Received: 18 December 2021 | Accepted: 12 May 2022

DOI: 10.1111/rda.14159

SHORT COMMUNICATION

Reproduction in Domestic Animals WILEY

Localization and in silico-based functional analysis of miR-202 in bull testis

Bushra T. Mohammed[1,2] | F. Xavier Donadeu[2]

[1]Department of Pathology and Microbiology, College of Veterinary Medicine, University of Duhok, Duhok City, Iraq

[2]The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Roslin, UK

Correspondence
Bushra T. Mohammed, Department of Pathology and Microbiology, College of Veterinary Medicine, University of Duhok, Duhok City, Iraq.
Email: bushrat.mohammed@uod.ac; mzgeeni@yahoo.com

Funding information
BTM was funded by College of Veterinary Medicine, University of Duhok, Kurdistan Region, Iraq. The Roslin Institutes receives funding from The Biotechnology and Biological Sciences Research Council through an Institute Strategic Program Grant.

Abstract

Bull fertility is pivotal to the prosperity of the cattle industry worldwide. miR-202 has been shown to be gonad specific and to have key roles in gonad function in different species. To further understand the involvement of miR-202 in bull reproduction, this study aimed to establish its localization in bovine testicular tissue and to identify putative biological functions using bioinformatics approaches. We assessed the miR-202 expression in paraffin-embedded tissue samples collected form an abattoir using in situ hybridization. miR-202 was present in Sertoli cells and in germ cells at different stages of development. Using available databases, a total of 466 predicted gene targets of miR-202 were identified. Functional annotation revealed that miR-202 target genes were mainly associated with protein modification and phosphorylation processes as well as longevity regulating pathway. Moreover, genes in the longevity regulating pathway mapped to PI3K/Akt/mTOR pathway which is involved in promoting proliferation of testicular cells and spermatogenesis. These findings suggest that miR-202 plays important roles in regulating proliferation and viability of testicular cells including somatic and germ cells.

KEYWORDS
bull, in situ hybridization, miR-202, testicular cells

1 | INTRODUCTION

Semen quality is an important determinant of reproductive success in cattle herds, whether AI or natural service are used. (Barth, 2018) Reduced bull fertility is a major cause of poor reproductive performance of herds worldwide. Studies indicate that 20%–40% of bulls are subfertile. (Khatun et al., 2013; Kastelic, 2013) Apart from economic implications, bull subfertilty leads to reduced animal welfare through an increased need for breeding and culling of repeat breeder cows. (Kastelic, 2013) Thus, a good understanding of the molecular mechanisms underlying testes function and spermatogenesis, and how these determine the production of high-quality sperm, is essential to achieve high levels of productivity in the cattle industry. (Taylor et al., 2018; Rexroad et al., 2019)

Spermatogenesis is a highly organized process within the seminiferous tubules in the testes. Sertoli cells and Leydig cells play a pivotal role in the initiation and maintenance of sperm development as well as in regulation of male hormone production. (Phillips et al., 2010) Spermatogenesis involves three main events, spermatocytogenesis, meiosis and spermiogenesis. During spermatocytogenesis, germ cells differentiate and give rise to spermatogonial stem cells, which actively undergo mitotic division to generate two set of diploid primary spermatocytes. These then undergo meiosis I to produce two haploid secondary spermatocytes. Each haploid secondary

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2022 The Authors. *Reproduction in Domestic Animals* published by Wiley-VCH GmbH.

spermatocyte differentiates into two haploid spermatids via meiotic cell division II, resulting in production of four haploid spermatids. During spermiogenesis, spermatids differentiate and become spermatozoa which migrate into the lumen of seminiferous tubules in a process called spermiation. (Staub & Johnson, 2018; Valli et al., 2015)

Testicular development and function are tightly regulated by microRNAs (miRNAs), which act by modulating the expression of a wide range of protein-coding genes involved in cell differentiation processes within the male reproductive system.(Barbu et al., 2021; Fernández-Pérez et al., 2018; Papaioannou, 2010) Mammalian testes express a large set of miRNAs including several tissue-specific sequences.(Gao et al., 2020; Rakoczy et al., 2013; Yang et al., 2013) Expression of the gonad-specific miRNA, miR-202, is highly conserved across species including mouse, human, bovine, boars, chicken, Zebra fish and salamander.(Wainwright et al., 2013; Dabaja et al., 2015; Bannister et al., 2011; Chen et al., 2017; Presslauer et al., 2017; Sontakke et al., 2014; Revay et al., 2015) miR-202 is expressed in the testicular somatic cell compartment(Chen et al., 2017) as well as in germ cells at different stages of development.(Chen et al., 2017; Jia et al., 2015) Studies showed that miR-202 was localized in Sertoli cells of mouse testes and that it mediated some of the effects of the testis-determining factor SOX9, involved in early gonad development.(Wainwright et al., 2013) A recent study showed that miR-202 was robustly expressed in Sertoli cells of fertile men but was absent in sterile men.(Dabaja et al., 2015) Moreover, Chen et al showed that miR-202 was expressed at high levels in mouse spermatogonial stem cells, and that CRISPR-Cas9-mediated miR-202 knockout resulted in premature cell differentiation with loss of stemness, as well as increased mitosis and apoptosis.(Chen et al., 2017) As regards to bovine, a recent study showed that miR-202 was highly enriched in sperm, and that sperm-borne miRNA regulates the first cleavage in bovine embryos.(Wang et al., 2021) The aim of this study was to determine the expression pattern of miR-202 in the bull testes, as well as to identify broader potential roles of this miRNA by using in silico target prediction.

2 | METHODS

2.1 | In situ hybridization

Testes from three healthy, 9-month-old bulls were obtained at an abattoir and transported in phosphate-buffered saline (PBS) at 4°C within an hour of collection. Once in the laboratory, the testes were dissected into small pieces and fixed in 4% PFA treated with diethylpyrocarbonate (DEPC) (Sigma-Aldrich,). After overnight fixation, tissue sections were cut at 6 μm and denatured with 5 μg/ml of proteinase K in 75 ml PBS then fixed in 4% PFA for 10 min and rinsed with 0.2% Glycine in PBS. The tissues were incubated with freshly prepared imidazole buffer then slides were placed in a humid chamber and freshly prepared 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was added to each slide for 1 hr at RT followed by 2 hr of pre-hybridization with 50% formamide and 5x SSC buffer at 25°C. Sections were then incubated overnight with Double Digoxigenin labelled LNA modified oligonucleotide probes (Exiqon,) against either bta-miR-202 (100% homologous sequence to human miR-202-5p; 80 nM), U6snRNA (positive control, 3 nM) or a scrambled sequence (negative control, 80 nM) in hybridization buffer. After the application of probe, slides were covered with gel bond film and heated to 60°C for 5 min, then placed in humidifying chamber at 50°C. After overnight incubation, slides were sequentially washed with 4x, 2x and 0.2x SSC post-hybridization buffer for 10 min at 50°C to avoid unspecific binding and then rinsed with 1x Tris-buffered saline (TBS). Slides were then incubated with blocking solution for 1 hr at RT. Anti-digoxigenin antibody (1:200) was added to slides for 2 hr at RT followed by colour development with NBT/BCIP at 4°C for up to 16 hr. The signal was analysed with a light microscope. Independent analyses were performed using three different testicular sections from each animal.

2.2 | Target prediction and gene enrichment analysis

miRNA target prediction was performed using three different algorithms, miRMap (v1.1), TargetScan 7.2 and miRWalk (v3.0). (Agarwal et al., 2015; Vejnar & Zdobnov, 2012) ClueGO (v2.5.7) plus Cluepedia (v1.5.7) software were used to identify Gene Ontology (GO) terms and Kyoto Encyclopedia of Gene and Genome (KEGG) pathways. ClueGO enables analysis of gene sets from organisms including bovine and considers many identifier types subtracted from a variety of sources including NCBI, UniPrtKB and Ensembl. (Bindea et al., 2009) Two-sided hypergeometric tests were used for enrichment analyses, Benjamini–Hochberg correction was used for p value correction and Kappa coefficient of 0.4 was used to indicate the resemblance of GO terms for associated genes. The resulting GO terms with $p < .01$ and KEGG pathways with $p < .05$ were considered significant. Furthermore, the results were visualized using Cytoscape (v3.8.2).

3 | RESULTS

3.1 | Localization of miR-202 in bull testes

Testes from three bulls were examined using ISH to establish the cellular location of miR-202. Different testicular cell types were identified based on position, size and shape. miR-202 was detected in both Sertoli and spermatogenic cells (Figure 1a,b). Staining of miR-202 was most pronounced in the cytoplasm of Sertoli cells, whereas staining in spermatogenic cells was localized mostly to the nucleus (Figure 1c). In germ cells, miR-202 signal was particularly strong in spermatogonia and primary spermatocytes located near the basal compartment of the seminiferous tubule, as compared to secondary spermatocyte and spermatids (Figure 1b). These results suggest changes in miR-202 expression at different developmental stages during spermatogenesis. No miR-202 signal was detected in blood vessels and interstitial cells (Figure 1c). In addition, whereas the positive control, RnU6, displayed a strong nuclear localization

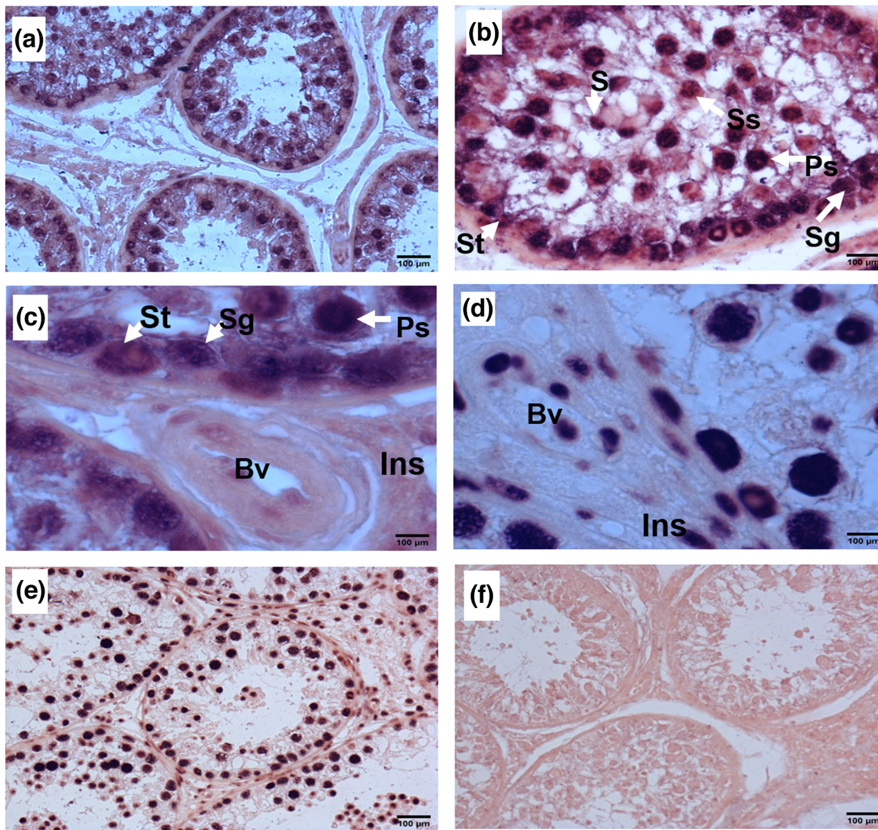

FIGURE 1 Representative images of in situ hybridization detection of miR-202 in sections of bull testes ($n = 3$ animals). Sertoli cells (St), spermatogonia (sg), primary spermatocytes (Ps), secondary spermatocytes (ss) and spermatids (S) are indicated by white arrows. Blood vessels (Bv) and interstitial cells (ins) are also shown. Sections hybridized with probes against miR-202 (a, b, c), U6B (d, e) and scrambled sequence controls (f) are shown. Original magnification 200x,400x and 1,000x. Scale bar, 100 μm

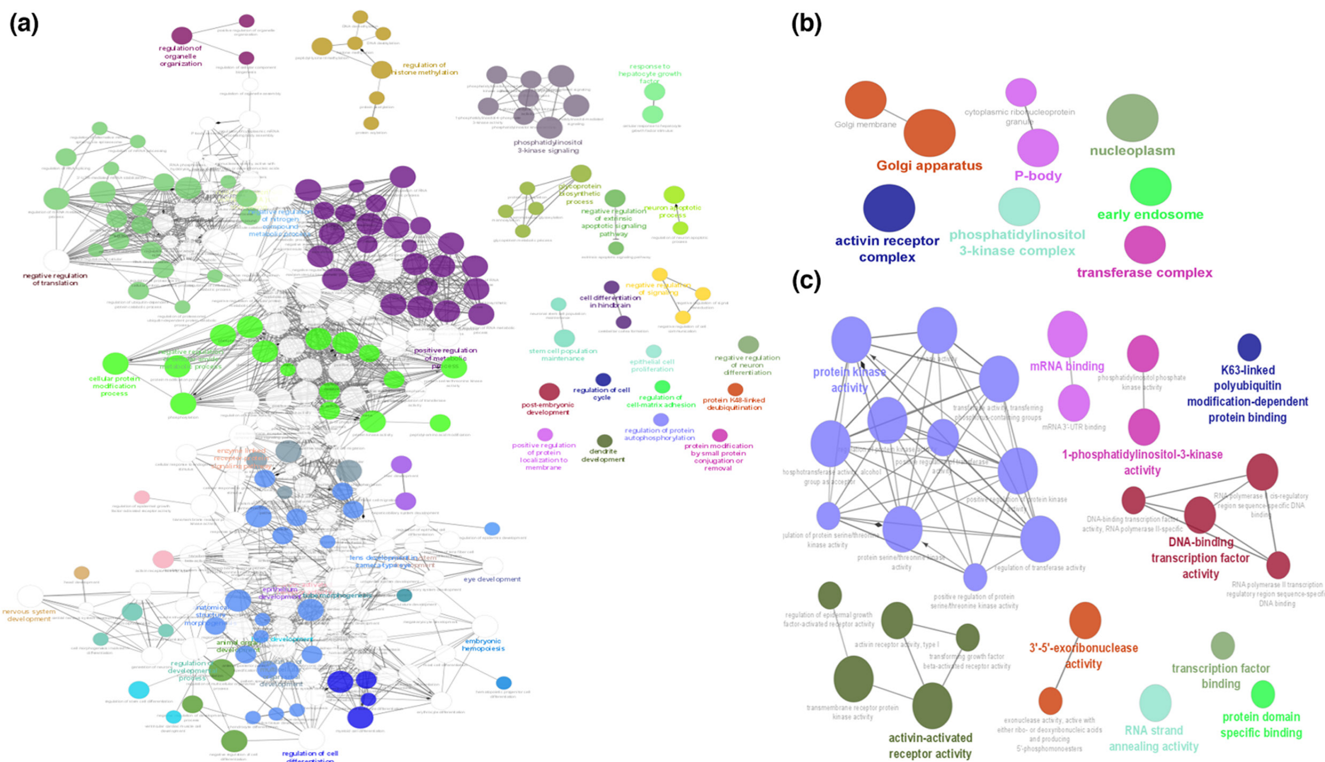

FIGURE 2 Functional GO terms enriched for target genes of miR-202 identified using ClueGO and CluePedia ($p \leq .01$). Different functional groups are represented by colours. Each node represents a GO term, and node size represents level of significance for term enrichment. Terms are connected based on shared genes. Enriched biological processes (a), cellular components (b) and molecular functions (c) of miR-202 targets

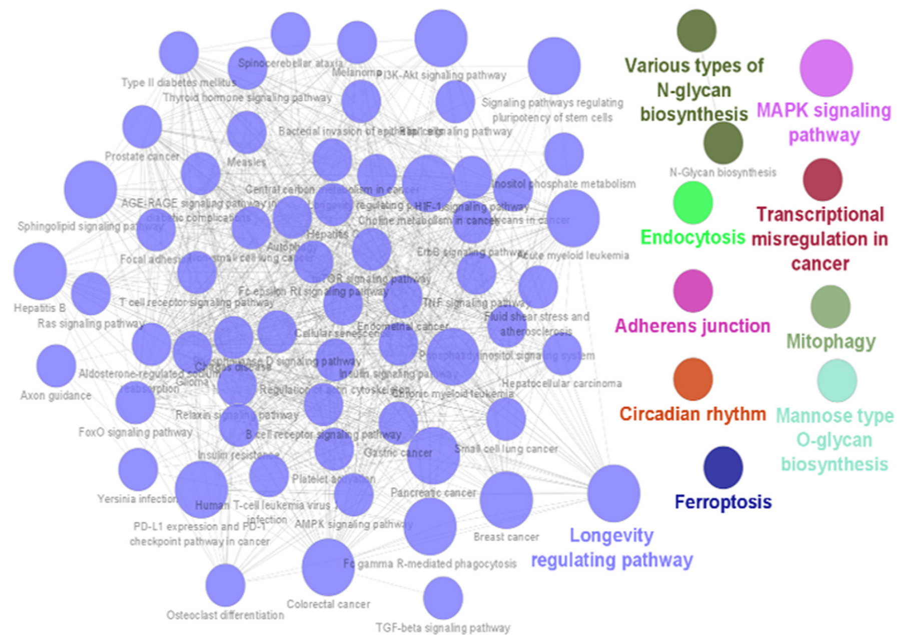

FIGURE 3 Functional KEGG pathways enriched for target genes of miR-202 identified using ClueGO and CluePedia ($p \leq .05$). Different functional groups are represented by colours. Each node represents a GO term, and node size represents level of significance for term enrichment. Pathways are connected based on shared genes

in all sections (Figure.1d,e), the negative control showed no signal (Figure 1f).

3.2 | miRNA target prediction and enrichment functional analysis

In order to gain insight into the roles of bta-miR-202 in bovine testis, miRNA target prediction tools were used. A total of 466 target genes were identified using miRMap, TargetScan 7.0 and miRWalk databases (Table S1). Gene ontology (GO) analysis using all identified targets showed that target genes were significantly enriched for several biological processes (BP), cellular components (CC) and molecular functions (MF) (Figure 2a–c). The most significant GO terms were protein modification process (GO:0036211, $p < 1.09E-08$), nucleoplasm (GO:0005654, $p < 5.04E-07$) and phosphotransferase activity, alcohol group as acceptor (GO:0016773, $p < 4.49E-05$), in BP, CC and MF categories respectively. Kyoto Encyclopedia of Genes and Genomics (KEGG) pathway enrichment showed a subset of significantly enriched pathways for bta-miR-202 target genes, the most significant of which was the longevity regulating pathway (KEGG:04211, $p < .0043$) (Figure 3). This pathway includes genes such as CREB1, EIF4E, PIK3CA, PIK3CB, PIK3R1, PRKAA1, RB1CC1 and RPS6KB1 which are involved in PI3K/Akt/mTOR signalling.

4 | DISCUSSION

The primary aim of the present study was to characterize the expression pattern of miR-202-5p in the bovine testis. The results indicate cell-type-dependent expression of miR-202 during spermatogenesis. miR-202 was localized in the cytosol of Sertoli cells, consistent with its involvement in post-transcriptional target gene regulation in those cells. In humans, miR-202-5p was highly enriched in Sertoli cells, its expression differed between fertile and sterile men, and a role was suggested in mediating the interaction between somatic and germ cells during spermatogenesis. (Dabaja et al., 2015) A different study reported that miR-202-5p was highly expressed in Sertoli cells in mouse and chicken embryonic gonads. (Bannister et al., 2011; Wainwright et al., 2013) We also showed that miR-202 was present in germ cells at all stages of development. In agreement with this finding, miR-202-5p was expressed throughout spermatogenesis in Medaka, demonstrating its involvement in male gamete development and differentiation. (Qiu et al., 2018) Another study demonstrated that miR-202-5p was significantly enriched in spermatozoa and developing male germ cells at different stages in zebrafish, (Jia et al., 2015) all together suggesting a conserved role in male germ cell function.

Our analyses identified 'protein-modification process' as a top predicted function of miR-202 target genes. In addition, the target genes of bta-miR-202 were significantly enriched in several molecular functions (GO terms) related to phosphotransferase and kinase activity which have a role in phosphorylation of many signalling proteins that are involved in regulation of mitochondrial activity, motility and apoptosis of testicular cells. (Gervasi & Visconti, 2017; Jankovičová et al., 2018; Silva et al., 2015) Furthermore, the top significant KEGG term enriched for miR-202 target genes was longevity-regulating pathway. The genes in the longevity regulatory pathway, including CREB1, EIF4E, PIK3CA, PIK3CB, PIK3R1, PRKAA1, RB1CC1 and RPS6KB1 are associated with several signalling pathways including PI3K/Akt/mTOR signalling. (Salas-Pérez et al., 2019) PI3K/Akt /mTOR pathway is implicated in many cellular processes such as cell growth, survival, metabolism and autophagy. (Deng et al., 2021) The proliferation of Sertoli cells, which play a key role during spermatogenesis by facilitating adjacent germ cells with access to nutrients and growth factors, (Deng et al., 2021; Kimmins et al., 2004) is stimulated by FSH signalling through PI3k/Akt/mTOR.

(Riera et al., 2012) Moreover, hyperactivation of phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR signalling is linked to different forms of cancer including testicular cancer. (Xu et al., 2017)

5 | CONCLUSION

Our findings demonstrate that miR-202 is expressed in Sertoli cells and, at varying levels, in different developmental stages of germ cells in bull testes. We also provide evidence suggesting the involvement of miR-202 in multiple protein regulation, metabolism and longevity regulating pathways in the testes. Overall, these findings are consistent with critical roles of miR-202 in regulating maturation and viability of testicular somatic and germ cells in bull testes.

AUTHOR CONTRIBUTIONS

BTM and FXD contributed to the study conception and design. Methods and data analysis were performed by BTM. BTM and FXD drafted the manuscript and all authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID

Bushra T. Mohammed https://orcid.org/0000-0002-0621-1103

REFERENCES

Agarwal, V., Bell, G. W., Nam, J. W., Bartel, D. P., (2015). Predicting effective microRNA target sites in mammalian mRNAs. *Elife [Internet]*, *4*:e05005. Available from: https://elifesciences.org/articles/05005

Bannister, S. C., Smith, C. A., Roeszler, K. N., Doran, T. J., Sinclair, A. H., & Tizard, M. L. V. (2011). Manipulation of estrogen synthesis alters MIR202* expression in embryonic chicken Gonads1. *Biology of Reproduction [Internet]*, *85*(1), 22–30. Available from: https://doi.org/10.1095/biolreprod.110.088476

Barbu, M. G., Thompson, D. C., Suciu, N., Voinea, S. C., Cretoiu, D., & Predescu, D. V. (2021). The roles of MicroRNAs in male infertility. *International Journal of Molecular Sciences*, *22*(6), 2910.

Barth, A. D., Review: The use of bull breeding soundness evaluation to identify subfertile and infertile bulls. *Animal [Internet]*. 2018;*12*:s158–s164. Available from: https://www.sciencedirect.com/science/article/pii/S1751731118000538

Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., … Galon, J. (2009). ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics*, *25*(8), 1091–1093. Available from: https://doi.org/10.1093/bioinformatics/btp101

Chen, J., Cai, T., Zheng, C., Lin, X., Wang, G., Liao, S., Wang, X., … Han, C. (2017). MicroRNA-202 maintains spermatogonial stem cells by inhibiting cell cycle regulators and RNA binding proteins. *Nucleic Acids Research*, *45*(7), 4142–4157. Available from: https://doi.org/10.1093/nar/gkw1287

Chen, R., Du, J., Ma, L., Wang, L. Q., Xie, S. S., Yang, C. M., … Dong, W. Z. (2017). Comparative microRNAome analysis of the testis and ovary of the Chinese giant salamander. *Reproduction*, *154*(3), 169–179. Available from: https://doi.org/10.1530/REP-17-0109

Dabaja, A. A., Mielnik, A., Robinson, B. D., Wosnitzer, M. S., Schlegel, P. N., Paduch, D. A., (2015). Possible germ cell-Sertoli cell interactions are critical for establishing appropriate expression levels for the Sertoli cell-specific MicroRNA, miR-202-5p, in human testis. *Basic and Clinical Andrology [Internet]*, *25*(1):2. Available from: https://doi.org/10.1186/s12610-015-0018-z

Deng, C. Y., Lv, M., Luo, B. H., Zhao, S. Z., Mo, Z. C., Xie, Y. J., (2021). The role of the PI3K/AKT/mTOR Signalling pathway in male reproduction [internet]. *Current Molecular Medicine*, *21*539–548. Available from: http://www.eurekaselect.com/article/112031

Fernández-Pérez, D., Brieño-Enríquez, M. A., Isoler-Alcaraz, J., Larriba, E., & Del Mazo, J. (2018). MicroRNA dynamics at the onset of primordial germ and somatic cell sex differentiation during mouse embryonic gonad development. *RNA*, *24*(3), 287–303.

Gao, Y., Wu, F., Ren, Y., Zhou, Z., Chen, N., Huang, Y., … Dang, R. (2020). MiRNAs expression profiling of bovine (Bos taurus) testes and effect of bta-miR-146b on proliferation and apoptosis in bovine male germline stem cells. *International Journal of Molecular Sciences*, *21*(11), 3846. Available from: https://doi.org/10.3390/ijms21113846

Gervasi, M. G., Visconti, P. E., (2017). Molecular changes and signaling events occurring in spermatozoa during epididymal maturation. *Andrology [Internet]*, *5*(2):204–218. Available from: https://pubmed.ncbi.nlm.nih.gov/28297559

Jankovičová, J., Michalková, K., Sečová, P., Horovská, Ľ., Maňásková-Postlerová, P., Antalíková, J., (2018). Evaluation of protein phosphorylation in bull sperm during their maturation in the epididymis. *Cell and Tissue Research [Internet]*, *371*(2):365–373. Available from: https://doi.org/10.1007/s00441-017-2705-x

Jia, K. T., Zhang, J., Jia, P., Zeng, L., Jin, Y., Yuan, Y., … Yi, M. (2015). Identification of MicroRNAs in zebrafish spermatozoa. *Zebrafish*, *12*(6), 387–397. Available from: https://doi.org/10.1089/zeb.2015.1115

Kastelic, J. P., (2013). Male involvement in fertility and factors affecting semen quality in bulls. *Animal Frontiers [Internet]*, *3*(4):20–25. Available from: https://academic.oup.com/af/article/3/4/20/4638651

Khatun, M., Kaur, S., & Kanchan, C. S. (2013). Subfertility problems leading to disposal of breeding bulls. *Asian-Australasian Journal of Animal Sciences*, *26*(3), 303.

Kimmins, S., Kotaja, N., Davidson, I., Sassone-Corsi, P., (2004). Testis-specific transcription mechanisms promoting male germ-cell differentiation. *Reproduction [Internet]*, *128*(1):5–12. Available from: https://rep.bioscientifica.com/view/journals/rep/128/1/1280005.xml

Papaioannou, M. D., & Nef, S. (2010). microRNAs in the testis: Building up male fertility. *Journal of Andrology [Internet]*, *31*(1), 26–33. Available from: https://doi.org/10.2164/jandrol.109.008128

Phillips, B. T., Gassei, K., & Orwig, K. E. (2010). Spermatogonial stem cell regulation and spermatogenesis. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *365*(1546), 1663–1678. Available from: https://doi.org/10.1098/rstb.2010.0026

Presslauer, C., Tilahun Bizuayehu, T., Kopp, M., Fernandes, J. M., & Babiak, I. (2017). Dynamics of miRNA transcriptome during gonadal development of zebrafish. *Scientific Reports*, *7*, 43850. Available from: https://doi.org/10.1038/srep43850

Qiu, W., Zhu, Y., Wu, Y., Yuan, C., Chen, K., Li, M., (2018). Identification and expression analysis of microRNAs in medaka gonads. *Gene [Internet]*, *646*:210–216. Available from: https://www.sciencedirect.com/science/article/pii/S0378111917311319

Rakoczy, J., Fernandez-Valverde, S. L., Glazov, E. A., Wainwright, E. N., Sato, T., Takada, S., ... Wilhelm, D. (2013). MicroRNAs-140-5p/140-3p modulate Leydig cell numbers in the developing mouse testis1. *Biology of Reproduction*, *88*(6), 143. Available from: https://doi.org/10.1095/biolreprod.113.107607

Revay, T., Quach, A. T., Maignel, L., Sullivan, B., King, W. A., (2015). Copy number variations in high and low fertility breeding boars. *BMC Genomics [internet]*, *16*(1):280. Available from: https://doi.org/10.1186/s12864-015-1473-9

Rexroad, C., Vallet, J., Matukumalli, L. K., Reecy, J., Bickhart, D., Blackburn, H., ... Wells, K. (2019). Genome to phenome: Improving animal health, production, and well-being – A new USDA blueprint for animal genome research 2018–2027. *Frontiers in Genetics*, *10*, 327. Available from: https://doi.org/10.3389/fgene.2019.00327

Riera, M. F., Regueira, M., Galardo, M. N., Pellizzari, E. H., Meroni, S. B., & Cigorraga, S. B. (2012). Signal transduction pathways in FSH regulation of rat Sertoli cell proliferation. *American Journal of Physiology - Endocrinology and Metabolism*, *302*(8), E914–E923.

Salas-Pérez, F., Ramos-Lopez, O., Mansego, M. L., Milagro, F. I., Santos, J. L., Riezu-Boj, J. I., & Martínez, J. A. (2019). DNA methylation in genes of longevity-regulating pathways: association with obesity and metabolic complications. *Aging*, *11*(6), 1874–1899. Available from: https://doi.org/10.18632/aging.101882

Silva, J. V., Freitas, M. J., Correia, B. R., Korrodi-Gregório, L., Patrício, A., Pelech, S., & Fardilha, M. (2015). Profiling signaling proteins in human spermatozoa: Biomarker identification for sperm quality evaluation. *Fertility and Sterility*, *104*(4), 845–856.e8. Available from: https://doi.org/10.1016/j.fertnstert.2015.06.039

Sontakke, S. D., Mohammed, B. T., McNeilly, A. S., & Donadeu, F. X. (2014). Characterization of microRNAs differentially expressed during bovine follicle development. *Reproduction*, *148*(3), 271–283.

Staub, C., Johnson, L., (2018). Review: Spermatogenesis in the bull. *Animal [Internet]*, *12*:s27–35. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1751731118000435

Taylor, J. F., Schnabel, R. D., & Sutovsky, P. (2018). Identification of genomic variants causing sperm abnormalities and reduced male fertility. *Animal Reproduction Science*, *194*, 57–62. Available from: https://doi.org/10.1016/j.anireprosci.2018.02.007

Valli, H., Phillips, B. T., Orwig, K. E., Gassei, K., Nagano, M. C., (2015). *Chapter 15 - Spermatogonial Stem Cells and Spermatogenesis*. In: Plant, T. M., Zeleznik, A. J. B. T. K. and NP of R (Fourth E, Editors. Academic Press. Pp. 595–635. Available from: http://www.sciencedirect.com/science/article/pii/B9780123971753000156

Vejnar, C. E., & Zdobnov, E. M. (2012). miRmap: Comprehensive prediction of microRNA target repression strength. *Nucleic Acids Res [Internet]*, *40*(22), 11673–11683. Available from: https://doi.org/10.1093/nar/gks901

Wainwright, E. N., Jorgensen, J. S., Kim, Y., Truong, V., Bagheri-Fam, S., Davidson, T., ... Wilhelm, D. (2013). SOX9 regulates MicroRNA miR-202-5p/3p expression during mouse testis differentiation1. *Biology of Reproduction*, *89*(2), 34. Available from: https://doi.org/10.1095/biolreprod.113.110155

Wang, M., Du, Y., Gao, S., Wang, Z., Qu, P., Gao, Y. ... Wang, Y. (2021). Sperm-borne miR-202 targets SEPT7 and regulates first cleavage of bovine embryos via cytoskeletal remodeling. *Development*, *148*(5), dev189670. Available from: https://doi.org/10.1242/dev.189670

Xu, H., Feng, Y., Jia, Z., Yang, J., Lu, X., Li, J., ... Chen, J. (2017). AXIN1 protects against testicular germ cell tumors via the PI3K/AKT/mTOR signaling pathway. *Oncology Letters*, *14*(1), 981–986. Available from: https://doi.org/10.3892/ol.2017.6214

Yang, Q., Hua, J., Wang, L., Xu, B., Zhang, H., Ye, N.,... Shi, Q. (2013). MicroRNA and piRNA profiles in normal human testis detected by next generation sequencing. *PLoS One*, *8*(6), e66809. Available from: https://doi.org/10.1371/journal.pone.0066809

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Mohammed, B. T., & Donadeu, F. X. (2022). Localization and in silico-based functional analysis of miR-202 in bull testis. *Reproduction in Domestic Animals*, *57*, 1082–1087. https://doi.org/10.1111/rda.14159