal, newborn, postnatal, and pubertal testes and ovaries, and adult ovary and lutein cells (Paranko *et al.*, 1996). Di Felice *et al.* (2005) found that HSP60 precursor expression was constant in the whole ovarian cycle. In this study, the level of HSP60 in ovary of estrus is higher than it in pregnancy. Although HSP60 has an anti-apoptotic effect, and is connected with infertility, multiple genital duct diseases and is up-regulated in ovarian cancer, the role of HSP60 in reproduction is currently unknown and requires further research.

Copper Metabolism Domain Containing 1 (COMMD1), as a COMMD family member, has multiple functions including copper metabolism and inhibits the activity of hypoxia-inducible factor 1 (HIF-1) (Van De Sluis *et al.*, 2010). Copper can promote estrogen and progestin secretion and is essential to the development of early embryos (Picco *et al.*, 2012). Van De Sluis *et al.* (2007) compared homozygous COMMD1 null (COMMD1/) mice with normal mice, they found that the HIF-1α of 9.5 days postcoitum (dpc) for COMMD1/embryos showed enhanced stability and the target gene exhibited higher transcriptional activity in COMMD1(-/-) mice, which induced a retardation of embryo development, placenta vascularization was absent

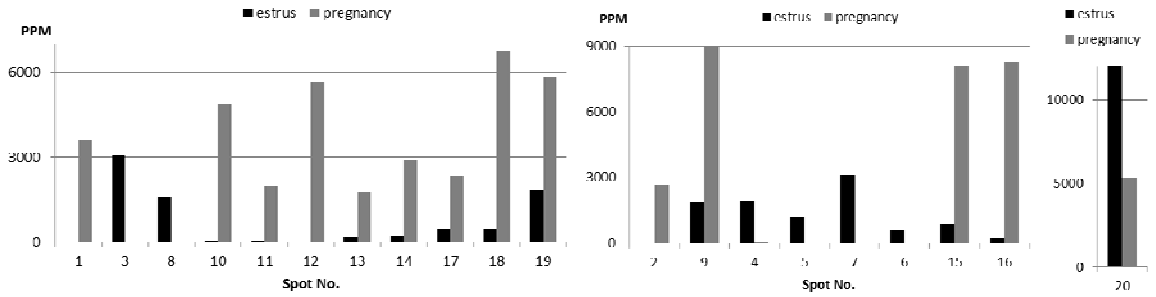


Fig. 2: Changes of the differentially expressed protein content between estrus and pregnancy. PPM is the relative volume intensities of protein spots calculated by PDQuest 8.0. Spot 1-20 are the differentially expressed proteins.2, 9 are 60 kDa heat shock protein; 4, 5, 7 are albumin; 6, 15, 16 are retinal dehydrogenase 1.

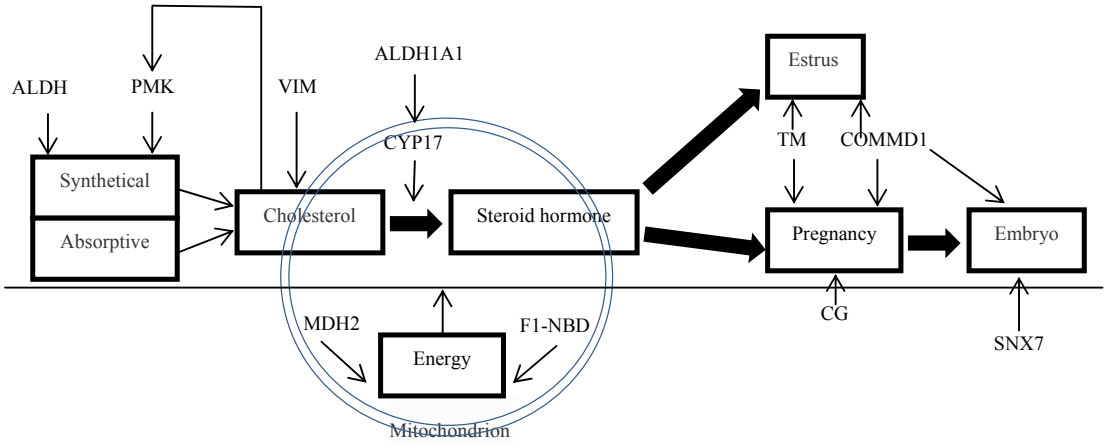


Fig. 3: A simple relationships diagram of some of differential expressed proteins.

and the embryo died in uterus between 9.5 and 10.5 dpc. Therefore, COMMD1 may regulate estrogen and progestin secretion by adjusting copper metabolism during estrus, and the up-regulation of COMMD1 in pregnant ovary indicates its potential role in maintaining pregnancy and embryo development of yak cows, but whether the copper metabolism is directly related to pregnancy and embryo development needs further study.

The function of sorting nexin (SNX) family proteins are mainly involved in protein transportation regulation during various processes, but many functions in vivo remained unknown. A study of zebrafish (Xu *et al.*, 2012) found that sorting nexin 7 (SNX7) is essential for embryonic liver development. The increased of SNX7 after pregnancy in this study suggests that SNX7 may also associate with early embryonic development of Tianzhu white yak.

Malate dehydrogenase (MDH), a key mitochondrial enzyme, catalyzes the reversible oxidation of malate to oxaloacetate in the process of citric acid cycle which generates energy. After white yak are pregnant, energy consuming in ovary was increased, and malate dehydrogenase 2 (MDH2) was up-regulated accordingly.

Aldehyde dehydrogenase (ALDH) is essential for retinoid metabolism. Retinoid is associated with steroid production, oocyte maturation, and early embryo development (Edassery *et al.*, 2010). In this study, ALDH was expressed during estrus and pregnancy suggests that it may be related to reproduction of yak cows by participate in retinoid metabolism. Large amounts of progestin are synthesized to maintain pregnancy, and retinoid can be used for progestin synthesis, which caused ALDH up-regulation after pregnancy.

Retinal dehydrogenase 1 (ALDH1A1) is an upstream regulator of gonadal hormone synthetase CYP17 (Livera *et al.*, 2004). The increasing of ALDH1A1 after pregnancy of Tianzhu white yak suggests that which may be related to steroid hormone. We detected a kind of ALDH1A1 in three differentially expressed spots, one of which was down regulated after pregnancy and the other two were up regulated. The three different ALDH1A1 spots may be related to post-translational modification.

Chorionic gonadotrophic hormone (CG), a gonadotropic hormone, was up regulated after pregnancy. CG is necessary for pregnancy, it stimulates corpora lutea to secret progestin and helps the uterus maintain a proper developmental state of embryonal nidation, promote the development of blastocysts, and sustain pregnancy.

F1-ATPase is the globular domain of the membrane-bound ATP synthase (F1F0-ATPase), and it can hydrolysis ATP to ADP and phosphate and vice versa. Further, 4-chloro-7-nitrobenzofurazan (NBD-Cl) inhibits ATP synthesis and hydrolysis by preventing subunit adoption to catalytic conformation (Orriss *et al.*, 1998). So, NBD-Cl covalently inhibited F1-ATPase (F1-NBD) was up-regulated after pregnancy, which may be correlated with enhance control of ATPase metabolism in the pregnant ovary.

Vimentin (VIM) is a type III intermediate filament protein belonging to cytoskeleton components. In human ovary, VIM was up-regulated at pregnancy phase compared with estrus (Wang *et al.*, 2005). The similar result has been found in Tianzhu white yak, the VIM level was increased

after pregnancy. Injected pregnant mare serum gonadotropin and human chorionic gonadotropin stimulation after genetically ablated mice's vimentin gene (Vim(-/-)), Shen *et al.* (2012) found that adrenal and ovarian steroidogenesis was markedly defected, the level of progestin production and corticosterone production decreased 70% and 50% in female Vim(-/-) mice, respectively. In isolated adrenal and granulosa cells from Vim (-/-) cell in vitro, steroidogenesis was also defected and the transport of cholesterol from cytosol to mitochondria was deficiency. Cholesterol transportation, as the bottle neck of steroid hormone synthesis, may be regulated by a protein (Yang *et al.*, 2005). VIM may be related to regulate steroid hormone synthesis by controlling the transport of cholesterol from cytosol to mitochondria (Shen *et al.*, 2012). The level of VIM was also increasing as the steroid hormone synthesis increasing after pregnancy.

Conclusion: This is the first report of ovarian proteomics study of Tianzhu white yak during estrus and pregnancy. Results suggest VIM and PMK were down-regulated after pregnancy, and those two proteins were related to oocyte maturation and follicular development. Specifically expressed and up-regulated proteins after pregnancy may be associated with synthesis and modification of steroid hormones, energy metabolism, maintenance of pregnancy and embryo development. Some of these proteins could be related through a simple workflow diagram (Fig. 3). ALDH and PMK can promote the synthesis of steroid hormone precursor, which is feedback regulated by PMK. The transportation of these hormone precursor into mitochondrial is regulated by VIM. ALDH1A1 promoted the synthesis of steroid hormone by regulating CYP17, which take effect in the ovary during estrus and pregnancy. TM, COMMD1, SNX7 and CG play a part in estrous ovary /and pregnancy /and embryo. MDH2 and F1-NBD were responsible for energy metabolism during the whole process.

Acknowledgment: The project was financially supported by the National Natural Science Foundation of China (31001095) and National 863 Plan Project of China (2013AA102505-3).

REFERENCES

Amikura R, M Kashikawa, A Nakamura and S Kobayashi, 2001. Presence of mitochondria-type ribosomes outside mitochondria in germ plasm of *Drosophila* embryos. Proc Natl Acad Sci USA, 98: 9133-9138.

Angelucci S, D Ciavardelli, F Di Giuseppe, E Eleuterio, M Sulpizio, GM Tiboni, F Giampietro, P Palumbo and C Di Ilio, 2006. Proteome analysis of human follicular fluid. Biochimica et biophysica acta, 1764: 1775-1785.

Ben-Ze'ev A, G Baum and A Amsterdam, 1989. Regulation of tropomyosin expression in the maturing ovary and in primary granulosa cell cultures. Dev Biol, 135: 191-201.

Boonsri P, TS Neumann, AL Olson, S Cai, TJ Herdendorf, HM Miziorko, S Hannongbua and DS Sem, 2012. Molecular docking and NMR binding studies to identify novel inhibitors of human phosphomevalonate kinase. Biochem Biophys Res Commun, 430: 313-319.

Chang Q, XX Yan, SY Gu, JF Liu and DC Liang, 2008. Crystal structure of human phosphomavelonate kinase at 1.8 A resolution. Proteins, 73: 254-258.

Di Felice V, S David, F Cappello, F Farina and G Zummo, 2005. Is chlamydial heat shock protein 60 a risk factor for oncogenesis? Cell Mol Life Sci, 62: 4-9.

Edassery SL, SV Shatavi, JP Kunkel, C Hauer, C Brucker, K Penumatsa, Y Yu, JA Dias and JL Luborsky, 2010. Autoantigens in ovarian autoimmunity associated with unexplained infertility and premature ovarian failure. Fertil Steril, 94: 2636-2641.

Elzanaty S, J Erenpreiss and C Becker, 2007. Seminal plasma albumin: Origin and relation to the male reproductive parameters. Andrologia, 39: 60-65.

Grieshaber NA, C Ko, SS Grieshaber, I Ji and TH Ji, 2003. Follicle-stimulating hormone-responsive cytoskeletal genes in rat granulosa cells: Class I beta-tubulin, tropomyosin-4, and kinesin heavy chain. Endocrinology, 144: 29-39.

Hales K, J Meredith and R Storti, 1994. Transcriptional and post-transcriptional regulation of maternal and zygotic cytoskeletal tropomyosin mRNA during *Drosophila* development correlates with specific morphogenic events. Dev Biol, 165: 639-653.

Livera G, C Pairault, R Lambrot, M Lelievre-Pegorier, J Saez, R Habert and V Rouiller-Fabre, 2004. Retinoid-sensitive steps in steroidogenesis in fetal and neonatal rat testes: In vitro and in vivo studies. Biol Reprod, 70: 1814-1821.

Ma XS, 2009. Analysis the reasons for the low fertility and livability in Tianzhu white yak. China Cattle Sci, 35: 84-85.

Mears JA, MR Sharma, RR Gutell, AS McCook, PE Richardson, TR Caulfield, RK Agrawal and SC Harvey, 2006. A structural model for the large subunit of the mammalian mitochondrial ribosome. J Mol Biol, 358: 193-212.

Miarelli M and F Signorelli, 2010. Differential expression of liver proteins in Chianina and holstein young bulls. J Anim Sci, 88: 593-598.

Orriss GL, AG Leslie, K Braig and JE Walker, 1998. Bovine F1-ATPase covalently inhibited with 4-chloro-7-nitrobenzofurazan: The structure provides further support for a rotary catalytic mechanism. Structure, 6: 831-837.

Paranko J, J Seitz and A Meinhardt, 1996. Developmental expression of heat shock protein 60 (HSP60) in the rat testis and ovary. Differentiation, 60: 159-167.

Picco SJ, DE Rosa, JP Anchordoquy, JM Anchordoquy, A Seoane, GA Mattioli and CC Furnus, 2012. Effects of copper sulphate concentrations during in vitro maturation of bovine oocytes. Theriogenology, 77: 373-381.

Qian Y, M Xie, Z Hou, Y Meng, J Liu, Y Zhai, Y Zhu, Z Zhou, J Sha and J Liu, 2004. A two-dimensional reference map of mouse ovary proteins. J Peking Univ (Health Sci), 36: 581-586.

Rho S, S You, Y Kim and D Hwang, 2008. From proteomics toward systems biology: Integration of different types of proteomics data into network models. BMB Reports, 41: 184-193.

Satoh M, M Tokoro, H Ikegami, K Nagai, Y Sono, SW Shin, S Nishikawa, K Saeki, Y Hosoi and A Iritani, 2009. Proteomic analysis of the mouse ovary in response to two gonadotropins, follicle-stimulating hormone and luteinizing hormone. J Reprod Dev, 55: 316-326.

Sharma MR, EC Koc, PP Datta, TM Booth, LL Spremulli and RK Agrawal, 2003. Structure of the mammalian mitochondrial ribosome reveals an expanded functional role for its component proteins. Cell, 115: 97-108.

Shen WJ, SK Zaidi, S Patel, Y Cortez, M Ueno, R Azhar, S Azhar and FB Kraemer, 2012. Ablation of vimentin results in defective steroidogenesis. Endocrinology, 153: 3249-3257.

Van De Sluis B, X Mao, Y Zhai, AJ Groot, JF Vermeulen, E Van Der Wall, PJ Van Diest, MH Hofker, C Wijmenga and LW Klomp, 2010. COMMD1 disrupts HIF-1α/β dimerization and inhibits human tumor cell invasion. J Clin Invest, 120: 2119-2130.

Van De Sluis B, P Muller, K Duran, A Chen, AJ Groot, LW Klomp, PP Liu and C Wijmenga, 2007. Increased activity of hypoxia-inducible factor 1 is associated with early embryonic lethality in COMMD1 null mice. Mol Cell Biol, 27: 4142-4156.

Wang L, YF Zhu, XJ Guo, R Huo, X Ma, M Lin, ZM Zhou and JH Sha, 2005. A two-dimensional electrophoresis reference map of human ovary. J Mol Med, 83: 812-821.

Wiener G, H Jialin and L Ruijun, 2003. The yak. 2 Ed, Regional Office for Asia and the Pacific Food and Agriculture Organization of the United Nations, Bangkok, Thailand, pp: 87-108.

Xu L, W Yin, J Xia, M Peng, S Li, S Lin, D Pei and X Shu, 2012. An antiapoptotic role of sorting nexin 7 is required for liver development in zebrafish. Hepatology, 55: 1985-1993.

Yu SJ and BX Chen, 1993. Traditional systems of management of yak cows in China and observations on reproductive characteristics using milk and plasma progesterone. Vienna, pp: 127-135.

Yu SJ, XH Ju, LB Wang and JF Fan, 2007. Successful embryo transfer in Tianzhu white yak using standard protocol. Sci China Series C: Life Sci, 50: 655-659.