Animal Reproduction Science 208 (2019) 106109

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Characterization of gonadotropin releasing hormone receptor gene in Sokoto and Kalahari Red goats

Adamu Mani Isa[a,b,c,*], Martha N. Bemji[a], Mathew Wheto[a], Tolulope J. Williams[d], Eveline M. Ibeagha-Awemu[e]

[a] Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria
[b] Department of Animal Science, Usmanu Danfodiyo University, Sokoto, Nigeria
[c] Key Laboratory of Animal Genetics, Breeding and Reproduction, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, 100193, PR China
[d] Department of Animal Physiology, Federal University of Agriculture, Abeokuta, Nigeria
[e] Agriculture and Agri-Food Canada, Sherbrooke Research and Development Center, QC, Canada

ARTICLE INFO

Keywords:
GnRHR
Goat
Litter size

ABSTRACT

The objective of this study was to characterize *GnRHR* gene in Sokoto ($n = 70$) and Kalahari Red ($n = 70$) goats. Three SNPs, (g.-29T > G, g.48 G > A and g.209 T > G), were detected in Sokoto Red (SR) and one (g.48 G > A) in Kalahari Red (KR) goats. All the mutations occurred within the 5′UTR and Exon one of the gene and the g.209 T > G was non-synonymous and, therefore, resulted in an amino acid change from methionine to arginine at Position 70 of the GnRHR polypeptide. The homozygous mutant genotypes at the three SNP loci were not detected in both breeds but minor allele frequencies were ≥ 0.1 for the three SNP loci in SR goats. Frequency of the T allele, however, was 0.93 at the only SNP locus detected in KR goats. There was a strong linkage disequilibrium (LD; $r^2 > 0.98$) among the detected mutations in SR goats resulting in two haplotypes (T-G-T and G-A-G) with a frequency of 86% and 13%, respectively. There was no significant association between genotypes at the polymorphic loci and litter size ($P > 0.05$) in the two breeds. The non-synonymous mutation (g.209T > G) appears to have changed the nucleotide binding region and area spanning exposed/buried regions on the predicted secondary structure of the two variants of the receptor. This change led to variation in the tertiary structure between the two variants of the receptor and can influence the function of the transmembrane receptor. Comparison of the GnRH receptor domains for goats, sheep, cattle and swine confirmed that the seven transmembrane domains of the receptor are conserved in all the farm animals considered.

1. Introduction

Goats receive less attention than other livestock species in research (Marai et al., 2002). Goats, however, represent an important genetic resource because of the meat and milk they provided mainly as a resource for economically challenged farmers in developing countries. Along with sheep, goats provide the main means of survival and food security in the semiarid regions of the world (Devendra, 1999). There are more than 387 million goats in Africa, supplying over 1.2 million metric tons of meat and almost 4 million metric tons of milk annually (FAOSTAT, 2016). In Nigeria, the population of goats was estimated to be above 73.8 million (FAOSTAT, 2016).

* Corresponding author at: Department of Animal Science, Usmanu Danfodiyo University, Sokoto, Nigeria.
E-mail address: isa.adamu@udusok.edu.ng (A.M. Isa).

https://doi.org/10.1016/j.anireprosci.2019.106109
Received 7 March 2019; Received in revised form 18 May 2019; Accepted 25 June 2019
Available online 26 June 2019

Devendra (2010) estimated that there are about 1,156 different goat breeds with varied specialty product provisions globally. There, however, are fewer of these such specialized breeds that are native to Africa. The Sokoto Red (SR) breed is one of the three major Nigerian goat breeds. Animals of this breed are predominantly reared in the savannah regions of the country and are reliable sources of meat, milk and high quality skin. The SR breed like the other two indigenous goat breeds of Nigeria is genetically unimproved. Average litter size at birth is 1.8 (FAO, 1991; Awemu et al., 1999). Multiple births are extremely common and was reported to be greater than 67% (FAO, 1991). The Kalahari Red (KR) breed, however, is a hardy, well-muscled commercial meat-breed from South Africa (Kotze et al., 2004; Mdladla et al., 2017) and was imported into Nigeria less than a decade ago (Bemji et al., 2014) for breeding purposes. The breed originated from the Kalahari Desert region bordering South Africa, Botswana and Namibia and represents between 10% and 17% of goats in South Africa (Mdladla et al., 2017). Twinning is the only type of multiple birth in this breed with a rate of about 40% even when fed a concentrate diet for maintaining an optimal body condition of animals for reproductive functions (Oderinwale et al., 2017).

Goat herd productivity is determined by number of kids weaned or the weaning weight of kids per litter (number of kids born/doe) or both. Litter size is an indicator of reproductive efficiency (Susilorini et al., 2017) and the most important determinant for number of kids weaned. Litter size is markedly affected by a doe's fertility and prolificacy and is the main trait with a significant effect on overall profitability of the goat industry (Mellado et al., 2011). Litter size is determined by ovulation rate and consequently by gonadotropin (follicle stimulating hormone and luteinizing hormone) actions with the secretion of these hormones being controlled by gonadotropin releasing hormone (GnRH). The GnRH functions mainly on the anterior pituitary lobe via a specific GnRH receptor (GnRHR) on the plasma membrane, where it induces the synthesis of alpha and two separate beta chains of the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) thereby stimulating gonadal production of sex steroids (Avet et al., 2018). The GnRHR, therefore, controls synthesis and secretion of FSH and LH, and through the actions of these hormones, it regulates ovulation.

Although the genetic composition of RS and KR goat breeds has been characterized using microsatellite markers and mitochondrial sequences (Pieters et al., 2009; Awotunde et al., 2015) there has been no research focused on characterization of *GnRHR* gene in the breeds and association of the SNPs with reproductive traits in SR and KR goat breeds.

2. Materials and methods

2.1. Experimental animals and animal management

Does (n = 70 for each breed) from SR and KR breeds were used in this study. The goats were in their 4th parity with litter size ranging from one to three. Method of management of the animals was reported in Isa et al. (2017). The experimental animals of SR and KR were managed, respectively, at the National Animal Production Research Institute, Zaria and Institute of Food Security, Environmental Resources and Agricultural Research of the Federal University of Agriculture, Abeokuta, Nigeria.

2.2. Blood sample collection and genomic DNA isolation

The ethical guidelines of the College of Animal Science and Livestock Production of the Federal University of Agriculture, Abeokuta were followed in conducting this study. Blood samples were collected from each doe via the jugular vein into EDTA-coated vacutainer tubes. Genomic DNA was extracted from the samples using Nucleospin® Genomic DNA kit (Nucleospin Blood, Macherey-Nagel, Germany) according to the guide of the manufacturer. Quantity and quality of purified DNA was assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technolgies, Wilmington, DE, USA).

2.3. Primers and PCR amplification

The *GnRHR* sequence (Accession Number NC_022298.1, from 81631057 to 81649727) was retrieved from NCBI Genbank and used to design the primers. Three pairs of PCR primers were used to amplify the promoter region, all exons and the flanking introns. Primer sequences and product size of the amplicons are reported in Table 1.

The PCR reaction was performed in a 25 µl volume containing 1 µM each primer (forward and reverse), 10X PCR buffer (including 1.5 mM $MgCl_2$), 200 µM dNTPs and 1 unit of *Taq* DNA polymerase (Bioline Life Sciences, London, UK). About 60 ng of genomic DNA was used as template. Thermocycling conditions consisted of 35 cycles of initial denaturation at 95 °C for 5 min, denaturation at 94 °C for 30 s; primer annealing at (58–62 °C) for 1 min; primer extension at 72 °C for 1 min with a final extension at 72 °C for 10 min. The

Table 1
Primer sequences used in the amplification of coding regions of *GnRHR* gene.

Primer name	Primer length (bp)	Primer sequence	Region	Amplicon size (bp)	Tm (^{0}C)
GnRHR_659F	20	TTTGCTTTAGCACCCTGTTG	Exon one	849	58
GRHR_1507R	22	GAGCCCAAGCTCTCAGAGATAA			
GnRHR_14098F	20	TTGGTGATTGTTCTGCAAGG	Exon two	810	62
GnRHR_14907R	20	GCCCATGCTTCTACTGGGTA			
GnRHR_17851F	20	TTCAACCCCAGTCCACTCTC	Exon three	943	62
GnRHR_18793R	20	TAAGCCCCTTTGCAGAGAAA			

PCR products were separated in 1.5% agarose gel in 1x TBE electrophoresis buffer. The gels were stained and viewed using UV light in an Alphamager® 2200 version 5.5 gel documentation systems (Alpha Innotech, San Leandro, CA, USA).

2.4. Sequencing, SNP identification and genotyping

Samples were pooled (10 samples per pool) and sequenced to screen for mutations in the coding and regulatory regions of the *GnRHR* gene in the two goat populations using BigDye® Terminator chemistry technology on ABI3730XI (Applied Biosystems, Foster City, CA, USA) DNA analyzer. Pooling of samples was conducted within population only. The SNP positions identified from the sequences generated using the Sanger sequencing technique were used to genotype all the does at the polymorphic loci using the SequenomiPLEX Gold Technology on MassARRAY platform (Sequenom Inc. San Diego, CA, USA) at McGill University and Genome Quebec Innovation Centre (https://genomequebec.mcgill.ca/).

2.5. Data analyses

Genotypes of individuals were analyzed for genotypic and allelic frequencies, heterozygosity (He), polymorphism information content (PIC) and linkage disequilibrium (LD) using the genetics package (Warnes and Leisch, 2005) using R statistical software. Haplotype frequencies in SR breed where more than one SNP was identified were computed using the haplo.stat (Sinwell and Schaid, 2005) package also of the R statistical software.

A generalized linear fixed effect model was used to investigate the effect of genotype at the polymorphic loci on litter size. Data were analyzed using SAS version 9.1 (SAS Institute, Cary, NC, USA). The model used in the analysis is provided with the following description;

$$Yij = \mu + Gi + eij$$

Where, $Yij$is the litter size (trait) of i^{th} doe, μ is the population mean, Gi is the fixed effect associated with i^{th} genotype (1, 2) and $eij$is the residual error.

2.6. Protein structure and domain prediction

Prediction of secondary structure of the two variants of the GnRH receptor was conducted using PredictProtein (Rost et al., 2004; https://www.predictprotein.org/) procedures. Tertiary structure was predicted using SWISS-MODEL (Waterhouse et al., 2018) and structure assessment procedures was conducted using the *MolProbity* algorithm (Chen et al., 2010). Domain prediction of the receptor structure in goats and other species was conducted using the SMART online tool (Letunic and Bork, 2017).

3. Results and discussion

3.1. Identified SNPs within GnRHR gene in SR and KR goats

Three mutations were detected within the 5′UTR and Exon one of *GnRHR* gene. The SNPs were g.-29T > G, g.48 G > A and g.209 T > G. Numbering occurred based on the goat *GnRHR* sequence (GeneBank: NC_022298.1) relative to the translation start site. Two of these mutations were transversions (g.-29T > G and g.209 T > G) while the other (g.48 G > A) was a transition. Only the transition mutation was detected in KR goats while all the three SNPs were identified in the SR breed. One of the transversion SNPs (g.209 T > G) resulted in an amino acid change from methionine to arginine at Position 70 on the gonadotropin releasing hormone receptor polypeptide (Table 2) which can lead to modifications in the advanced (secondary, tertiary or quaternary) structure of the receptor. It seems the other exons of the *GnRHR* gene were conserved in the two populations of goats. In a similar investigation, Yang et al. (2011) reported two mutations within Exon one (g.757 G > A and g.819 T > G) in Boer goats. An et al. (2009) also reported that there was a deletion (A714/) and an insertion (/731 G) in Exon one of the gene in Xinong Saanen and Boer goats. Another SNP (T > A) in Exon two was also documented for Boer and Shaanan goat breeds (Li et al., 2011). In cattle, Derecka et al. (2010) reported seven transition mutations within the bovine *GnRHR* in dairy cows; two within promoter (g.-331A > G and g.-108T > C) and five within Exon one (g.206 G > A, g.260C > T, g.341C > T, g.388C > T and g.410C > T). Unlike in the present study, however, all the exonic SNPs reported by Derecka et al. (2010) were synonymous.

The data for the observed frequency of genotype and allele distribution at the polymorphic loci in goats of the SR and KR breed are presented in Table 3. At all the sites, there were only homozygous wild type and heterozygous genotypes. Frequency of the TT

Table 2
SNPs identified within *GnRHR* gene in SR and KR goats.

Region	Mutation	Codon Change	Type of Substitution	Breed where polymorphism occurs
5'UTR	g.-29T > G	–	–	SR
Exon one	g.48 G > A	GCG /GCA	16 Ala > Ala	SR and KR
Exon one	g.209 T > G	ATG/AGG	70 Met > Arg	SR

Table 3
Genetic structure of polymorphic sites of *GnRHR* gene in SR and KR goats.

SNP	Breed	Genotypic frequencies	Allelic frequencies	He	PIC
g.-29T > G	SR	TT TG	T G	0.24	0.21
		0.73 0.27	0.87 0.13		
g.48 G > A	SR	GG GA	G A	0.20	0.01
	KR	0.22 0.78	0.89 0.11	0.13	0.12
		0.86 0.14	0.93 0.07		
g.209 T > G	SR	TT TG	T G	0.24	0.21
		0.73 0.27	0.87 0.13		

He = heterozygosity, PIC = polymorphism information content.

genotype was high (0.73) in SR goats at the two tranversion sites (g.-29T > G and g.209 T > G). The minor allele (G) had a frequency of 0.13 at the two SNP sites. Conversely, the frequency of the heterozygote genotype was high (0.78) at the transition SNP site in SR breed. In KR breed, however, the homozygote wild type genotype (GG) was of a greater frequency (0.86). Based on heterozygosity and PIC, the two breeds had little genetic diversity (< 0.25; Table 4).

3.2. Linkage disequilibrium and haplotype frequency

Based on pairwise linkage disequilibrium measures (D' and r^2), the three SNPs identified in SR goats were in strong LD ($r^2 > 0.88$; Table 4) which may be due to the proximity to one another (< 300bp). This is consistent with genome wide reports that highlighted persistence of LD between SNPs that are less than 50 kb apart (Goddard et al., 2006; Mdladla et al., 2016).

Two haplotypes of the gene were observed in the SR goats with G-A-G haplotype accounting for more than 86% of the total (Table 5). There was a strong LD between loci which was the likely cause because it ensures restricted recombination between markers and would be transmitted to the next generation as linked markers.

3.3. Association of polymorphisms in GnRHR with litter size in goats of the SR and KR breeds

Gonadotropin releasing hormone receptor as a result of its unique capacity to transmit gonadotropin activity has a significant function in regulating number of oocytes released and consequently litter size in livestock. There was no significant ($P > 0.05$) association between SNPs and litter size in the two goat breeds (Table 6). This may be due to small sample size used in the study. In another study, however, Yang et al. (2011) reported that there was a significant association between g.757 G > A and g.891 G > T mutations with litter size in Boer goats at the fourth and third parity, respectively. Mutations reported by Yang et al. (2011) occurred within coding region of the gene. Recently, the SNP detected within 5′UTR in the current study (g.206 G > A) was reported to be associated with litter size in West African Dwarf goats (Bemji et al., 2018). Similarly, An et al. (2009) reported that there were associations between the AB genotype with a larger litter size in Xinong Saanen and Boer does. Another mutation (T > A) detected within Exon two in Shaanan and Boer goats was reported to be associated with litter size, and does with AA genotypes had more kids than the heterozygote does (Li et al., 2011). The results of these studies indicate that GnRH receptor has an important function in regulating the number of oocytes released during ovulation in goats.

3.4. GnRH receptor structure prediction

The mutation of the nucleotide from T to G at Position 209 of the *GnRHR* gene in SR goats resulted in a change in both the secondary and tertiary structure of the receptor.

Based on the predicted secondary structure of the receptor, g.209 T > G mutation takes place within the low complexity region of the transmembrane protein. This results in change in the RNA binding region in the methionine variant of the polypeptide to a DNA binding region in the arginine variant of the receptor (Fig. 1). The two variants, however, contain protein binding and another RNA binding region. While the RNA binding region in the methionine variant spans only a single base, the DNA binding region that was replaced in the arginine variant spans five bases such that there is a greater probability for interaction with the nucleotide. Another alteration predicted to have been caused by the g.209 T > G mutation to the secondary structure of the receptor was in the exposed and buried regions. The arginine variant of the receptor has a greater exposure and more accessible region than its

Table 4
Estimates of linkage disequilibrium parameters (D'/r^2) for pair of SNPs identified in *GnRHR* gene in SR goats.

Pairs of SNPs	D'	r^2	*P*-value
g.-29T > G and g.48 G > A	0.9992	0.8847	1.97e-09
g.-29T > G and g.209 T > G	0.9995	0.9995	5.69e-13
g.48 G > A and g.209 T > G	0.9992	0.8847	1.97e-09

D'= Lewontin parameter, r^2 = correlation coefficient.

Table 5
Haplotype frequency observed in *GnRHR* in SR goats.

Haplotype	g.-29T > G	g.48 G > A	g.209 T > G	Frequency
Haplotype 1	T	G	T	0.1346
Haplotype 2	G	A	G	0.8654

Table 6
Least square means and standard error for litter size of different genotypes of *GnRHR* gene in Sokoto and Kalahari Red goats.

SNP	Breed	Genotype	Average litter size	*P*-value
g.-29T > G	SR	TT	1.47 ± 0.14	0.74
		TG	1.57 ± 0.03	
g.48 G > A	SR	GG	1.50 ± 0.15	0.77
	KR	GA	1.60 ± 0.04	0.084
		GG	1.42 ± 0.47	
		GA	1.00 ± 0.17	
g.209 T > G	SR	TT	1.47 ± 0.14	0.74
		TG	1.57 ± 0.03	

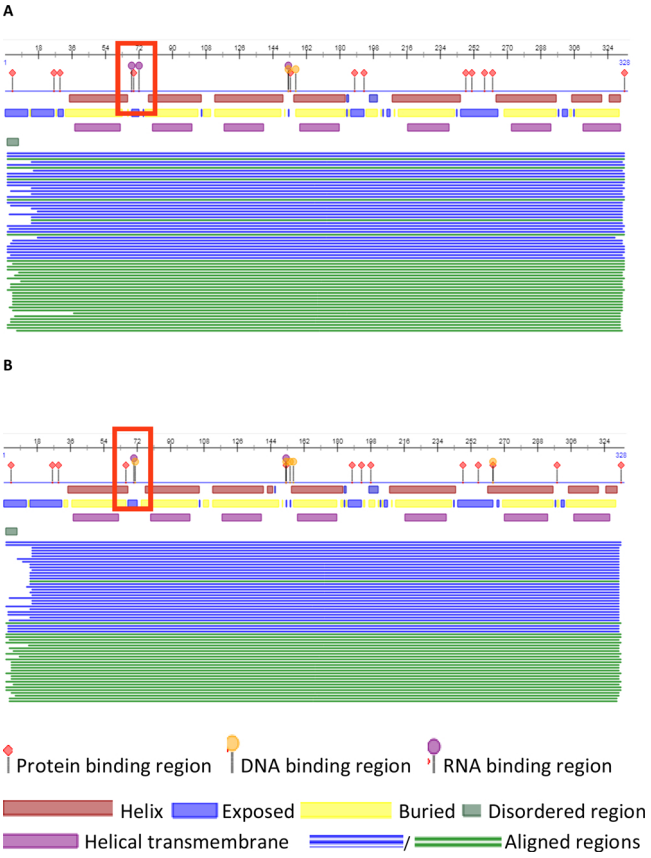

Fig. 1. Secondary structure of caprine GnRH receptor depicting two variants of g.209 T > G mutation based on its predicted primary protein structure; (A) Secondary structure of the receptor with methionine at Position 70 of the polypeptide (B) Secondary structure of the receptor with arginine at Position 70 of the polypeptide.

methionine counterpart. These variations may have ultimately resulted in a change in the tertiary structure of the two variants of the receptor (Fig. 2).

An important factor in determining the folding in the tertiary structure of a protein is the distribution of polar and non-polar amino acids. Methionine is an amphipathic amino acid and its presence in a protein structure often leads to formation of a binding ligand with metal ions being a component while arginine is a charged amino acid and is associated with salt bridge formation in the resulting protein and, is therefore hydrophilic. The methionine variant of the receptor is more compact with fewer intra-sheet loops

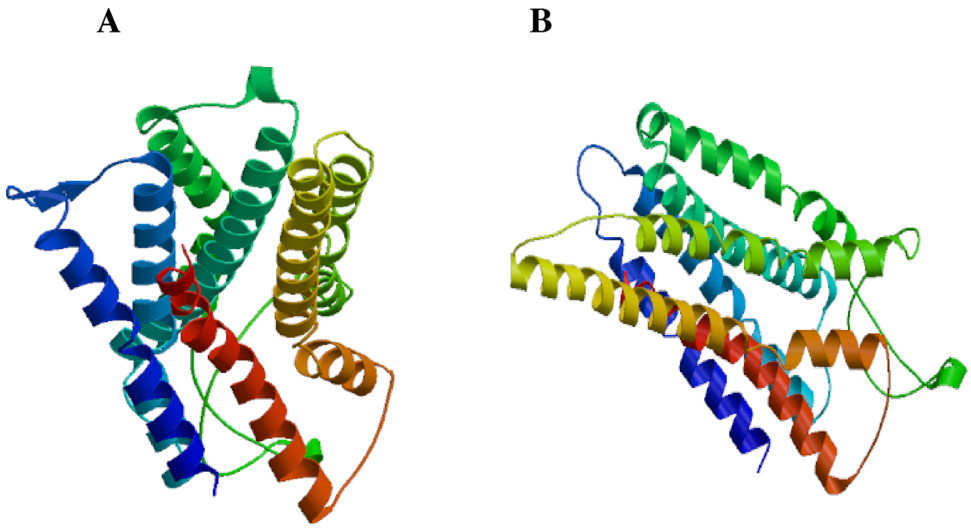

Fig. 2. Tertiary structure of caprine GnRH receptor variants; (A) Tertiary structure of GnRH receptor with methionine at Position 70 of the polypeptide (B) Tertiary structure of GnRH receptor with arginine at Position 70 of the polypeptide.

than the arginine variant which may likely have biological (functional) implications (Fig. 2). This may be a result of variation in the polarity index of the methionine and arginine at Position 70 of the two variants of the transmembrane receptor. Quality metrics scores including clash scores, percent Ramachandra and Rotamer outliers used in the assessment of structure prediction are in the range of 1.44% and 1.64%, 1.03% and 1.38%, and 0.37% and 0.75%, respectively. Clash scores and percent Rotamer outlier are within the range for protein structure prediction with high quality (Shao et al., 2017). Arginine variant predicted structure has the greatest limits for these two quality metrics. Percent Ramachandra outliers for the arginine variant predicted structure, however, indicates there is an increase of 0.35% compared to the methionine variant but are both within the range for a poorly predicted structure. This causes a corresponding reduction of percentage Ramanchandra favored from 95.2% in the methionine variant to 94.5% in the arginine variant of the receptor. The deviation of the percentage Ramachandra outliers from the other two quality parameters might be due to local errors inherent in the *MolProbity* algorithm inferred by the developers of the software (Chen et al., 2010) because the three quality metrics used in the structure assessment in the present study were reported to be positively and strongly correlated (Shao et al., 2017).

Although percent identity of the methionine and arginine variants of the GnRH receptor in RS goats is high (99.7%), the two variants differ in both secondary and tertiary structure due to differences in polarity of methionine and arginine capable of changing the loops and folds observed in the tertiary structure of the receptor. As a membrane protein, there is very little variation in structure of GnRHR that may have implications on the signal strength of the hormone it transmits because of varying flexibility of the protein is important for protein functions (Kufareva and Abagyan, 2012).

When the two variants of the GnRHR detected in RS goats were classified using protein subfamily domain architecture approaches of the NCBI (Marchler-Bauer et al., 2017), the receptor was again classified as being a member of the seven transmembrane receptor family. According to the constituent KEGG pathway analysis of the protein characterization technique, the receptor contributes to the GnRH signaling and neuroactive ligand-receptor interaction pathways. Domain prediction analysis using the SMART tool approach indicated there were only two confidently predicted domains; seven transmembrane regions and a single low complexity domain. The seven transmembrane regions are conserved among ruminants (goats, sheep, cattle, and buffalo) and non-ruminant (swine) farm animals. Cattle, however, have a small unclassified region (spanning amino acid 232–269) between transmembrane five and six which is absent in the other farm animals. There is a low complexity domain prediction in ruminants but not in swine (Fig. 3) and likely contributes to functions that lead to variations between the ruminant species and swine in the mode of ovulation and prolificacy.

Findings in the present study indicate that the 5′UTR and Exon one region of the *GnRHR* gene in SR and KR goats is polymorphic while the rest of the coding and regulatory regions (Exons two and three, and 3′UTR) are conserved. There are two variants of the GnRHR polypeptide due to substitution of methionine by arginine at Position 70 of the receptor peptide detected in RS goats. Although three SNPs were detected in the GnRHR gene of RS goats, only two haplotypes instead of eight were observed due to very strong LD among the three detected SNPs. The two variants of the receptor in SR goats appear to have altered secondary and tertiary structures even though there is a greater percent identity in their primary structure. Results of this study are also consistent with the GnRH receptor having seven transmembrane domains in goats, sheep, cattle and swine with an uncharacterized region between Domain five and six present only in cattle. Swine GnRHR lacks a low complexity domain present at the beginning of the receptor in ruminant species.

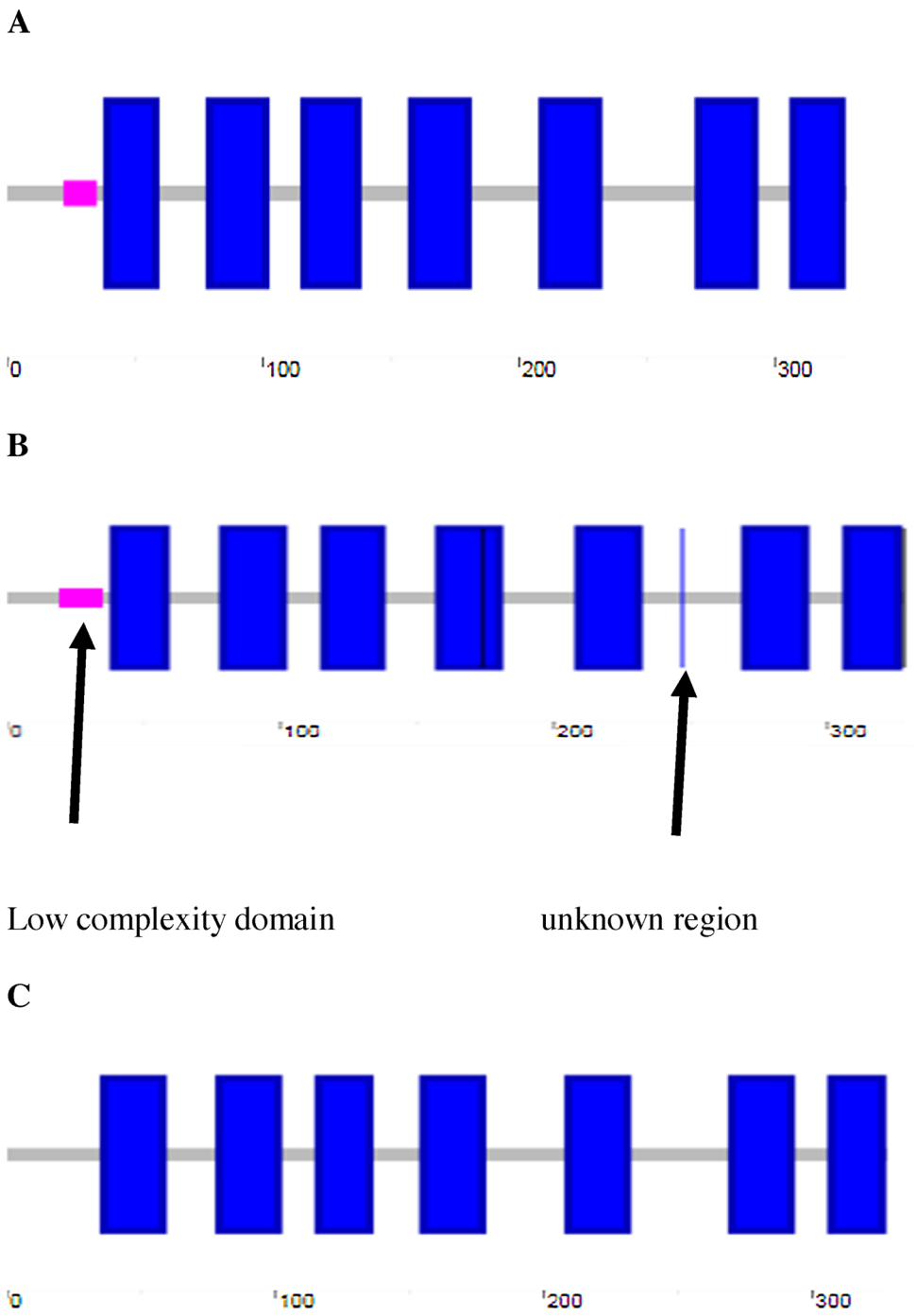

Fig. 3. Domain prediction for GnRH receptor in; (A) Goat and Sheep (B) Cattle (C) Swine.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

We sincerely appreciate National Animal Production Research Institute, Zaria and Institute of Food Security, Environmental Resources and Agricultural Research of the Federal University of Agriculture, Abeokuta, Nigeria for access to their goat herds and

making available their records during data collection. We are sincerely grateful to the anonymous reviewers of this manuscript for their insightful comments which led to improvement in the quality of the paper.

References

An, X.P., Han, D., Hou, J.X., Li, G., Wang, J.G., Yang, M.M., Song, Y.X., Zhou, G.Q., Wang, Y.N., Ling, L., Yan, Q.M., Cao, B.Y., 2009. *GnRHR* gene polymorphisms and their effects on reproductive performance in Chinese goats. Small Rumin. Res. 85, 130–134. https://doi.org/10.1016/j.smallrumres.2009.09.00.

Avet, C., Denoyelle, C., L'Hoˆte, D., Petit, F., Guigon, C.J., Cohen-Tannoudji, J., Simon, V., 2018. GnRH regulates the expression of its receptor accessory protein SET in pituitary gonadotropes. PLoS One 13 (7), e0201494. https://doi.org/10.1371/journal.pone.0201494.

Awemu, E.M., Nwankalor, L.N., Abubakar, B.Y., 1999. Environmental influences on pre weaning and reproductive performance of Red Sokoto does. Small Rumin. Res. 34, 161–165. https://doi.org/10.1016/S0921-4488(99)00058-9.

Awotunde, E.O., Bemji, M.N., Olowofeso, O., James, I.J., Ajayi, O.O., Adebambo, A.O., 2015. Mitochondrial DNA sequence analyses and phylogenetic relationships among two Nigerian goat breeds and the South African Kalahari Red. Anim. Biotechnol. 26 (3), 180–187. https://doi.org/10.1080/10495398.2014.977907.

Bemji, M.N., Awotunde, E.O., Olowofeso, O., Adebambo, A.O., 2014. Phylogenetic relationships among two Nigerian goat breeds and Kalahari Red goat of South Africa. Proceedings 10th World Congress of Genetics Applied to Livestock Production 17–22 Paper No. 870.

Bemji, M.N., Isa, A.M., Ibeagha-Awemu, E.M., Wheto, M., 2018. Polymorphisms of caprine GnRHR gene and their association with litter size in West African Dwarf goats. Mol. Biol. Rep. 45 (1), 63–69. https://doi.org/10.1007/s11033-017-4141-0.

Chen, V.B., Arendall 3rd, W.B., Headd, J.J., Keedy, D.A., Immormino, R.M., Kapral, G.J., Murray, L.W., Richardson, J.S., Richardson, D.C., 2010. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr. D Biol. Crystallogr. 66, 12–21. https://doi.org/10.1107/S0907444909042073.

Derecka, K., Ahmad, S., Hodgman, T.C., Hastings, N., Royal, M.D., Woolliams, J.A., Flint, A.P.F., 2010. Sequence variants in the bovine gonadotropin releasing hormone receptor gene and their associations with fertility. Anim. Genet. 41, 329–331. https://doi.org/10.1111/j.1365-2052.2009.01996.x.

Devendra, C., 1999. Goats: challenges for increased productivity and improved livelihoods. Outlook Agr. 28 (4), 215–226. https://doi.org/10.1177/003072709902800404.

Devendra, C., 2010. Concluding synthesis and the future for sustainable goat production. Small Rumin. Res. 89, 125–130. https://doi.org/10.1016/j.smallrumres.2009.12.034.

FAO, 1991. Small Ruminant Production and Small Ruminant Genetic Resources in the Tropics. Anim. Prod. Health Paper 88. Rome Italy. www.fao.org/3/t0376e/t0376e00.htm.

FAOSTAT, 2016. Food and Agricultural Organization Statistical Database. (Accessed 03.09. 2018). http://faostat3.fao.org.

Goddard, M.E., Hayes, B., McPartlan, H., Chamberlain, A.J., 2006. Can the same genetic markers be used in multiple breeds? Proceedings of the 8th World Congress on Genetics Applied to Livestock Production 13–18. pp. 22-16. http://www.wcgalp8.org.br.

Isa, A.M., Bemji, M.N., Wheto, M., Williams, T.J., Ibeagha-Awemu,, E.M., 2017. Mutations in inhibin alpha gene and their association with litter size in Kalahari Red and Nigerian goats. Livest. Sci. 203, 106–109. https://doi.org/10.1016/j.livsci.2017.07.012.

Kotze, A., Swart, H., Grobler, J.P., Nemaangani, A., 2004. A Genetic profile of Kalahari Red goat breed from South Africa. South Afr. J. Anim. Sci. 34, 10–12.

Kufareva, I., Abagyan, R., 2012. Methods of protein structure comparison. Methods Mol. Biol. 857, 231–257. https://doi.org/10.1007/978-1-61779-588-6_10.

Letunic, I., Bork, P., 2017. 20 years of the SMART protein domain annotation resource. Nucleic Acids Res. 46 (4), 493–496. https://doi.org/10.1093/nar/gkx922.

Li, G., Wu, H., Fu, M., Zhou, Z., 2011. Novel single nucleotide polymorphisms of *GnRHR* gene and their association with litter size in goats. Archiv Tierzucht Dummerst. 54 (6), 618–624. https://doi.org/10.5194/aab-54-618-2011.

Marai, I.F.M., Abou-Fandoud, E.I., Daader, A.H., Abu-Ella, A.A., 2002. Reproductive doe traits of Nubian (Zairabi) goats in Egypt. Small Rumin. Res. 46, 201–205 https://doi.org /10.1016/S0921-4488(02)00195-5.

Marchler-Bauer, A., Bo, Y., Han, L., He, J., Lanczycki, C.J., Lu, S., Chitsaz, F., Derbyshire, M.K., Geer, R.C., Gonzales, N.R., Gwadz, M., Hurwitz, D.I., Lu, F., Marchler, G.H., Song, J.S., Thanki, N., Wang, Z., Yamashita, R.A., Zhang, D., Zheng, C., Geer, L.Y., Bryant, S.H., 2017. CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. Nucleic Acids Res. 45, D200–D203. https://doi.org/10.1093/nar/gkw1129.

Mdladla, K., Dzomba, E.F., Huson, H.J., Muchadeyi, F.C., 2016. Population genomic structure and linkage disequilibrium analysis of South African goat breeds using genome-wide SNP data. Anim. Genet. 47 (4), 471–482. https://doi.org/10.1111/age.12442.

Mdladla, K., Dzomba, E.F., Muchadeyi, F.C., 2017. Characterization of the village goat production systems in the rural communities of the Eastern Cape, KwaZulu-Natal, Limpopo and North West Provinces of South Africa. Trop. Anim. Health Prod. 49 (3), 515–527. https://doi.org/10.1007/s11250-017-1223-x.

Mellado, M., Cesa, A., Jesus, R., Maria, A., De Santiago, M., Alvaro, R., Juan, R.L., Francisco, G.V., 2011. Relationship between litter birth weight and litter size of five goat genotypes. Anim. Prod. Sci. 51, 144–149 https://doi.org /10.1071/AN10112_ER.

Oderinwale, O.A., Oluwatosin, B.O., Sowande, O.S., Bemji, M.N., Amosu, S.D., Sanusi, G.O., 2017. Concentrate supplementations of grazing pregnant Kalahari Red goats: effects on pregnancy variables, reproductive performance, birth types and weight of kids. Trop. Anim. Health Prod. 49 (6), 1125–1133. https://doi.org/10.1007/s11250-017-1303-y.

Pieters, A., Marle-Koster, E.V., Visser, C., Kotze, D., 2009. South African developed meat goat: a forgotten animal genetic resource? Anim. Genet. Res. Info. 44, 33–43. https://doi.org/10.1017/S1014233900002844.

Rost, B., Yachdav, G., Liu, J., 2004. The PredictProtein server. Nucleic Acids Res. 32 (Suppl_2), W321–W326. https://doi.org/10.1093/nar/gkh377.

Shao, C., Yang, H., Westbrook, J.D., Young, J.Y., Zardecki, C., Burley, S.K., 2017. Multivariate analyses of quality metrics for crystal structures in the PDB archive. Structure 25 (3), 458–468. https://doi.org/10.1016/j.str.2017.01.013.

Sinwell, J.P., Schaid, D.J., 2005. *haplo.stat*: Statistical Analysis of Haplotypes With Traits and Covariates When Linkage Phase Is Ambiguous. R Package Version.

Susilorini, T.E., Kuswati, Maylinda, S., 2017. The effects of non-genetic factors on the birth weight, litter size and pre-weaning survive ability of Etawah cross-breed goats in the breeding village center in Ampelgading District. Res. J. Life Sci. Bioinform. Pharm. Chem. Sci. 4 (3), 184–189. https://doi.org/10.21776/ub.rjls.2017.004.03.4.

Warnes, G., Leisch, F., 2005. Genetics: Population Genetics R Package Version 1.2.0.

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F.T., de Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T., 2018. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 46, 296–303. https://doi.org/10.1093/nar/gky427.

Yang, W., Tang, K., Zhang, C., Xu, D., Wen, Q., Yang, L., 2011. Polymorphism of the *GnRHR* gene and its association with litter size in Boer goats. South Afr. J. Anim. Sci. 41 (4), 398–402. https://doi.org/10.4314/sajas.v41i4.10.