На основании полученных результатов можно заключить, что у половозрелых кобелей при бабезиозе патоморфологические изменения в семенниках характерны для аутоиммунного орхита, развившегося вследствие разрушения гематотестикулярного барьера.

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СЕКЦИЯ «АКТУАЛЬНЫЕ ПРОБЛЕМЫ ОБЩЕЙ И ЧАСТНОЙ ЗООТЕХНИИ»

UDK 636.2

STUDY OF THE ROLES OF STEROL REGULATORY ELEMENT-BINDING PROTEIN-1/2 (SREBP-1/2) ON FSH AND LH SECRETION LEVEL AND OVARIAN FOLLICLE IN HIGH AND LOW FERTILITY OF BUFFALOES

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Abstract: *The concentration of phospholipids in blood and the synthesis of reproductive hormones are highly associated with lipids regulated by sterol regulatory element-binding protein (SREBP) transcription factors, which is a master part of improving reproductive performance and ovulation rate. In addition, the cholesterol binding protein is fundamental creation for steroids hormone such as follicle-stimulating hormone FSH, luteinizing hormone LH and Estrogen which pack impact the follicles production.*

Keywords*: SREBP-1, 2, Gene expression, FSH, LH, Buffaloes*

Introduction

SREBP plays a fundamental role as transcription elements for participate in biosynthesis of cholesterol, fatty acids and phospholipid which is directly correlated to synthesis of all reproduction and steroid hormones through controlled organization work of acyl-coenzyme A oxidase and carboxylase, 3HMG-CoA and Peroxisome proliferator-activated receptor (S. Akbar, 2013). Studies varied transcript SREBP-1/2 genes in eukaryotic mammalian as mice and cow and prokaryotic as yeast which affect a rate of limiting enzyme, that elongation of saturated and monounsaturated fatty acids. In addition, these include studies on hepatic lipogenic gene expression in genetically modified mice characterized by over expression or disruption of SREBP as well as studies on physiological changes of SREBP levels in modified mice after treatment (J. Kim et al., 2015). In mouse and human, the hepatic is coordinate SREBP gene expression by the synergistic action of response to insulin in a mediator of glucose encouraged gene expression. In addition, metabolism and insulin sensitivity process is corner pivot and was carried by elongation of very long chain fatty acids protein 6 (ELOVL6) through of chaining rate enzyme for the extension of saturated and essential fatty acids which impacted key role in energy required for consists of phospholipids and remained unclear this regulation in the chicken which need to be more investigation (Y.A. Moon et al., 2014; J.S. Bae et al., 2016).

The mechanisms of regulation polypeptide hormonal depended on metabolic pathway of cholesterol. Thus, correlation positive between activity in this cholesterol reaction and benefit on biosynthesis of reproduction hormones and the evidence is that treatment by cholesterol, insulin and glucagon raised to SREBP-2 expression as the confluence positive (W. Tang, 2019). Insulin and Luteinizing hormone (LH) stimulate by SREBP transcriptional activity and when detected this role in swine it found that receptor of granular cells which associated with ovum and become mature to directly ovulation (R. Dalbies-Tran et al., 2020). Steroid formulated under the

stimulation of gonadotropin Gn-RH and cytokine to FSH and LH. Gonadotropin and FSH were ended of the track of steroid output from cholesterol metabolic way. Ovarian tissues have granular cells in grafe follicles which to need step of steroid shape to biosynthesis and secretion estrogen and these cells were adapt with residual amount of free cholesterol expected binding protein and can detection through these cells for SREBP gene. The demand very active for steroid formed estrogen this is the result for highly gene expression, as a superior commercial line which increase stimulation of gonadotropin FSH pituitary ovary axis through pattern of SREBP-1/2 transcription (W.A. Lai et al., 2013; C. Boiti et al., 2021). It is widely accepted deficiencies in determining gene function and interpretation of the axes work in the chicken. Although, prove its effectiveness in many animal species.

Material and methods

1. Collection sample from buffalos.

2. SNP identification and association analysis with low and high fertility

The PCR sequencing method will be used to identify SREBP-1, 2 SNPs. Briefly, DNA obtained from each breed will be pooled and amplified using SREBP-1, 2 gene using specific PCR primers. Then, the PCR products will be gel purified and sequenced. The obtained sequences will be aligned to screen SNPs based on their differences. PCR-restriction fragment length polymorphism (PCR-RFLP) technology will be used to test SNP genotypes. PCR products will be digested by restriction enzymes following the manufacturer's instructions, and then detected through agarose gel electrophoresis. The association analysis using mixed model approach will be conducted to examine the association between the ovarian follicle and the SNPs. Each trait will be corrected for fixed effects. All statistical tests will be carried out using SAS.

3. Expression analysis of SREBP-1, 2 and its relationship with FSH, LH, and Estrogen level

Fresh buffalos liver will be collected immediately from high reproductive performance buffalos as a downer. The collected liver tissue will rapidly dissect into small pieces using a sterile scalpel, immediately will store at -80ºC until RNA extraction. Total RNA will extract from the liver using the RNA Mini Kit (Qiagen, Austin, TX, USA) following manufacturer's recommendations. RNA will be quantified using Nano drop technology with the Epoch Multi Volume System. The total RNA will be transcribed into cDNA via specific reverse transcription using the Transcript First-Strand cDNA Synthesis Super Mix according to the manufacturer's instructions (Tiangen Biotech (Beijing) Co., Ltd). Quantitative real-time PCR (RT-PCR) (34cycles) will be employed to analyze its relationship with FSH, LH, and Estrogen level using the gene-specific primers forward and reverse. The expression level of β-actin will be used as an endogenous reference gene.

4. Molecular isolation and cloning of SREBP-1, 2 genes

SREBP-1 and SREBP 2: 3' UTR will be amplified with the use of forward and reverse primer. Site-directed mutation will be used to introduce a 2-base substitution into the SREBP1, 2 genes binding site by mutagen primers. The Vector will be digested by enzymes. To cut the vector, the vector will be incubated with the enzyme,

digestion buffer and dH2O overnight. Loading dye will be added to the digested product and loaded to 1 % agarose gel. Electrophoresis of the sample will be done at 120 V for 20min. Following electrophoresis, the gel will be checked under the UV light. DNA fragment will be cut from agarose gel with a clean, sharp scalpel. EZNA gel extraction kit protocol will be followed to retrieve and purify the DNA fragment. The 3 'UTR of SREBP-1, 2 and mutant will be separately double digested with the same enzymes. The 3'UTR of SREBP-1, 2 and mutant will be separately inserted into Vector between the enzyme sites. Finally, the samples will be centrifuged briefly and incubated overnight.

5. Transformation, Pick up Clones, and Isolation of Plasmid DNA

Competent cells from transgene will be used to perform the transformation. The competent cells thawed on ice and cells will be added into each tube holding ligation products of pre-chilled tubes and mixed gently. The tubes will be incubated on ice. Followed by a precise heat shock used water bath then chilled on ice. The samples will be added into Eppendorf tubes having SOC medium without antibiotic and incubated at 37°C, while shaking at 250–300 rpm. Thereafter, the samples will be removed and centrifuged at 5000 rpm. The supernatant will be discarded and the remaining amount vortexed. The transformation mixture will be spread on LB agar plates. The plates will be incubated overnight. The plate will be checked for clone growth next day. Several clones will be picked to grow in LB medium plus antibiotic at 37°C while shaking at 250–300 rpm. PCR and gel electrophoresis will be performed to check whether ligation was successful. Plasmid will be sequenced to check the orientation of the insert and detecting of any mutation. Plasmid DNA will be purified with EZNA Endo-free Plasmid Mini Kit (Omega biotek) and checked on 1 % agarose gel.

6. Co-transfection of Plasmid DNA and SREBP-1, 2 Mimic Along with into granulosa cells

A day prior to transfection, granulosa cells will be seeded to each well of a 24-well plate. Approximately 70–80% confluence of the cells is anticipated at the time of transfection. Cells will be incubated at 37°C with 5% CO2 overnight.

7. Genome-wide surveys of SREBP-1, 2 gene regulation by Chip Seq

Form library preparation and sequencing. The libraries will be prepared using TruSeq ChIP Library Preparation Kit. Sequencing will be performed using HiSeq 3000/HiSeq 4000 Systems.

8. Immunohistochemistry

Slices of fresh tissue such as brain, liver, ovary, testes and kidney between pure and transgenic chickens will be placed in either B5 fixative or 10% formalin or paraffin embedded. The Paraffin sections created with a microtome, will be subjected to antigen-retrieval methods and immunohisto-chemical techniques.

Conclusion

The SREBP-1, 2 genes have one of functions affected on reproductive efficiency and may be useful as molecular breeding markers for improving follicle production and were appeared proved in many studies on several species but few studies on Buffalo. So, should be need deeply study in buffalo.

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UDK 636.2

GENOME-WIDE ASSOCIATION STUDY FOR MILK PRODUCTION AND MILK COMPOSITIONS TRAITS IN HOLSTEIN CATTLE

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Abstract: *Genome-wide association studies (GWAS) have made possible the screening of several single nucleotide polymorphisms (SNPs) in genes associated with milk production and milk compositions traits in dairy cattle. In the present review, we focus on candidate genes that have been related with milk production and milk composition traits in Holstein cattle.*

Key words: *GWAS, Holstein, SNPs, Milk production, Milk compositions.*