Proceedings of the World Congress on Genetics Applied to Livestock Production, 11.256

Liver proteomics to study the onset of puberty in Brahman heifers

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Summary

Bos indicus (B. indicus) breeds are better adapted to harsh environments and tropical regions, but they often present a late puberty onset in comparison to *Bos taurus (B. taurus)*. An understanding of the mechanisms that regulate puberty onset in *B. indicus* is important in order to increase cattle productivity. Liver plays a prominent role in metabolism in mammals, and considerable evidence supports a link between liver function and female fertility in cattle. Hence, it might be helpful to the study of puberty to investigate protein expression patterns in liver of Brahman heifers (a *B. indicus* breed). We identified 777 proteins expressed in liver and 70 of these were differentially expressed (DE) between pre- and post-pubertal heifers. The functional analysis of the DE proteins showed enriched pathways that might regulate puberty onset, such as steroid hormone biosynthesis, PPAR signaling pathway, tyrosine metabolism, arginine and proline metabolism and tryptophan metabolism. In conclusion, the proteomics approach was effective in identifying DE proteins and furthering our understanding the biological link between liver function and puberty onset.

Keywords: Puberty, proteomics, Brahman heifers, HSD11B1, mass spectrometry

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Introduction

Bos indicus (*B. indicus*) breeds are better adapted to tropical and subtropical climate regions, where *Bos taurus (B. taurus)* breeds are less tolerant to heat stressors (Burrow, 2012). However, the onset of puberty in *B. indicus* breeds generally occurs later than in *B. taurus* (Plasse et al., 1968). Reducing the age at puberty to increase cattle productivity is a major aim for *B. indicus* breeders.

The liver plays an important role in metabolism and can contribute to reproduction (Rui, 2014). Insulin-like growth factor 1 (IGF1), secreted mainly by the liver, has a direct action on the anterior pituitary and gonads, suggesting its role in reproductive physiology (Daftary &Gore, 2005). Hepatic ERα has been demonstrated as a sensor of metabolic signals which is capable of tuning energy metabolism on reproductive needs (Della Torre et al., 2011). Hence, study of liver proteomics might provide insight into the onset of puberty in *B. indicus.*

The objective of the study was to identify DE proteins and molecular pathways involved in puberty of Brahman heifers by characterizing the liver proteome of 6 pre- and 6-post-pubertal heifers. Our data may provide valuable candidate proteins and context to their biological functions which could help to understand the links between metabolic signals in the liver and the onset of puberty in *B. indicus* breeds.

Materials and methods

Heifers used in this study were managed, handled and euthanized as per approval of the Animal Ethics Committee of the University of Queensland, Production and Companion Animal group (certificate number QAAFI/279/12). Twelve Brahman heifers of similar age, weight less than 250 kg, were selected from commercial Brahman heifers from two breeders as previously described (Fortes et al., 2016). These heifers were managed as one cohort as they consumed a pasture-based diet at the Gatton Campus facilities of the University of Queensland.

Pubertal status was defined using the observation of first *corpus luteum* (CL). Serum progesterone concentrations were also measured to confirm a functional CL in post-pubertal heifers (2.0 ± 0.7 ng/mL, mean ± SE). Pre-puberty heifers were randomly selected from the group that had never ovulated (plasma progesterone concentration 0.4 ± 0.2 ng/mL, mean ± SE) and paired with post-pubertal animals in slaughter days.

The entire liver was removed from the animal and 3 samples of 1 cm^3 were dissected from the liver and snap frozen in liquid nitrogen. In total, 6 pre- and 6 post-pubertal liver tissues were processed separately for protein identification and quantification as described (Xu et al., 2014). Briefly, liver proteins were diluted, alkylated, precipitated and digested with trypsin. Peptides were desalted using C18 Ziptips (Millipore) and analyzed by liquid chromatography electrospray ionization tandem mass spectrometric (LC-ESI-MS/MS). ProteinPilot (AB SCIEX), searching UniProt database of *B. taurus* reference was utilized to identify peptides. The ProteinPilot data (false discovery rate (FDR) < 1%) were then used as

ion libraries for sequential window acquisition of all theoretical mass spectra (SWATH) analysis. SWATH was performed on all samples using an IDA library prepared from 1 randomly chosen pre-pubertal sample and 1 randomly chosen post-pubertal sample. Statistical analyses were performed using MSstats (v2.6) in R (Choi et al., 2014).

For a better description of protein function, the list of DE proteins in liver was then uploaded to STRINGv10 database (http://string-db.org) for pathway enrichment analyses.

Results and discussions

Mass spectrometry was able to identify 777 proteins expressed in the liver (FDR < 1%), and quantified 630 proteins using relative quantitative SWATH analysis. Among these proteins, 70 had significantly different levels in the comparison between pre- and post-pubertal heifers (FDR < 10^{-5}). Of these DE proteins, 29 had higher levels in post- compared to pre-pubertal heifers, while 41 had lower levels in post- compared to pre-pubertal heifers (FDR < 10^{-5}).

We identified 22 overrepresented pathways for the set of DE proteins between pre- and post-pubertal heifers. Among these KEGG pathways, we found that most of the pathways (FDR < 0.05) were associated with the metabolism of tyrosine, tryptophan, arginine and proline. Study of urine metabonomics data of Chinese girls noted that abnormal metabolism of aromatic amino acid like tryptophan and tyrosine might have a close correlation with central precocious puberty by inhibiting hypothalamus-pituitary-adrenal (HPA) axis and activating hypothalamus-pituitary-gonadal (HPG) axis (Yang et al., 2012). This study also demonstrated that two pathways: tyrosine metabolism and tryptophan metabolism were upstream of HPG and HPA axis, whereas arginine and proline metabolism was downstream of these axes (Yang et al., 2012). In our current study, the up-regulation of tyrosine metabolism and the down-regulation of arginine and proline metabolism were detected. Our results suggest that cattle puberty onset may be influenced by similar metabolic factors, suggested in cited human studies.

The *PPAR signaling pathway* was also identified in the enrichment analyses. Peroxisome proliferator-activated receptors (PPARs) are members of ligand-activated nuclear receptor family and play an important role in energy homeostasis (Yang et al., 2008). Among three isotypes of PPARs, PPARγ can regulate the expression of leptin gene that is a metabolic hormone affecting reproduction in heifers (Garcia et al., 2002). Collective studies of mouse model strongly suggested the role of PPARs in female reproduction (Yang et al., 2008).

The liver is the site of steroid hormone inactivation, and we also discovered the *steroid hormone biosynthesis* as a significantly enriched KEGG pathways in our liver proteomics (FDR = 0.01). DE proteins in this pathway were HSD11B1, HSD17B6 and LOC511498. Among these 3 DE proteins, HSD11B1 and LOC511498 were down-regulated DE proteins, while HSD17B6 was an up-regulated DE protein post-puberty. The decrease of HSD11B1 expression level after puberty was also detected in monkeys (Stute et al., 2012). Further, HSD11B1 protein is responsible for glucocorticoids regeneration (Draper et al., 2003) and the glucocorticoids secretion level was positively correlated with the age at puberty onset in humans (Shi et al., 2011). Therefore, the down-regulation of HSD11B1 in post-pubertal heifers seen in our study was consistent with other studies and suggested its role in

B. indicus puberty.

In conclusion, our study provided a list of 70 candidate proteins involved in the process of puberty onset. The functional analysis of DE proteins showed numerous enriched pathways (FDR < 0.05) that might regulate the occurrence of puberty, such as *steroid hormone biosynthesis*, *PPAR signalling* pathway or specific amino acid metabolisms. The candidate proteins identified in this study might be useful for understanding molecular mechanisms of the onset of puberty in *B. indicus* breeds.

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