Title: Multi-trait meta-analysis identified genomic regions associated with sexual precocity in tropical beef cattle[1]

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ABSTRACT: Multi-trait meta-analyses are a strategy to produce more accurate genome-wide association studies (GWAS), especially for complex phenotypes. We carried out a meta-analysis study for traits related to sexual precocity in tropical beef cattle (Nellore and Brahman) aiming to identify important genomic regions affecting these traits. The traits included in the analyses were age at first calving (AFC), early pregnancy (EP), age at first *corpus luteum* (AGECL), first postpartum anoestrus interval (PPAI) and scrotal circumference (SC). The traits AFC, EP and SC_N were measured in Nellore cattle, while AGECL, PPAI and SC_B were measured in Brahman cattle. Meta-analysis resulted in 108 significant single nucleotide polymorphisms (SNP), at an empirical threshold *P*-value of $1.39x10^{-5}$ (FDR < 0.05). Within 0.5 Mb of the significant SNP, candidate genes were annotated and analyzed for functional enrichment. Most of the closest genes to the SNP with higher significance in each chromosome have been associated with important roles in reproductive function, these are *TSC22D2*, *KLF7*, *ARHGAP29*, *7SK*, *MAP3K5*, *TLE3*, *WDR5*, *TAF3*, *TMEM68*, *PPP1R15B*, *NR2F2*, *GALR1*, *SUFU* and *KCNU1*. We did not observe any significant SNP in BTA5, BTA12, BTA17, BTA18, BTA19, BTA20, BTA22, BTA23, BTA25 and BTA28. Although the majority of significant SNP are in BTA14, it was identified significant associations in multiple chromosomes (19 out of 29 autosomes), which is consistent with the postulation that reproductive traits are complex polygenic phenotypes. Five proposed association regions harbor the majority of the significant SNP (76 %) and were distributed over four chromosomes ($P < 1.39x10^{-5}$, FDR < 0.05): BTA2 (5.55%) from 95 to 96 Mb, BTA4 (5.55%) from 94.1 to 94.8 Mb, BTA14 (59.26%) from 24 to 25 Mb and 29 to 30 Mb, and BTA21 (5.55%) from 6.7 Mb to 11.4 Mb. These regions harbored key genes related to reproductive function. Moreover, these genes were enriched for functional groups associated with immune response, maternal-fetal tolerance, pregnancy maintenance, embryo development, fertility and response to

stress. Further studies including other breeds and precocity traits could confirm the importance of these regions and identify new candidate regions for sexual precocity in beef cattle.

Key words: *Bos indicus*, Brahman, early puberty, GWAS, meta-analysis, Nellore

INTRODUCTION

Reproductive traits are important for economic success in beef cattle production (Formigoni et al., 2005; Brumatti et al., 2011). Despite its economic importance, genetic gains of reproductive traits are generally slow. These traits are considered polygenic and highly influenced by environmental factors (Cardoso et al., 2015). Genomic selection has allowed higher genetic gains for fertility traits in beef and dairy cattle (Zhang et al., 2014; Garcia-Ruiz et al., 2016). In parallel with genomic selection, genome-wide association studies (GWAS) have detected quantitative trait loci (QTL) affecting reproductive traits aiming to better understand their genetic mechanisms (Hawken et al., 2012; Irano et al., 2016; Melo et al., 2017). However, these traits are controlled by many QTL of small effect and GWAS can produce high false positive rates. Validation for the identified QTL requires further investigation. To improve QTL detection, GWAS meta-analyses corroborate evidence from independent studies of related traits by identifying polymorphisms that produce variation in common among complex traits (Bolormaa et al., 2014; Ramayo-Caldas et al., 2016). The aim of the current study was to carry out meta-analysis across two independent *Bos indicus* populations to improve QTL detection for reproductive traits. Specifically, we aimed to: 1) identify genomic regions associated with sexual precocity traits measured relatively early in a cow's life and the related bull trait scrotal circumference; 2) identify candidate genes in those genomic regions; and 3) investigate biological roles of the identified candidate genes. The traits measured in Nellore were age at first calving (AFC), early pregnancy (EP) and scrotal circumference (SC_N) and in Brahman were age at the first *corpus luteum* (AGECL), first postpartum anoestrus interval (PPAI) and scrotal circumference (SC_B).

MATERIAL AND METHODS

Nellore Phenotypes and Genotypes

Information of animals from Alliance Nellore dataset, born in eight different farms distributed in Northwest, Southwest and Midwest of Brazil were used. Heifers were either artificially inseminated or naturally mated. Usually farmers apply two breeding seasons, in the early breeding season females are exposed to the first mating at around 16 months of age and, those females that failed to conceive have a second chance in the later breeding season, at about 26 months of age. Females that did not conceive in the first neither in the second chance were culled and were not considered in the analysis of AFC. More details about the dataset are described in Costa et al. (2015) and Irano et al. (2016).

Summary statistics of Nellore phenotypes – AFC, EP and SC_N – are presented in Table 1. The phenotype AFC was defined as the number of days from birth to first calving; EP was defined as success (1) for heifers that had the first calf with less than 31 months of age, i.e., heifers that got pregnant in the first breeding season, or failure (0) for those heifers that had the first calf after 31 months of age. In both populations, SC was measured at about 18 months of age, at the widest point of the scrotum with a standard metal tape, in cm.

The contemporary groups (CG) for AFC, EP and SC_N were defined by concatenating the information of herd, year and season of birth, weaning and yearling management groups. Groups with less than 4 animals for AFC and EP or less than 3 animals for SC_N were excluded. For AFC and SC_N, we considered only data in the interval of CG ± 3 standard deviation from the mean of each group. For EP CG without

variability, i.e., presenting only females with the same categorical response were excluded. For SC the linear effect of the age of the animal at the recording was included as a covariate in the model. It was considered in the analysis just animals presenting age at recording from 10 to 24 months of age. It was excluded a total of 20 animals that presented ages out of this interval.

For Nellore traits, phenotypes were previously corrected for CG effects to avoid biased fixed effect estimates, since the whole Nellore dataset contained both genotyped and non-genotyped animals. CG estimates used to pre-correct the phenotypes were obtained under a regular mixed animal model (Henderson, 1984). Then, these corrected phenotypic traits for genotyped animals were used in GWAS approach.

Animals were genotyped with high-density Illumina Bovine HD Assay (Illumina, San Diego, CA, USA) and GeneSeek Genomic Profiler Indicus HD - GGP75Ki (Neogen Corporation, Lincoln, NE, USA), which have 777,962 and 74,677 (73,941 in common with HD) SNP markers, respectively. Genotype imputation to Bovine HD was performed for animals genotyped with GGP75Ki using FImpute software (Sargolzaei et al., 2014), taking into account pedigree information. The imputation accuracy is expected to be higher than 0.98 (Carvalheiro et al., 2014). Quality control was performed, removing animals with call rate < 0.90 and removing SNP with minor allele frequency (MAF) < 0.01, call rate < 0.95, GC score < 0.15, Hardy-Weinberg equilibrium test *p*-value < 10^{-5}, SNP in non-autosomal regions, and unmapped SNP. After quality control 2,923 females with genotypes for 412,993 SNP and 5,078 males with genotypes for 477,317 SNP remained for the analyses. To construct the genomic relationship matrix (G), SNP with MAF > 0.1 were considered.

Brahman Phenotypes and Genotypes

Data from the Cooperative Research Centre for Beef Genetic Technologies (Beef CRC) were used in this study. The Beef CRC data for male and female Brahman cattle was previously described (Johnston et al., 2009; Burns et al., 2013; Corbet et al., 2013). The number of observations and descriptive statistics for each trait are presented in Table 1. The traits evaluated were AGECL, PPAI and SC_B. When the heifers achieved the weight of 200 Kg, an ovarian ultrasound was carried out at every 4 to 6 weeks to detect the first CL and calculate AGECL. To get PPAI phenotypes the number of days from calving to the first ovulation postpartum was observed. Ovarian ultrasound was used to observe CL presence after calving to indicate an ovulatory event.

For AGECL and PPAI, CG were formed by concatenating information of cohort, year of birth and management group. For SC_B, CG included the effects of cohort and year of birth. Age of young bull at recording was used as a covariate for SC_B.

Brahman animals were genotyped using the Illumina BovineSNP50 versions 1 or 2, and posteriorly imputed for high-density panel using Beagle software (Browning and Browning, 2009). To allow imputation, representative animals of the Beef CRC population were genotyped using the HD chip from Illumina as described by Fortes et al. (2013). Quality control excluded animals with call rates < 98%, SNP with call rates < 85% and MAF < 0.02. After quality control remained 660,433 SNP for PPAI, 659,845 for AGECL and 465,644 for SC_B.

Genome-wide association methods and meta-analyses

A single-trait single-marker model was used to perform GWAS for all traits using the genomic mixed model proposed by Kang et al. (2010), which accounts for population substructure, fitted as follows for Nellore (1) and Brahman (2):

$$\boldsymbol{y} = \mathbf{1}\mu + \boldsymbol{sa} + \boldsymbol{Zu} + \boldsymbol{\varepsilon}, \quad (1)$$

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{sa} + \boldsymbol{Zu} + \boldsymbol{\varepsilon}, \quad (2)$$

where $\boldsymbol{y}$ is a vector containing the phenotypic information, corrected for the fixed effects in Nellore data (equation 1), $\mathbf{1}$ is a vector of ones, μ is an overall mean, $\boldsymbol{X}$ is an incidence matrix relating fixed effects (CG) in $\boldsymbol{\beta}$ with the phenotypes in $\boldsymbol{y}$, $\boldsymbol{s}$ is the vector containing the genotypes coded as 0, 1 or 2 according to the number of B allele copies, $\boldsymbol{a}$ is the vector with the SNP additive genetic substitution effects, $\boldsymbol{Z}$ is the incidence matrix of polygenic random effects of the animals in $\boldsymbol{u}$ and $\boldsymbol{\varepsilon}$ is the vector of residuals. $\boldsymbol{u}$ and $\boldsymbol{\varepsilon}$ followed normal distribution with $\boldsymbol{u} \sim N(0, \boldsymbol{G}\sigma^2_a)$ and $\boldsymbol{\varepsilon} \sim N(0, \boldsymbol{I}\sigma^2_e)$, respectively, where $\boldsymbol{G}$ is the genomic relationship matrix for all individuals and SNP (except the SNP considered in $\boldsymbol{a}$), calculated as described in the first method proposed by VanRaden (2008), σ^2_a is the additive genetic variance, $\boldsymbol{I}$ is an identity matrix and σ^2_e is the residual variance. The SNP effect estimates were computed using the SNP & Variation Suite (SVS) software (Release 8.3.0, Golden Helix, Inc., 2014) under the model previously described and the EMMAX method (Kang et al., 2010).

Multi-trait meta-analyses were performed using the method described by Bolormaa et al. (2014). This method consists of a statistic test following a χ^2 distribution with n degrees of freedom, where n is the number of traits included in meta-analysis, calculated as:

$$Multi\text{-}trait\ \chi^2 = \boldsymbol{t'_i} V^{-1} \boldsymbol{t_i}, \quad (3)$$

where t_i is a vector 6x1 of the $i^{\text{-th}}$ SNP effect estimates for the 6 traits divided by their respective standard errors, t'_i is the transpose vector of t_i, and V^{-1} is an inverse of the 6X6

correlation matrix of the correlations between the *t*-values of the six traits across the 387,971 SNP considered in the model. The *t*-values vector was computed as:

$$\boldsymbol{t_i} = \boldsymbol{a_i}/SE(\boldsymbol{a_i}), \quad (4)$$

Where $SE(a_i)$ is the standard error of a_i (SNP effect vector). Just common SNP in both Nellore and Brahman panels were considered in the analysis. False discovery rate (*f*) was calculated as:

$$f = m\alpha/s, \quad (5)$$

Where *m* corresponds to the number of tests (markers) considered in the meta-analysis, α is the significant threshold and *s* is the number of tests where $p < \alpha$, where *p* is the correspondent observed *p*-value for each marker. To select α values producing a false discovery rate lower than 0.05, the *p*-value rank position procedure (Benjamini and Hochberg, 1995) was used. In summary, the Equation 5 was changed to obtain $\alpha = fs/m$, and *s* was defined as the largest *p*-value rank position *i* that satisfies $p_i \leq f_i/m$ with $f = 0.05$ (Pereira et al., 2016).

Gene annotation and enrichment analysis

The genes within 0.5 Mb of significant SNP ($P < 1.39 \times 10^{-5}$) were annotated as candidate genes. These candidate genes were used to perform gene ontology (GO) and enrichment analyses, using DAVID bioinformatics resources (Huang da et al., 2009).

RESULTS AND DISCUSSION

Significant SNP

Heritabilities were previously reported for the traits and populations studied here, they were 0.08 for AFC, 0.30 for EP, 0.41 for SC_N, 0.57 for AGECL, 0.52 for PPAI and 0.75 for SC_B, (Forni and Albuquerque, 2005; Irano et al., 2016; Johnston et al., 2009; Johnston et al., 2010; Corbet et al., 2013).

Meta-analysis resulted in 108 significant SNP, at an empirical threshold *P*-value of 1.39×10^{-5} (Fig. 1). This number of significant SNP was the maximum rank position which *P*-value satisfied the condition $P \leq \alpha$ with *FDR* = 0.05 (Supplementary Table S1). Most of the significant SNP (59.3%) were mapped to chromosome 14, producing a strong association peak between 24.3 and 26 Mb (Fig. 2), in which 14 genes were harbored (*XKR4*, *TRNAT-AUG*, *TMEM68*, *TGS1*, *LYN*, *RPS20*, *MOS*, *PLAG1*, *CHCHD7*, *SDR16C5*, *SDR16C6*, *PENK*, *LOC101907667*, *IMPAD1*). Another region in BTA14, from 29.5 to 29.6 Mb presented a smaller peak, in which four SNP were significant. All those SNP (*rs109465877*, *rs111023138*, *rs134875123* and *rs42534153*) were located in an intronic region of the gene *NKAIN3*, which was in a genomic window explaining 1% of the genetic variance of early pregnancy in Nelore heifers (Oliveira Junior et al., 2017).

The peak observed in chromosome 14 is close to significant regions that were associated with Brahman and Nellore reproductive traits (Fortes et al., 2012; Fortes et al., 2013; Irano et al., 2016). The *PLAG1* gene (mapped to 25,000,459-25,052,403 base pairs), a key gene associated with several traits of economic importance in different cattle breeds (Karim et al., 2011; Littlejohn et al., 2012; Nishimura et al., 2012; Fortes et al., 2013; Utsunomiya et al., 2013; Saatchi et al., 2014; Juma et al., 2016; Pereira et al., 2016) is located in this region. One of the most significant SNP (*P*-value = 6.39×10^{-6}), the *rs109815800* was mapped in an intronic region of *PLAG1*.

Fortes et al. (2012) also found a region in chromosome 14 associated with AGECL and the age in which bull reach 26 cm of SC – phenotypes that represent the age at puberty for both females and males – for the same Brahman population used in this study. One of the most significant SNP also was close to *SDR16C6* and *PLAG1* genes.

Despite this evident peak in chromosome 14, the most significant SNP (*P*-value = 3.21×10^{-14}) of the meta-analysis was located in chromosome 9 at 75.6 Mb. This SNP is in an intronic region of the *MAP3K5* gene, which was identified as an estrogen receptor in human (Mirkin et al., 2005).

In Table 2 are presented the most significant SNP of each chromosome, its position and the closest gene. In BTA1, *TSC22D2* emerged as a candidate gene in our meta-analysis study. This gene was high-expressed in human oocytes (Kakourou et al., 2013). In BTA2, *KLF7* was the closest gene to the top SNP (*rs134877457*). It was associated with growth traits in Chinese cattle (Ma et al., 2011) and presents an important role in mouse neurogenesis (Laub et al., 2001; Caiazzo et al., 2010), olfactory neurogenesis (Kajimura et al., 2007), and play a role in adipogenesis regulation in human (Kawamura et al., 2006).

In BTA3 the top SNP (*rs109478958*) suggested *ARHGAP29* as a candidate gene. Its protein was up-regulated in bull semen with good quality, compared with bad quality semen (Singh, et al., 2018). The top SNP for chromosomes 6 (*rs43492923*) and 7 (*rs135631400*) were close to *7SK*, a small nuclear ribonucleoprotein coding gene (snRNA). This snRNA play role in DNA transcription and was found close to a significant SNP for bull fertility in chromosome 10 (Suchocki and Szyda, 2015), has a critical role in the control of primordial germ cells proliferation of mouse embryos (Okamura et al., 2012), and was detected in seminal plasma, ejaculated sperm and

epidydimal sperm in pigs with a significant biological role in spermatogenesis (Chen et al., 2017).

In BTA9, the gene *MAP3K5* was the closest to the top SNP (*rs110257163*) and was previously associated with regulation of apoptosis in porcine ovarian granulosa cells, the expression of *MAP3K5* was increased when the levels of FSH hormone increased in those cells (Sirotkin et al., 2008). Also, this gene was a strong candidate associated with growth and reproduction traits in transcriptome analysis of bovine pituitary gland (Pareek et al., 2016), and it presented some interactions with estrogen receptors in rat primary cortical neurons (Singer et al., 1999).

In BTA10, *TLE3* (closest to the top SNP *rs110458186*) was expressed in mouse mesoderm embryonic cells (Pfeffer et al., 2017). The expression of this gene was associated with FSH hormone increased levels in bovine granulosa cells (Nivet et al., 2018) and with estrogen receptors in human breast cancer cell line (Jangal et al., 2014). This gene was associated with mouse embryo survival. *TLE3* mutants presented placenta defects (Gasperowicz et al., 2013) and this gene acted as a co-regulator of adipogenesis in mouse (Villanueva et al., 2011). This gene also affects the embryonic stem cell differentiation (Laing et al., 2015).

In BTA11, the candidate gene *WDR5* presents an important role in porcine early embryo development (Ding et al., 2017) and was essential for vertebrate development (Wysocka et al., 2005; Gori et al., 2006). In BTA13 the most significant SNP (*rs136854801*) was located in an intronic region of the *TAF3* gene. This gene harbor a SNP marker associated with the Major histocompatibility complex (MHC) in an Italian human population (Pistis et al., 2013). The importance of the MHC for reproduction was previously discussed in Ziegler et al. (2010). Besides that, this gene was associated with

endoderm differentiation in mouse embryonic stem cells (Liu et al., 2011), was significantly up-regulated in human oocyte, and was enriched in the estrogen receptor signaling pathway (Kocabas et al., 2006).

In BTA14, *TMEM68* was identified as a candidate gene. This gene was expressed in human epididymis (Belleannée et al., 2012), was up-regulated in heifer blastocyst (Carter, et al., 2010) and was associated with feed intake and growth traits in cattle (Lindholm-Perry et al., 2011). In BTA16, *PPP1R15B* was the gene closest to the top SNP (*rs109871859*). It was expressed in bovine cumulus oocyte cells (Abd El Naby et al., 2011) and in bovine granulosa cells under FSH stimulation (Nivet et al., 2018). *PPP1R15* gene knockout mouse embryos survived, but had several problems as grown retardation (Harding et al., 2009). In BTA21, the emergent candidate, *NR2F2*, was previously associated with Leydig cell steroidogenesis in mice testis cells (Mendoza-Villarroel et al., 2014). Bauersachs et al. (2006) concluded that this gene was associated with embryo implantation in cows. In BTA24 the gene closest to the top SNP (*rs133759831*) was *GALR1*. This gene was differentially expressed in ovine hypothalamus in pre-pubertal age compared to foetal and adult ages, suggesting a regulation role in the beginning of reproduction (Whitelaw et al., 2009). Also, it was located near a significant SNP associated with oligozoospermia and azoospermia in human (Aston and Carrell, 2009), was associated with the regulation of ovarian steroids in rats (Mitchell et al., 2004) and interacted with testosterone in male rat brain (Bouret et al., 2000). In BTA26, *SUFU* was mapped close to the top SNP (*rs135708259*). This gene was a candidate gene associated with spermatogenesis in mouse and it was found in spermatocytes (Szczepny et al., 2005). In BTA27, the *KCNU1* was the closest gene to the top SNP (*rs135961785*). This gene presents an important role in semen quality (Santi et al., 2010), which could be observed

in a knockout mouse experiment (*SLO3*, null mutation). Also Zeng et al., 2011 reported the importance of *SLO3* for male fertility, by evaluating its role in the normal morphology and sperm motility in mouse. This gene was enriched in the pathway "Vascular smooth muscle contraction", which was activated by progesterone treatment in neonatal mouse uterus, inhibiting its development (Filant et al., 2012).

On chromosomes 4, 8, 15 and 29 the top SNP were close to uncharacterized proteins, their identification are presented in Table 2. No significant SNP were observed in BTA5, BTA12, BTA17, BTA18, BTA19, BTA20, BTA22, BTA23, BTA25 and BTA28. Evidence from the current meta-analysis seems to corroborate with previous knowledge of gene function for the discussed genes. Although the majority of the significant SNP were located in chromosome 14, significant SNP were also mapped in multiple chromosomes, which is consistent with the idea that reproductive traits are complex polygenic phenotypes.

Functional annotation and enrichment analyses

A total of 389 genes were located within 0.5 Mb of the 108 significant SNP ($P < 1.39x10^{-5}$). From these genes, functional annotation and enrichment analyses were performed and 333 genes were identified by DAVID software (Huang da et al., 2009), using bovine genome as a background gene list. Enrichment analyses identified 48 functional gene groups as significant in DAVID resources, using high astringency criterion. The top 5 functional gene groups, with higher enrichment score, are presented in Table 3.

Groups 1 (S100/CaBP-9k-type, calcium binding) and 2 (domain: EF-hand 1) were related to calcium binding pathway. S100 proteins are a family protein expressed just in vertebrates. They participate of several biological events, as regulation of proliferation, differentiation, apoptosis and inflammation (Donato et al., 2013). Calbindin-D9k (CaBP-9k-type) is a vitamin-D-dependent Ca^{+2} binding protein, member of the S100 family. This protein is mainly found in intestinal tissue, but was also found in other tissues as uterus and placenta (Krisinger et al., 1995; Emam et al., 2016; Mathieu et al., 1989). All the genes clustered in group 1 were members of S100A family. This gene family was expressed in ovary, prostate and testis of human and rats (Wicki et al., 1996) and in thyroid gland, placenta and prostate (Cannon et al., 2011). Their roles are generally associated with immunological function, promoting the sterility of reproductive tissues against pathogens (Teijeiro and Marini, 2015; Germeyer et al., 2008), however they were also related to embryo implantation process (Gray et al., 2006; Smits et al., 2018), to response to temperature stress (Landriscina et al., 2001), cell proliferation and apoptosis (Jin et al., 2011).

The majority of genes clustered in group 2 were also in group 1. This functional group, EF-hand 1, presents a group of proteins that share a similar structure, which is commonly found in calcium binding proteins. Besides of some genes in common with group 1, other two genes were enriched in this group, *CAPS*, *NCS1*. *CAPS* gene was expressed in human epididymis and endometrium during follicular fase (Thimon et al., 2007; Blockeel et al., 2010), while *NCS1* was expressed in bovine blastocysts cultured in-vitro under stimulation of thyroid hormones, presenting an important role in mammalian neuron-memory development (Ashkar et al., 2014).

Group 3 (Cell migration), is a fundamental process in the development and maintenance of multicellular organisms. Several reproductive events depend on this process, as embryo formation (Jovanović et al., 2010), immunological responses (Sánchez- Madrid and Pozo 1999), spermatogenesis (Smith et al., 2012) and oogenesis (Rørth et al., 2000). Genes clustered in this group were associated with daughter pregnancy rate, cow conception rate (Ortega et al., 2016), development of nervous system (Kapur et al., 1991; Kanaani et al., 2005; Jessberger et al., 2009), embryo lethality (Nourizadeh-Lillabadi et al., 2010), post-natal mortality and growth retardation (Pallares and Gonzalez–Bulnes, 2010), immunity (Bogdan, 2015), embryo differentiation (Rosa and Brivanlou, 2011), peri-natal mortality (Brites et al., 2003).

The group 4 clustered genes associated with the Immunoglobulin G (IgG). This is the most common isotype in human blood and extracellular fluid (Janeway et al., 2007), and the most abundant antibody class present in healthy individuals. This antibody was found in the mucus of woman reproductive tract in Fahrbach et al. (2013), which could suggest a role in the fortification of mucus barriers in the female reproductive tract. Besides that, mouse experiments were performed with a specific monoclonal IgM and demonstrated that females immunized with this antibody presented gestational failure (Sthoeger et al., 1993). A similar effect was observed in another mouse experiment, by Ornoy et al., 2003, where the administration of a purified IgG in pregnant mouse reduced the yolk sac and the embryo growth. This pathway enriched the genes *FCER1G*, *FCGR3A*, *FCGR2B*, which were associated with daughter pregnancy rate, cow conception rate (Ortega et al., 2016), embryo-maternal recognition in cattle (Mamo et al., 2012) and infertility in female mouse (Wetendorf et al., 2017).

The 5th top group was "Positive regulation of secretion". As the group 3, this group is related to a large variety of biological mechanisms, mainly mediated by regulatory hormones. Genes clustered in this group were associated with positive regulation of immune response (Macen et al., 1993; Kiba, 2016), neurogenesis (Jessberger et al., 2009) and response to stress (Faria et al., 2012).

David results presented gene clusters that were mostly associated with immune events. Earlier studies with *Bos indicus* cattle have found genes playing roles in immune responses, adaptability and reproduction (Bahbahani et al., 2018). Fayemi (2005) observed that the presence of anti-bodies (IgG) in bulls' sperm was related with fertility. Our results are in agreement with previous studies with *Bos indicus* cattle, pointing the importance of the immune competence to guarantee reproductive success in *Bos indicus* cattle.

Besides the most of significant SNP were located in chromosome 14, our meta-analysis identified genomic regions harboring significant SNP associations in the majority of the autosome chromosomes (19 out of 29), as expected for polygenic traits. The genes mapped within these associated regions are plausible candidate genes because of their position and due to previous evidence suggesting their functional association with reproduction, as discussed above. The candidate genes identified could be grouped in functional categories that seem to affect immune system and consequently, mammalian reproduction.

In summary, five regions distributed over four chromosomes presented the majority of the most significant SNP (76%): BTA2 (5.55%) from 95 to 96 Mb, BTA4 (5.55%) from 94.1 to 94.8 Mb, BTA14 (59.26%) from 24 to 25 Mb and 29 to 30 Mb, and BTA21 (5.55%) from 6.7 Mb to 11.4 Mb. Two independent populations were studied here,

animals from different breeds, born and bred in different geographic regions. The genes identified as candidates in the meta-analysis are probably affecting physiological mechanisms that control sexual precocity in both breeds, since we found key genes expressed in reproductive tissues and playing important roles in immune response (including in reproductive tract), maternal-fetal tolerance, pregnancy maintenance, embryo development, fertility and response to stress, or were located in previously reported QTL regions for reproductive traits in bovine. Also, we found some candidate genes associated with important hormones for the regulation of puberty in mammalian, as the FSH, estrogens and testosterone. The importance of these hormones for sexual precocity is expected to be conserved among mammalians.

In short, the genes presented here are both positional and functional candidates for sexual precocity in *Bos indicus* cattle. Future works could fine-map and validate the identified genomic regions and elucidate which genes are in fact harboring the mutations that could explain the proposed QTL.

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TABLES AND FIGURES

Table 1. Summary statistics of the traits age at first calving (AFC), early pregnancy (EP) and scrotal circumference (SC_N) in Nellore cattle and age at first *corpus luteum* (AGECL), first postpartum anoestrus interval (PPAI) and scrotal circumference (SC_B) in Brahman cattle.

Trait	Number of observations[1]	Mean ± SE	Minimum	Maximum
AFC, days	1,796	1068.2 ± 118.5	778.9	1292.8
EP, %	1,849	28.3	--	--
SC_N[2], cm	4,248	26.57 ± 2.56	17.47	36.17
AGECL, days	1,007	750.6 ± 142.14	394	1211
PPAI, days	629	180.11 ± 108.71	17	484
SC_B[3], cm	1,203	26.51 ± 2.76	18.25	39.25

[1] Number of observations considered in analyses: animals with available genotypes and phenotypes.

[2] SC_N measured at approximately 521 days of age.

[3] SC_B measured at approximately 540 days of age.

Table 2. Number of significant SNP ($P < 1.39x10^{-5}$) per *Bos taurus* autosome (BTA), the most significant SNP in each autosome (top SNP), its position and its distance from the closest gene in base pairs (bp).

BTA	Number of SNP	Top SNP	Position, bp	Gene Symbol	Distance, bp
1	1	*rs110366479*	118,606,312	*TSC22D2*	5,852
2	6	*rs134877457*	95,917,958	*KLF7*	14,648
3	3	*rs109478958*	49,436,731	*ARHGAP29*	0
4	3	*rs136050748*	110,440,534	*Uncharacterized Protein (ENSBTAG00000048097)*	530,702
6	1	*rs43492923*	118,436,689	*7SK – misc RNA*[1]	2,828
7	6	*rs135631400*	94,710,749	*7SK – misc RNA*[1]	510,435
8	1	*rs136590180*	68,307,110	*Uncharacterized Protein (ENSBTAG00000012266)*	16,590
9	1	*rs110257163*	75,613,158	*MAP3K5*	0
10	3	*rs110458186*	16,769,219	*TLE3*	132,436
11	2	*rs108980439*	104,936,387	*WDR5*	0
13	2	*rs136854801*	16,097,639	*TAF3*	0
14	64	*rs109748092*	24,710,609	*TMEM68*	718
15	1	*rs110493922*	9,064,376	*Uncharacterized Protein (ENSBTAG00000047425)*	141,535
16	1	*rs109871859*	1,929,989	*PPP1R15B*	36,732
21	7	*rs110478544*	11,435,365	*NR2F2*	628,702

24	1	*rs133759831*	2,276,768	*GALR1*	129,893
26	2	*rs135708259*	23,405,679	*SUFU*	47,301
27	1	*rs135961785*	31,926,152	*KCNU1*	0
29	2	*rs109184359*	9,174,027	*Uncharacterized Protein (ENSBTAG00000046374)*	1,952

[1]miscRNA = Miscellaneous RNA

Table 3. Top 5 most significant functional gene groups enriched in DAVID v.6.7 bioinformatics resources (https://david.ncifcrf.gov/home.jsp). Gene Ontology (GO) terms, enrichment scores (ES) and false discovery rates (FDR) are reported for each functional group; genes in each group are also reported.

Functional groups	Top GO term, code ~ name	Ontology	ES	FDR	Genes
1	IPR001751~ S100/CaBP-9k-type, calcium binding		7.06	5.2E-6	*S100A13, S100A1, S100A14, S100A16, S100A2, S100A3, S100A4, S100A7*
2	UP_SEQ_FEATURE~ domain: EF-hand 1		2.79	1.2E-1	*S100A13, S100A1, S100A14, S100A16, S100A2, S100A4, CAPS, NCS1, S100A7*
3	GO:0016477~ Cell migration	Biological Process	2.59	2.2E0	*FCER1G, cdk5, DBH, MP14, NOS3, NR2F2, pex7, LOC515718*
4	GO:0019864~ IgG Binding	Molecular Function	1.99	1.7E0	*FCER1G, FCGR3, FCGR2B*
5	GO:0051047~ Positive regulation of secretion	Biological Process	1.65	1.6E1	*FCER1G, CHRNB2, cdk5, serp1*

Figure captions

Figure 1. Manhattan plot of the meta-analysis for sexual precocity traits in Nellore and Brahman cattle. The y-axis represents the log inverse *P*-values for SNP associations and the x-axis represents the position in base pairs from chromosome 1 to 29. The blue line

indicates genome-wide significance, a *P*-value cutoff of ($P < 1.39x10^{-5}$), which is equivalent of a false discovery rate lower than 5%.

Figure 2. Zoom in BTA14, region of the strongest peak. SNP above the red line were significant ($P < 1.39x10^{-5}$). In the bottom, genes mapped in this region (Total = 87).

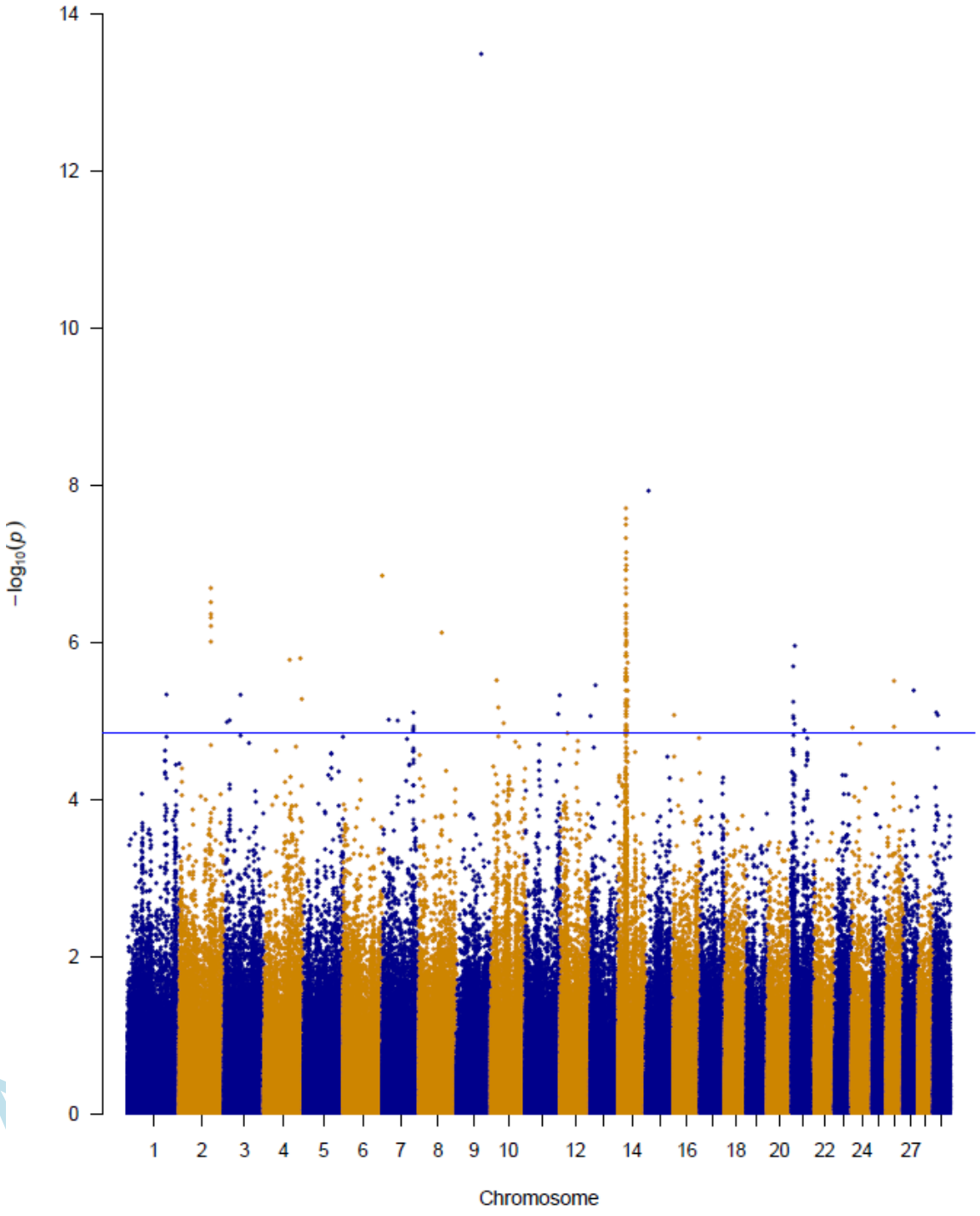

Figure 1

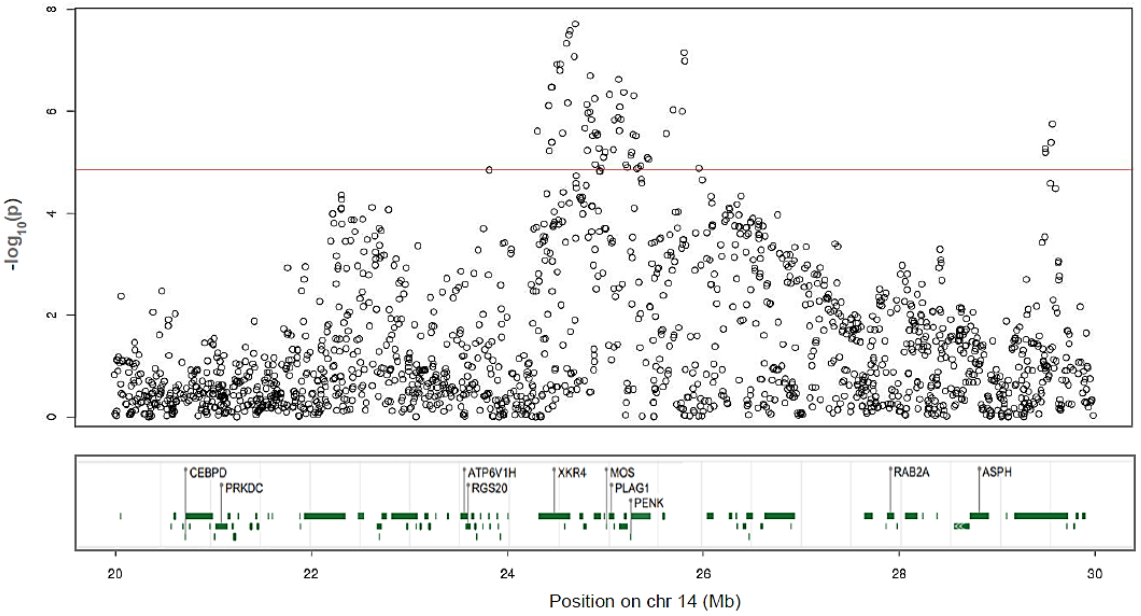

Figure 2