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# New molecular variants of hypothalamus–pituitary–gonad axis genes and their association with early puberty phenotype in *Bos taurus indicus* (Nellore)

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**Abstract**

Endocrine system plays a major role in the control of reproductive functions which are regulated by the hypothalamus–pituitary–gonad axis and its interactions. *FSH* and *LH* receptor genes are expressed at the gonads and *GnRH* receptor gene is expressed at the anterior pituitary gland. Misense mutations of the *FSH, LH* or *GnRH* receptors, activating or inactivating their functions in mammals, are potentially useful to allow the understanding of the role of this group of gonadotropins in reproductive phenotypes as early puberty and birth interval length. In the present study, polymorphisms in bovine exon 11 and 3′UTR of *LHR*, exon 10 and 3′UTR of *FSHR* and *GnRHR* genes were characterized with some of them resulting in changes in the aminoacidic chain. These polymorphic sites were found in a *Bos taurus indicus* (Nellore) female population by means of PCR–SSCP and DNA sequencing. Association between nucleotidic/aminoacidic changes and early puberty were determined by Chi-square analysis. It was found association between *FSHR* 3′UTR polymorphisms at position 2181, 2248 and 2249 bp and early puberty phenotype ($p<0.05$). The presence of these new molecular markers might be considered in further studies to validate its correlation with early puberty or other reproduction associated phenotypes in cattle breeds.


*Keywords:* *LHR*; *FSHR*; *GnRHR*; Early puberty; Bovine; Cattle

## 1. Introduction

*Bos taurus indicus* breeds are known to be the most adapted breeds for tropical regions and have great importance beyond animal production in developing countries. Heat tolerance and the ability to survive under limited food resources, partially explain the vast

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population of this sub-specie around the world. Nevertheless, they are considered to have lower reproductive efficiency, presenting lower fertility and delayed puberty when compared to *Bos taurus taurus* breeds (Lôbo, 1998). Early puberty is an economically interesting phenotype because it increases the reproductive life of females. In taurine breeds (*Bos taurus taurus*), puberty signs as ovulation, estrus and subsequent positive pregnancy occur between 10 and 14 months age and in *Bos taurus indicus* breeds only around 24 months age (Milazzotto et al., 2002).

Some of the molecular events controlling physiological pathways related to these phenotypes still remain unknown, since the current methodologies used to improve livestock breeding and selection are based mostly on the direct measurement of phenotype's expression or statistical predictions based on these measurements (Avendano et al., 2003). Some advances can be noticed by the introduction of genetic analysis into the field of endocrinology allowing the identification of single gene mutations as the primary cause of many endocrine hereditary syndromes, opening a new era on the understanding of the molecular basis of reproductive endocrine control (Themmen et al., 1997).

Regulation of reproductive function involves the hypothalamus–pituitary–gonad axis and its interactions. *FSH* and *LH* receptor (*FSHR* and *LHR*) genes are expressed at the gonads and *GnRH* receptor (*GnRHR*) gene is expressed at the anterior pituitary gland (Themmen and Hutaniemi, 2000), being all of them members of the G protein coupled receptor family (Segaloff and Ascoli, 1993). Upon hormone binding, the receptor activates the adenylcyclase and the phosphatidylinositol mediated signal transduction pathways and both play a crucial role in reproductive physiology including initiation and maintenance of spermatogenesis and ovarian follicle development (Gudermann et al., 1992; Leung et al., 1996; Chan, 1998). Mutations in these receptors that either activate or inactivate their functions were reported in humans as responsible for several reproductive genetic disorders (David et al., 1984; Shenker et al., 1993; Yano et al., 1995; Kraaij et al., 1995; Kosugi et al., 1995; Laue et al., 1995; Aittomaki et al., 1995; Gromoll et al., 1996; Rosenthal et al., 1996; Simoni et al., 1997; De Roux et al., 1997; Layman et al., 1998; Latronico et al., 1998a, b, 2000; Arnhold et al., 1999; Batista et al., 2000; Kottler et al., 2000).

Thus, the aims of the present study were i) to search for single nucleotide polymorphisms in the gonadotropin receptors genes (*LHR* and *FSHR*) and gonadotropin releasing hormone receptor gene (*GnRHR*) in *Bos taurus indicus* cattle (Nellore) and ii) to relate them with early puberty phenotype.

## 2. Materials and methods

### 2.1. Identification of DNA polymorphism in FSHR, LHR and GnRHR

#### 2.1.1. Animals and DNA extraction

Genomic DNA was obtained by phenol/chloroform method from 100 *Bos taurus indicus* females presenting (39) or not presenting (61) early puberty phenotype, kept under the same feeding and management conditions since post-weaning period. The age of puberty was determined by exposing the females to multiple sire lots at the age of 12–16 months old and measuring the positive pregnancy 60 days after the end of a 90 days breeding season by transretal examination. The classification criterion for early puberty was to be pregnant

Table 1
Primer sequences used for PCR amplification and DNA sequencing of *FSHR*, *LHR* and *GnRHR* gene fragments

| Primer | Sequence |
|---|---|
| LHRP6 | 5′ TTATTCTGCCATCTTTGCTGAGA 3′ |
| LHRP8 | 5′ CAAACTGACAGTCCCCCGCTTT 3′ |
| LHRP9 | 5′AGAAGTCTGCAAAGGAGAGGTTG 3′ |
| LHRP10 | 5′ CAACTGGACCAAAAGCTGCGA 3′ |
| LHRP11 | 5′ CCTCCGAGCATGACTGGAATGGC 3′ |
| LHRP12 | 5′ TTTATTTTGCAGTTCAAAATCCAG 3′ |
| LHRP13 | 5′ TCTTTGTATCTTTGTTGGTAGC 3′ |
| LHRP14 | 5′ CATGCGCCAATCCGTTTCTGTAC 3′ |
| LHRP15 | 5′ CTCAGCAACAAAAGAAATCCCT 3′ |
| LHRP16 | 5′ TTAACATTCCTTATAGCAAGCT 3′ |
| LHRP17 | 5′ CATGGATTGGAAGAATCAATAT 3′ |
| LHRP18 | 5′ TTCAGCTGTCCTGGACAAGA 3′ |
| FSHRP9 | 5′CTCTGACCTTCATCCAATTTGCA 3′ |
| FSHRP26 | 5′ACTAGGATGTTCCCAGTGATGG 3′ |
| FSHRP25 | 5′TGATATGGTTTATTAGCATCCT 3′ |
| FSHRP11 | 5′CATTCGAGCTGCATGGCATGGG 3′ |
| FSHRP10 | 5′ATCACGCTGGAAAGATGGCATACC 3′ |
| FSHRP14 | 5′GACATTGAGCACAAGGAGGGAC 3′ |
| FSHRP15 | 5′CCCCTTGTCACAACTCTATGTCA 3′ |
| FSHRP16 | 5′ATCTTTGACTTGGACACACAGTGAT 3′ |
| FSHRP17 | 5′CTGCCTCCCTCAAGGTGCCCCTC 3′ |
| FSHRP12 | 5′AGTTCTTGGCTAAATGTCTTAGGGGG 3′ |
| FSHRP18 | 5′TAATGGTTCCAATTACACACTTAT 3′ |
| FSHRP13 | 5′TGAAGTCTTCAGTTTCCTAATGAATC 3′ |
| GnRHRP1 | 5′ATGGCAAACAGTGACTCTCCT 3′ |
| GnRHRP2 | 5′ACAATCAGAGTCTCCAGCAG 3′ |
| GnRHRP3 | 5′AACATTTGACTTTAGCCAAC 3′ |
| GnRHRP4 | 5′CTGTGGTCCAGCAAAGATGC 3′ |
| GnRHRP5 | 5′CTATACATCTTTGGGATGAT 3′ |
| GnRHRP6 | 5′GTGGGGATCCTGATGAAGGA 3′ |
| GnRHRP7 | 5′AAACTACAACTGAATCAGTC 3′ |
| GnRHRP8 | 5′TAGAGAGAAATATCCATATA 3′ |