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INVESTIGATION THE POLYMORPHISM OF BONE MORPHOGENETIC PROTEIN 15 (BMP-15) GENE IN IRAQ COW.

Hayder Abdul-Kareem Hasan AL-Mutar

Department of Surgery and Obstetrics , College of Veterinary Medicine,– University of Baghdad ,Baghdad, Iraq

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ABSTRACT

Bone Morphogenetic Protein 15 (BMP-15) was known to regulate functions of ovary in mammals. The aim of this research was tocompare the BMP15 expression of ovaries incow and calf todetermine a relationship between the level of BMP15 genes and the developmental low competence of calfoocytes.Oocyte collected from 15cattle and 10 calves. Extracted DNA by intron kit (Korea).The BMP15 expression in Oocytes surrounded completely andpartiallyby cumulus cells of adult ovaries was higher significantly than that incalf ovaries. Oocytepolymorphism in protein calves compared with adults

INTRODUCTION

BMP15 geneis X-linked in oocytes expressed involved in granulose cell regulation and differentiation by mitosis granulose cell, repression follicle stimulating hormone receptor expression and engaged in the stimulation of kit ligand expression, All of which contributing a significant role in female fertility in mammals (1,2). Currently the hypothesis based on the non-covalently bond homo and hetero dimers of the proteins BMP15 regulated the fertility in bovine (3). The biological roles of Bone Morphogenetic Protein 15 (BMP15) are not completely understood even if gene to regulate function of granulose cells, and the BMP15play an important role in the process of follicular development and oocyte maturation (4). BMP15 known as prolificacy candidate genes, play key roles in regulating ovarian functions in animals, BMP15 mRNAs in cumulus granulose cells can be used as molecular markers forpredicting oocyte developmental potential.(1,5).

MATERIALS AND METHODS

Cattles ovaries were collected from Al-shulla slaughter, from 10 calves, 9-11 months and from 15 cattle,2-5 years. Oocyte recovery from follicles by aspiration methods (6,7). DNA was extracted by using the standard protocol by intron kit procedure. After extraction of genomic DNA, gel electrophoresis was used to detect the presence and integrity of the extracted DNA and presence of PCR product. Two conserved primers (forward primer: 5'-CTCTGAGACCAAACCGGGTA -3' and

reverse primer: 5'-CATGCCACCAGA ACTCAAGA-3') (3). thermal cycling conditions were done as follows: Denaturation at 95 °C for 5 min, followed by 35 cycles of 94 °C for 35s, 55°C for 35s and 72 °C for 35s with final incubation at 72 °C for 10 min using a thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). The Polymerase chain reaction (PCR) products were extracted by 2% agarose gel electrophoresis and visualized by contact with ultra violet light (302nm).Sequence of nucleotides of BMP-15 gene by using BioEdit program, which was performed by National Instrumentation Center for Environmental Management (NICEM) online at (http://nicem. Snu .ac. kr/main/ ?en _ skin =index.html), biotechnology lab, machine is DNA sequencer 3730XL, Applied Biosystem), Homology look for was conducted using Basic Local Alignment Search Device(BLAST) program which was available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov).Data were submitted to statistical analysis using chi-Square test, spss program (8).

RESULT AND DISCUSSION

The analysis of *BMP-15* gene polymorphism was carried out using PCR method. Genomic DNA of cattle was successfully amplified by pair of primer that covers entire coding sequence of *BMP-15* gene. Genomic DNA of white blood cells was also used for amplification of *BMP-15* gene using PCR specific primers. The amplified fragment which is yielded of single band of the desired product with a molecular weight of 350 base pair appeared sharp in agarose gel through Gel electrophoreses technique and loaded with (100-1000bp) DNA ladder (figure 1).

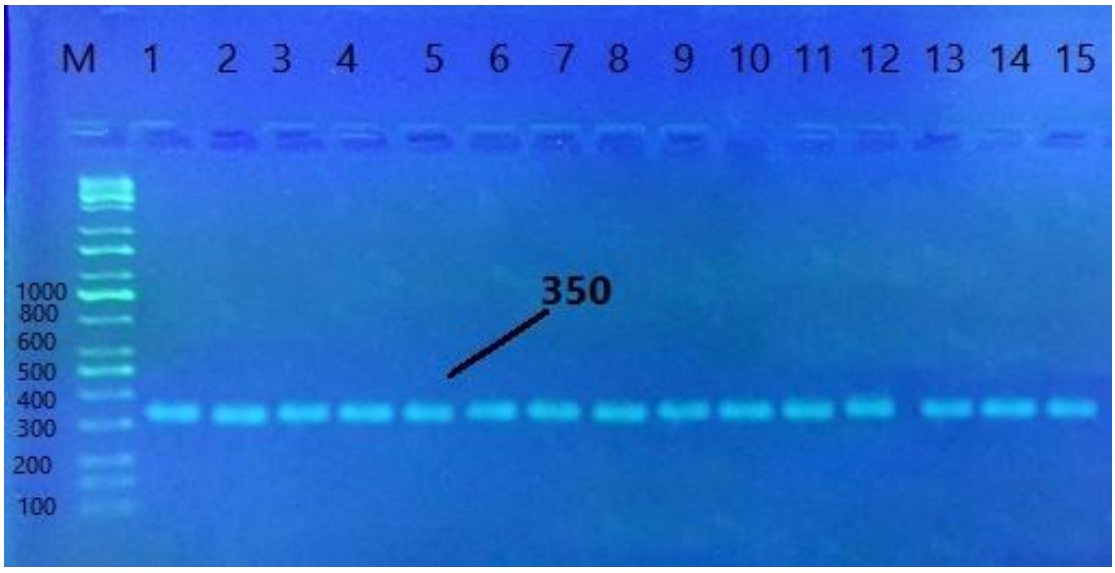

Figure (1): The product was electrophoresis on 2% agarose gel at 5 volt/cm^2, 1x TBE buffer for 2 hours. M: DNA ladder (100-10000bp), Lane 1-7 product for GnRHR gene of adult cow and Lane 8-15 product of calf, PCR product of band size 350bp. visualized under U.V light after staining with red stain safe.

The sequencing of amplified product of *BMP-15* gene from calf , out of them appeared 98% compatibility with standard Bostaurus breed bone morphogenetic protein 15 (BMP15) gene from 5054 to 5301 number of nucleotide from gene of gene Bank results as shown in Figure (2), Sequence ID: gb|EU712722.1|, and have number score (236) bits.

bone morphogenetic protein 15, partial [Bostaurus]

Sequence ID: emb|CAD58882.1|Length: 96Number of Matches: 1

Range 1: 7 to 88GenPeptGraphicsNext MatchPrevious Match

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Score          Expect Method                        Identities Positives  Gaps      Frame

165 bits(418)  8e-52  Compositional matrix adjust.  80/82(98%)  80/82(97%)  0/82(0%)  +3

Query  3    RHQLHLTHSHLSCHVEPWVQKSPTNHFPSSGRGSSKPSLLPKTWTEMDIMEHVGQKLWNH
182

            RHQLHLTHSHLSCHVEPWVQKSPTNHFPSSGRGSSKPSLLPKWTEMDIMEHVGQKLWNH

Sbjct  7    RHQLHLTHSHLSCHVEPWVQKSPTNHFPSSGRGSSKPSLLPKAWTEMDIMEHVGQKLWNH  66


Query  183  KGRRVLRLRFVCQQPRGSEVLE  248

            KGRRVLRLRFVCQQPRGSEVE

Sbjct  67   KGRRVLRLRFVCQQPRGSEVRE  88
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R H Q L H L T H S H L S C H V E P W V Q K S P T N H F P S S G R G S S K P S L L P K T W T E Met D I Met E H V G Q K L W N H K G R R V L R L R F V C Q Q P R G S E V L E S G

Bostaurus bone morphogenetic protein 15 (BMP15) gene, exons I, II and partial cds

Sequence ID: gb|EU712722.1|Length: 5829Number of Matches: 1

Range 1: 5054 to 5301GenBankGraphicsNext MatchPrevious Match

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Score                Expect     Identities          Gaps            Strand

431 bits(233)        4e-117     243/248(98%)        0/248(0%)       Plus/Plus

Query  1     ACCGCCATCAGCTTCACCTAACTCATTCCCACCTCTCCTGCCATGTGGAGCCCTGGGTCC
60

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  5054  ACCGCCATCAACTTCACCTAACTCATTCCCACCTCTCCTGCCATGTGGAGCCCTGGGTCC
5113


Query  61    AGAAAAGCCCAACCAATCACTTTCCTTCTTCAGGAAGAGGCTCCTCAAAGCCTTCCCTGT
120
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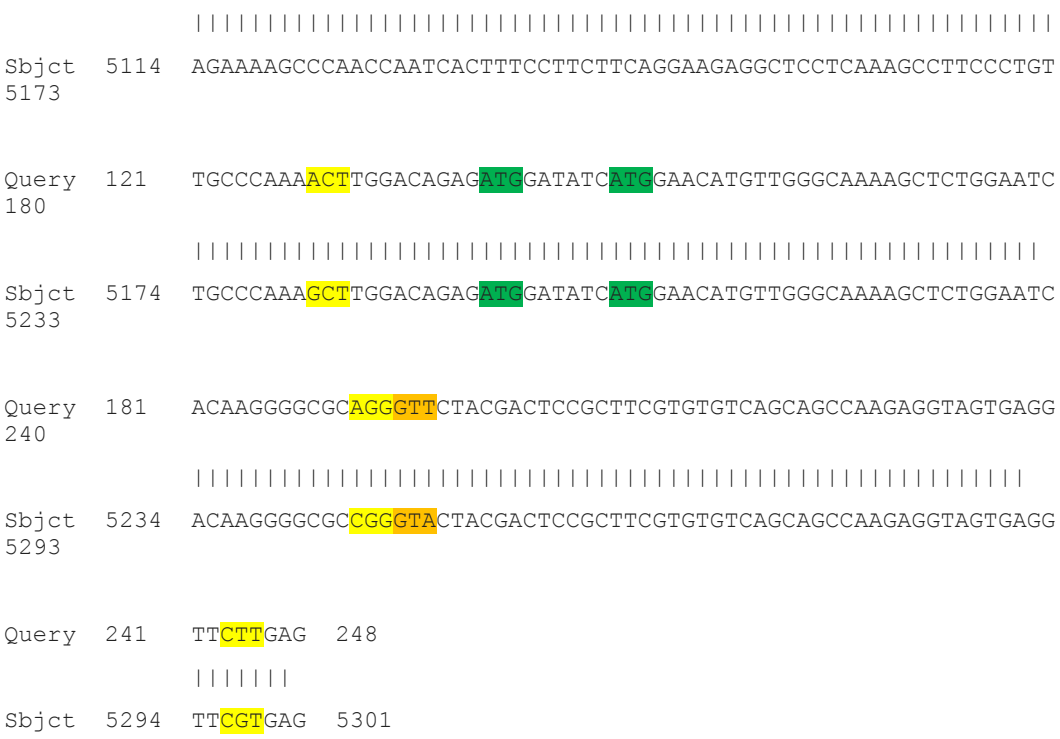

Figure (2): Sequencing of sense flanking the *BMP-15* gene, for cases of calf, obtained from Gene Bank. Query represents of sample; Sbject represent of database of National Center Biotechnology Information (NCBI).

Table 1: Type of polymorphism and amino acid change in sense of *BMP-15* gene in cattle's.

location of gene bank	Nucleotide change	Amino acid change	Predicted effect	Type of mutation
A5064 G	CAA>CAG	Glutamine> Glutamine	Silent	Transition
G5182A	GCT>ACT	Alanine>Threonine	Missense	Transition
C5245A	CGG>AGG	Arginine> Arginine	Silent	Transversion
A5250T	GTA>GTT	Valine>Valine	Silent	Transversion
G5297T	CGT>CTT	Arginine>Leucine	Missense	Transition

In total cases of cattle's had two type of transversion substitution in location 5245 C>A, 5250 A>T and three types of transition substitution in 5064 A>G, 5182 G>A and 5297 G>T, of BMP15 gene shown as in table 1. In this research, data showed the presence ofbovine BMP15 DNA and protein expression,and occur polymorphism of calf BMP15 compare adult in cumulus oocyte may be showed differences inpatterns of BMP15 incumulus cells and oocytes between cow and calf ovariesrelated to the follicle atresia status or to the estrous cycle **(9).**There was no difference in the oocyteBMP15 between calf and cow, and was smallerin calf cumulus cells than in cow cumulus cells**(10).**In follicles isgreater in cows than in

calves**(11,12).** The lower developmentalcompetence of calf oocytes may be partially explainedby a deficiency of BMP15 in cumulus cells**(13).(14)**Evaluating transcript abundance of bovine oocytes of different developmental competence found no difference in BMP15 gene expression. The oocyte can store histone mRNA and proteins during oogenesis **(15,16).** Additionally, studies have reported that a relationship exists between the amount of histones and oocytes competence in bovine. BMP15 were positively associated with age (**17**). BMP15 can stimulateoocyte development **(18)**The BMP15 genes contain two exons separated by a single intron that encode a rough endoplasmic reticulum signal peptide. The signal peptide region was encoded by the first exon, the proregion by segments of both exons and the mature peptide region by the second exon (**19**).Oocytewas a major source of both BMP15 and GDF9 and the major target for these factors was the granulose cells (**20).**

التحري عن التغايرات الوراثية لجين BMP-15 في الابقار العراقية

حيدر عبد الكريم حسن المطر

كلية الطب البيطري، فرع الجراحة والتوليد، جامعة بغداد،بغداد ،العراق.

الخلاصة

(BMP15) يعرف بتنظيموظائفالمبيضفيالثدييات . الهدف من هذا البحث هو مقارنة التغايرات الوراثية في المبايض للجين BMP15 بين العجول الصغيرة والابقار وتحديد العلاقة بين مستوى هذه الجينات ونخفاض تطور البيوض في العجلات الصغيرة . تم جمع البيوض من 15 بقرة و10 عجلات . وتم استخلاص الحامض النووي بستخدام كت الانترون (كوري المنشاء) . التغايرات الوراثية للجين في البويضات المحاطة بشكل كلي وجزئي في خلايا الركمة المبيضية في الابقار تكون بنسبة اعلى من العجلات الصغيرة . التغايرات الوراثية البروتينية في العجلات اعلى منها في الابقار الكبيرة.

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