Short Communication

Identifying sequence variation in cation channel sperm associated genes in Cape mountain zebra (Equus zebra zebra)

R.M. Smith1,2, A. Kotzé1,2, J.P. Grobler2 & D.L. Dalton1,3#
1National Zoological Garden, South African National Biodiversity Institute, P.O. Box 754, Pretoria, 0001, South Africa
2Department of Genetics, University of the Free State, P.O. Box 339, Bloemfontein, 9300 South Africa
3Department of Zoology, University of Venda, University Road, Thohoyandou 0950, Republic of South Africa

(Submitted 18 December 2019; Accepted 25 November 2020; Published 7 December 2020)

Abstract

The Cape mountain zebra (Equus zebra zebra) has recovered from near extinction over more than eight decades. While their numbers have increased, populations remain isolated with low genetic diversity. With more than 75 new populations being founded and more than 4800 extant animals, conservation management strategies are being implemented to mitigate risk of losses in genetic diversity and reproductive fitness. One objective is to identify reproductive characteristics that may improve population growth. Cation channel sperm (CatSper) genes play an important role in hyperactivation of sperm during fertilization. Mutations in these genes lead to reduced fertility and even infertility. Ten male zebras were sampled from a group that were translocated in 2016 in order to found a new population. Single nucleotide polymorphisms (SNPs) were identified in three of the CatSper genes (1-3). Lack of variation was observed in all exons, with only four SNPs being identified in the intronic regions in close proximity to exons 1, 2, 7, 8, and 9 of CatSper 1. These results may contribute to the pre-identification of males for new founder populations to ensure population growth and viability, and may be a useful tool for selection against low-producing individuals.

Keywords: endangered species, genetic variation, reproduction, single nucleotide polymorphism

Cape mountain zebra (CMZ) (Equus zebra zebra) is a sub-species of mountain zebra that is distributed only in South Africa (Novellie et al., 2002). These animals have recovered from a severe population bottleneck, which took place in the 1930s (Hrabar & Kerley, 2013), to a current estimated population size of more than 4800 (Birss et al., 2018). Because of reported low genetic diversity (Moodley & Harley, 2005), a biodiversity management plan was developed that focused on strategies and actions to strengthen overall population performance, distribution and genetic diversity to ensure fitness and resilience of the meta-population within the natural distribution range (Birss et al., 2018). Since the 1950s, the number of Cape mountain zebra has increased gradually through the founding of new populations to ensure continued population growth (Novellie et al., 2002). To date, the overall population occurs in more than 75 localities, which include 30 national and provincial parks. As many as 90% of the founding CMZ were sourced from Mountain Zebra National Park and 50% of all populations were founded with fewer than the recommended number of founding animals (Moodley & Harley, 2005).

Low reproductive success in CMZ mares (foaling rate of 32%) has been reported in Mountain Zebra National Park (Penzhorn, 1985) and in De Hoop Nature Reserve (DHNR) (Birss, 2018, pers. comm.). In addition, abnormal sperm heads because of a weak head-neck junction have been identified in a CMZ stallion (Penzhorn & Van der Merwe, 1988). Whereas several genes control sperm motility, the calcium channel of sperm (CatSper) is studied most (Ren et al., 2001). The CatSper protein family consists of specialized calcium (Ca2+) channel proteins that are expressed exclusively in the sperm flagellum (Hildebrand et al., 2010) and thus are directly involved in hyperactivation of the spermatozoa and penetration ability of the zona pellucida (Stauss et al., 1995). The CatSper complex is reported to include four subunits...
(CatSper 1 - 4) and three auxiliary subunits, namely CatSperβ, CatSperδ and CatSperγ (Navarro et al., 2008; Wang et al., 2009; Chung et al., 2011). CatSper 1 - 4 are expressed in spermatozoa and are functional on the principal piece of the sperm tail (Qi et al., 2007). This action is achieved through the use of Ca²⁺ ions, which control swimming behaviour through the ion pump action in the flagellum (Armon & Eisenbach, 2011). CatSper has been identified as a necessary component for reproductive success in mice (Ren et al., 2001; Carlson et al., 2003; Qi et al., 2007), human beings (Avenarius et al., 2009; Hildebrand et al., 2010; Strünker et al., 2011; Saha et al., 2015), and horses (Lou et al., 2013). Mutations leading to infertility have been reported in all four subunits of CatSper. In CatSper 1, two insertion mutations (c.539-540insT and c.948-949insATG) were reported to lead to infertility in humans (Avenarius et al., 2009). Mutations in the CatSper 2 gene also lead to low sperm counts in humans (Zhang et al., 2009) and a copy number variation was identified that caused infertility (Luo et al., 2019). CatSper 3 and 4 mutations in mice were shown to cause infertility (Jin et al., 2007). Mutations that lie within the functional domain of CatSper 3 (c.193T>C) and CatSper 4 (c.247A>G, c.157T>C, c.992G>A) genes were identified in humans and are associated with asthenozoospermia (Visser et al., 2011). The current study was undertaken to screen the CatSper 1 - 3 genes to determine nucleotide variations in CMZ as potential DNA markers associated with improved sperm motility. The genotype of an individual may serve as a criterion when selecting animals to be translocated to ensure population growth and viability.

Ethics submissions were approved by the University of the Free State Animal Ethics Committee (UFS-AED2017/0011) and Research Ethics and Scientific Committee of the National Zoological Garden, South African National Biodiversity Institute (NZG SANBI, NZG/RES/P17/19). The Department of Agriculture, Forestry and Fisheries of South Africa granted a permit under Section 20 of the Animal Diseases Act of 1984 (Ref: 12/11/1/1/8). Samples were collected under a Threatened or Protected Species Regulations Permit (No. 07507) through the Department of Environmental Affairs of South Africa.

Blood samples from 10 male CMZ were collected from DHNR. Two males were identified as foals, based on their size, presence of fluffy coat and deciduous teeth. Another two males were designated sub-adult because of the presence of undescended testicles. Six males were identified as adult stallions.

Reference sequences from horse (Equus caballus) from Ensembl were used to design the primers, namely CatSper 1 (ENSECAG00000024405), CatSper 2 (ENSECAG00000020759) and CatSper 3 (ENSECAG00000014744). The primers were designed in flanking regions of each exon (Table 1). DNA was extracted from the whole blood using the Zymo Quick-DNA™ Universal kit (Zymo Research, Irvine, California, USA) according to the manufacturer’s instructions for biofluid and cells. Extracted DNA was stored at -20 °C until further analysis. The DNA fragments were amplified using Taq DNA polymerase Master Mix RED (AmpliQon A/S, Odense M, Denmark) in 15 µl reactions, which included forward and reverse primers (0.5 µM), 50 ng of genomic template and GC enhancer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). The reactions were run under these conditions: 95 °C for 5 min, 35 cycles of 95 °C for 30 seconds, annealing for 30 seconds, followed by elongation at 72 °C for 30 seconds with a final elongation step of 72 °C for 10 minutes. Polymerase chain reactions were carried out in a T100™ thermal cycler (Bio-Rad Laboratories Inc., Hercules, California, USA). The amplified fragments were purified with Exonuclease I (Thermo Fisher Scientific Inc.) and FastAP thermosensitive alkaline phosphatase (Thermo Fisher Scientific Inc.) in a thermal cycler at 37 °C for 15 minutes, followed by 85 °C for 15 minutes. Next, the fragments were used as a template for sequencing using the BigDye™ Terminator v3.1 cycle sequencing kit (Applied Biosystems Inc., Foster City, California, USA) according to the manufacturer’s instructions. Briefly, 1 µl of BigDye™ Terminator v3.1 ready reaction mix, 3.2 pmol of either the forward or reverse primer, and 1 x BigDye™ Terminator v1.1 & v3.1, 5X sequencing buffer, were prepared in a mastermix with 5 µl of the amplified PCR product made up with nuclease-free water to 10 µl. Sequencing was conducted in a thermocycler using these parameters: denaturation at 94 °C for 2 minutes, 40 cycles of 85 °C for 10 seconds, 53 °C for 10 seconds and 60 °C for 4 minutes. The resulting reaction was then purified using the BigDye™ XTerminator™ sequencing purification kit, as recommended by the manufacturer. The DNA products were sequenced on ABI 3130 genetic analysis (Applied Biosystems Inc.). The resulting outputs were analysed with sequencing analysis software v6.0 (Applied Biosystems Inc.).

The sequence files were inspected visually and the chromatograms were edited and assembled (forward and reverse sequences) using Geneious® v10.2.6 software (Kearse et al., 2012) and the default parameters. Low-quality sections at the ends of the sequences were trimmed manually. A multiple sequence alignment was carried out for all ten samples. Horse sequences for each of CatSper genes 1 - 3 were used as a reference. The resulting alignment was inspected visually for sequence variants such as insertions, deletions and base-pair variations. Single nucleotide polymorphisms between the horse reference sequence and zebra were not considered here.
Here, for the first time the authors report sequence analysis of CatSper 1–3 in CMZ to identify possible nucleotide variations associated with sperm motility. The sequence data covered a range of exons (Table 2), which provided complete and, in some cases, partial coverage for some of the exons. Partial fragments were obtained because of the selected primer regions or because the target regions were too long for the sequencing method.

The role of CatSper genes in sperm motility is widely reported and the products of these genes are recognized as the most important calcium channels required for fertility in mammals (Singh & Rajender, 2014). Single nucleotide polymorphisms in the CatSper genes associated with sperm motility have been identified in Vrindavani cattle (Sivakumar et al., 2017) and mice (Qi et al., 2007). Knock-outs of the CatSper genes may cause infertility in humans without affecting normal sperm production (Singh & Rajender, 2014).

This study revealed that CMZ males have exons that are highly conserved within the subspecies. A lack of SNPs in this study may be attributed to the presence of unsuitable habitat, with most CMZ grazing on only 30% of the total area of the reserve (Smith et al., 2007). The absence of SNPs in exons of CatSper genes 1–3. The absence of SNPs in this study may be attributed to the number of animals and to the low genetic diversity of the population (Moodley & Harley 2005; Kotzé et al., 2019). Since only a small proportion of animals from a single population were used here, it would be useful to compare variation between the isolated populations of CMZ. Use of additional populations will establish how well conserved these genes are within the subspecies. A lack of SNPs in this study may be attributed to the presence of unsuitable habitat, with most CMZ grazing on only 30% of the total area of the reserve (Smith et al., 2007).

Here, portions of introns were also sequenced, and the authors report four SNPs that were identified in the intronic regions of 1, 7, and 9 of CatSper 1 (Figure 1). These are G1547A, which is located 126 bp downstream from exon 2; G4675T, which is located 43 bp downstream from exon 7, and G5270A, which is located 206 bp downstream from exon 9. Studies have shown that SNPs within the introns of genes play a role in mRNA expression (Nott et al., 2003; Wang et al., 2011; Zhang et al., 2014) and determine the phenotypic expression of certain traits, such as eye colour in humans (Sturm et al., 2008). Thus, these SNPs may have a role in the expression of the CatSper 1 protein in the mid piece of the sperm tail. Additional analysis is required to determine whether the SNPs in these regions influence the expression of the CatSper 1 gene.

### Table 1 Primers used in polymerase chain reaction to amplify Cape mountain zebra CatSper genes 1 - 3

| Primer Name | Forward sequence | Reverse sequence | Annealing temperature (°C) |
|-------------|------------------|------------------|---------------------------|
| CAT1_1      | AACCCTCATGTGGCTAGAAG | GGACGGTGAGCAAGACCTCA | 62                        |
| CAT1_2      | TCAGAAACGCAAAGAGGTAG | GGCTCCCTGGTTTCTACCAC | 60                        |
| CAT1_3      | GCTGCAACTCTTGTACCTCT | CAGTCCCATCCCCCTGGACAG | 60                        |
| CAT1_4      | CCTCTGACTACGTTGGG | GGGGTGTCTGAACCTGTGA | 53                        |
| CAT1_5      | CTNTACCCTGCTACCCTGG | TTCAACCGAGAAACTCGAGT | 60                        |
| CAT2_1      | TGATCTTACACATATACTGT | ATCCTACTCCAGAGAGCA | 55                        |
| CAT2_2      | TCTGATCATTTCTCTCATATTCC | GTTCCATTTCTGTACCTC | 55                        |
| CAT2_3      | TCTGAGAGGTCTAGATCCTC | GAGCTGGAGGAATCTAACC | 55                        |
| CAT2_4      | TCACACTTCTGCTTTGATATAT | GTTTCTAGAAGGGCTGTGTA | 55                        |
| CAT2_5      | CCAATATCTTAAGATTGTA | GTATGGATTAGGGGCAAT | 55                        |
| CAT3_1      | GCAGACCTTTAGTTGCTAC | CATAGGTCTGGACTATTCC | 55                        |
| CAT3_2      | GCTCTGACGCTTTGATCTG | AGTCAGACACACCTTTCA | 55                        |
| CAT3_3      | GGCTGAGGACTCGAGATCT | CAGCCTGTAGTGCTCTC | 55                        |
| CAT3_4      | GGTTGTCATCTCTTTCTCATTGC | ACTGATGGTCTGGAGTCC | 55                        |
### Table 2 Coverage of exons obtained from primers that were designed in this study

| Gene   | Fragment | Exon covered | Partial/Full | Coverage (%) |
|--------|----------|--------------|--------------|--------------|
| CatSper 1 | CAT1_1   | Exon 1       | Partial      | 88           |
|         | CAT1_2   | Exon 2       | Full         | 100          |
|         | CAT1_3   | Exon 3       | Partial      | 41           |
|         | CAT1_3   | Exon 4       | Full         | 100          |
|         | CAT1_5   | Exon 7       | Full         | 100          |
|         | CAT1_5   | Exon 8       | Full         | 100          |
|         | CAT2_1   | Exon 1       | Full         | 100          |
|         | CAT2_1   | Exon 2       | Full         | 100          |
|         | CAT2_1   | Exon 3       | Full         | 100          |
|         | CAT2_2   | Exon 4       | Full         | 100          |
|         | CAT2_2   | Exon 5       | Full         | 100          |
|         | CAT2_2   | Exon 6       | Full         | 100          |
|         | CAT2_3   | Exon 7       | Full         | 100          |
|         | CAT2_3   | Exon 8       | Full         | 100          |
|         | CAT2_5   | Exon 9       | Full         | 100          |
|         | CAT2_5   | Exon 10      | Full         | 100          |
| CatSper 2 | CAT3_1   | Exon 2       | Full         | 100          |
|         | CAT3_2   | Exon 3       | Full         | 100          |
|         | CAT3_3   | Exon 4       | Full         | 100          |
|         | CAT3_3   | Exon 5       | Full         | 100          |
|         | CAT3_3   | Exon 6       | Full         | 100          |
|         | CAT3_4   | Exon 7       | Partial      | 59           |
|         | CAT3_4   | Exon 8       | Full         | 100          |
Figure 1 Representative sequence chromatograms showing positions of single nucleotide polymorphisms identified in the intronic regions of CatSper 1. These positions are a) G1547A, b) G2241A, c) C4675T and d) G5270A.

Future unforeseen environmental stochasticity may necessitate the use of artificial fertilization techniques in CMZ to increase the reproductive output in key reserves to maintain genetic diversity and population viability. Identifying SNP variations within the introns and exons of genes associated with fertility may provide a criterion for selecting suitable candidates. Further studies on a larger sample set could include additional genes such as glutamine-rich protein 2 and A-kinase anchoring protein 4, which have been reported to identify a loss of sperm function (Shen et al., 2019) and reduced sperm motility (Moretti et al., 2007). After functional correlations have been established, structural changes in the protein could be better understood. In future comparative studies between zebra species that characterize sperm and other physiological parameters may be useful to diagnose potential defects in stallions, should semen samples become available.

Acknowledgements

The authors would also like to thank CapeNature and Sanbona Nature Reserve for their role in the collection of the samples. They thank the Professional Development Programme of the National Research Foundation and Department of Science and Technology of South Africa for supporting a doctoral fellow and the National Zoological Garden, South African National Biodiversity Institute for project funding to execute this study.

Authors Contributions

RMS conceptualized the research question, collected the data for this research, made a leading contribution to original manuscript, and carried out the statistical analysis. AK provided the platform for research and contributed to

Acknowledgements

The authors would also like to thank CapeNature and Sanbona Nature Reserve for their role in the collection of the samples. They thank the Professional Development Programme of the National Research Foundation and Department of Science and Technology of South Africa for supporting a doctoral fellow and the National Zoological Garden, South African National Biodiversity Institute for project funding to execute this study.

Authors Contributions

RMS conceptualized the research question, collected the data for this research, made a leading contribution to original manuscript, and carried out the statistical analysis. AK provided the platform for research and contributed to
reviewing the manuscript. JPG reviewed the manuscript and DD also conceptualized the research question, carried out analysis, and contributed to the original manuscript

Conflict of Interest Declaration
The authors declare no conflict of interest

References
Arnon, L. & Eisenbach, M., 2011. Behavioral mechanism during human sperm chemotaxis: Involvement of hyperactivation. PLoS One 6. DOI: 10.1371/journal.pone.0028359
Avenarius, M.R., Hildebrand, M.S., Zhang, Y., Meyer, N.C., Smith, L.L.H., Kahrizi, K., Najmabadi, H. & Smith, R.J.H., 2009. Human male infertility caused by mutations in the CatSper1 channel protein. Am. J. Hum. Genet. 84, 505-510. DOI: 10.1016/j.ajhg.2009.03.004
Birss, C., Cowell, C., Hayward, N., Pienke, D., Hrabar, H. & Kotze, A., 2018. Biodiversity Management Plan for the Cape Mountain Zebra Equus zebra in South Africa 2, 1-30.
Carlson, A.E., Westenbroek, R.E., Quill, T., Ren, D., Clapham, D.E., Hille, B., Garbers, D.L. & Babcock, D.F., 2003. CatSper1 required for evoked Ca2+ entry and control of flagellar function in sperm. Proc. Natl. Acad. Sci. USA 100, 14864-14868. DOI: 10.1073/pnas.2536658100
Chung, J.J., Navarro, B., Krapivinsky, G., Krapivinsky, L. & Clapham, D.E., 2011. A novel gene required for male fertility and functional CatSper channel formation in spermatozoa. Nat. Commun. 2. DOI: 10.1038/ncomms1153
Hildebrand, M.S., Avenarius, M.R., Fellous, M., Zhang, Y., Meyer, N.C., Auer, J., Serres, C., Kahrizi, K., Najmabadi, H., Beckmann, J.S. & Smith, R.J., 2010. Genetic male infertility and mutation of CatSper ion channels. Eur. J. Hum. Genet. 18, 1178-1184. DOI: 10.1038/ajhg.2010.108
Hrabar, H. & Kerley, G.I.H., 2013. Conservation goals for the Cape mountain zebra Equus zebra zebra — security in numbers? Ornx 47, 403-409. DOI: 10.1017/S0030605311002018
Jin, J., Jin, N., Zheng, H., Ro, S., Tafolla, D., Sanders, K.M. & Yan, W., 2007. CatSper3 and CatSper4 are essential for sperm hyperactivated motility and male fertility in the mouse. Biol. Reprod. 77, 37-44. DOI: 10.1095/biolreprod.107.060186
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A., 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647-1649. DOI: 10.1093/bioinformatics/bts199
Kotzé, A., Smith, R.M., Moodley, Y., Luikart, G., Birss, C., Van Wyk, A.M., Grobler, J.P. & Dalton, D.L., 2019. Lessons for conservation management: Monitoring temporal changes in genetic diversity of Cape mountain zebra (Equus zebra zebra). PLoS One 14, e0220331. DOI: 10.1371/journal.pone.0220331
Lea, J.M., Kerley, G.I.H., Hrabar, H., Barry, T.J. & Shultz, S., 2016. Recognition and management of ecological refugees: A case study of the Cape mountain zebra. Biol. Conserv. 203, 207-215. DOI: 10.1016/j.biocon.2016.09.017
Loux, S.C., Crawford, K.R., Ing, N.H., González-Fernández, L., Macías-García, B., Love, C.C., Varner, D.D., Velez, I.C., Choi, Y.H. & Hinrichs, K., 2013. CatSper and the relationship of hyperactivated motility to intracellular calcium and pH kinetics in equine sperm. Biol. Reprod. 89, 123. DOI: 10.1095/biolreprod.113.111708
Luo, T., Chen, H., Zou, Q., Wang, T., Cheng, Y., Wang, H., Wang, F., Jin, Z., Chen, Y., Weng, S. & Zeng, X., 2019. A novel copy number variation in CatSper2 causes idiopathic male infertility with normal semen parameters. Hum. Reprod. 34, 414-423. DOI: 10.1093/humrep/dey377
Moodley, Y. & Harley, E.H., 2005. Population structuring in mountain zebras (Equus zebra zebra). J. Reprod. Fertil. 83, 371-382.
Novellie, P., Lindeque, M., Lindeque, P., Lloyd, P. & Koen, J., 2002. Status and Action Plan for the Mountain Zebra (Equus zebra zebra). In: P. Moehlman (ed.) Equids: Zebras, asses and horses. IUCN, Gland, Switzerland, p. 204.
Penzhorn, B. & Van der Merwe, 1998. Testsis size and onset of spermatogenesis in Cape mountain zebra (Equus zebra zebra). J. Reprod. Fertil. 93, 371-375.
Penzhorn, B.L., 1985. Reproductive characteristics of a free-ranging population of Cape mountain zebra (Equus zebra zebra zebra). J. Reprod. Fertil. 73, 51-57. DOI: 10.1530/j.1475-191x.1985.t01-21-00214.x
Qi, H., Moran, M.M., Navarro, B., Chung, J. a, Krapivinsky, G., Krapivinsky, L., Kirichok, Y., Ramsey, I.S., Quill, T.A. & Clapham, D.E., 2007. All four CatSper ion channel genes are required for male fertility and sperm cell hyperactivated motility. Proc. Natl. Acad. Sci. USA 104, 1219-1223. DOI: 10.1073/pnas.0610286104
Ren, D., Navarro, B., Perez, G., Jackson, A.C., Hsu, S., Shi, Q., Tilly, J.L. & Clapham, D.E., 2001. A sperm ion channel required for sperm motility and male fertility. Nature 413, 603-609. DOI: 10.1038/35098027
Saha, S., Talukdar, K. & Chakraborty, A.K., 2015. The role of CatSper1 and CatSper2 ion channels in male fertility and infertility. Procodia Mater. Sci. 10, 730-736. DOI: 10.1016/j.mspro.2015.06.088
Shen, Y., Zhang, F., Li, F., Jiang, X., Yang, Y., Li, X., Li, W., Wang, X., Cheng, J., Liu, M., Zhang, X., Yuan, G., Pei, X., Cai, K., Hu, F., Sun, J., Yan, L., Tang, L., Jiang, C., Tu, W., Xu, J., Wu, H., Kong, W., Li, S., Wang, K., Sheng, K., Zhao, X., Yue, H., Yang, X. & Xu, W., 2019. Loss-of-function mutations in QRICH2 cause male infertility with...
multiple morphological abnormalities of the sperm flagella. Nat. Commun. 10, 1-15. DOI: 10.1038/s41467-018-0812-x

Singh, A.P., & Rajender, S., 2014. CatSper channel, sperm function and male fertility. Reprod. Biomed. Online. DOI: 10.1016/j.rbmo.2014.09.014

Sivakumar, A., Kumar, S., Yathish, H.M., Mishra, C., Modi, R.P., Chaudhary, R., Khan, S., Sivamani, B., Ghosh, S.K. & Sarkar, M., 2017. Expression profiling and identification of novel SNPs in CatSper2 gene and their influence on sperm motility parameters in bovines. Anim. Biotechnol. 29, 34-40. DOI: 10.1080/10495398.2017.1294597

Smith, R.K., Marais, A., Chadwick, P., Lloyd, P.H. & Hill, R.A., 2007. Monitoring and management of the endangered Cape mountain zebra Equus zebra zebra in the Western Cape, South Africa. Afr. J. Ecol. 46, 207-213. DOI: 10.1111/j.1365-2028.2007.00893.x

Singh, A.P. & Rajender, S.S., 2014. CatSper channel, sperm function and male fertility. Reprod. Biomed. Online. DOI: 10.1016/j.rbmo.2014.09.014

Sivakumar, A., Kumar, S., Yathish, H.M., Mishra, C., Modi, R.P., Chaudhary, R., Khan, S., Sivamani, B., Ghosh, S.K. & Sarkar, M., 2017. Expression profiling and identification of novel SNPs in CatSper2 gene and their influence on sperm motility parameters in bovines. Anim. Biotechnol. 29, 34-40. DOI: 10.1080/10495398.2017.1294597

Smith, R.K., Marais, A., Chadwick, P., Lloyd, P.H. & Hill, R.A., 2007. Monitoring and management of the endangered Cape mountain zebra Equus zebra zebra in the Western Cape, South Africa. Afr. J. Ecol. 46, 207-213. DOI: 10.1111/j.1365-2028.2007.00893.x

Stauss, C.R., Votta, T.J. & Suarez, S.S., 1995. Sperm motility hyperactivation facilitates penetration of the hamster zona pellucida. Biol. Reprod. 53, 1280-1285. DOI: 10.1095/biolreprod53.6.1280

Strünker, T., Goodwin, N., Bronk, C., Kashkhar, N.D., Weyand, I., Seifert, R. & Kaupp, U.B., 2011. The CatSper channel mediates progesterone-induced Ca2+ influx in human sperm. Nature 471, 382-386. DOI: 10.1038/nature09769

Sturm, R.A., Duffy, D.L., Zhao, Z.Z., Leite, F.P.N., Stark, M.S., Hayward, N.K.K., Martin, N.G. & Montgomery, G.W., 2008. A single SNP in an evolutionary conserved region within intron 86 of the HERC2 gene determines human blue-brown eye color. Am. J. Hum. Genet. 82, 424-431. DOI: 10.1016/j.ajhg.2007.11.005

Visser, L., Westerveld, G.H., Xie, F., Van Daalen, S.K.M., Van Der Veen, F., Lombardi, M.P. & Repping, S., 2011. A comprehensive gene mutation screen in men with asthenozoospermia. Fertil. Steril. 95, 1020-1024.e9. DOI: 10.1016/j.fertnstert.2010.11.067

Wang, D., Guo, Y., Wrighton, S.A., Cooke, G.E. & Sadee, W., 2011. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. Pharmacogenomics J. 11, 274-286. DOI: 10.1038/tpj.2010.28

Wang, H., Liu, J., Cho, K.-H. & Ren, D., 2009. A novel, single, transmembrane protein CatSperG is associated with CatSper1 channel protein1. Biol. Reprod. 81, 539-544. DOI: 10.1095/biolreprod.109.077107

Zhang, X., Zhou, L., Fu, G., Sun, F., Shi, J., Wei, J., Lu, C., Zhou, C., Yuan, Q. & Yang, M., 2014. The identification of an ESCC susceptibility SNP rs920778 that regulates the expression of IncRNA HOTAIR via a novel intronic enhancer. Carcinogenesis 35, 2062-2067. DOI: 10.1093/carcin/bgu103

Zhang, Y., Malekpour, M., Al-Madani, N., Kahrizi, K., Zanganeh, M., Mohebsi, M., Mojahedi, F., Daneshi, A., Najmabadi, H. & Smith, R.J.H., 2009. Sensorineural deafness and male infertility: a contiguous gene deletion syndrome. Case Reports 2009, bcr0820080645-bcr0820080645. DOI: 10.1136/bcr.08.2008.0645