GLUTAMINE REPEAT POLYMORPHISM IN THE EXON 1 OF ANDROGEN RECEPTOR GENE IN GRASSCUTTER (*Thryonomys swinderianus*)

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ABSTRACT Grasscutter (*Thryonomys swinderianus*) has been a subject of domestication for the past several decades in West and Central Africa. In Ghana, attempts are being made to intensify the domestication efforts because grasscutter meat is a delicacy and huge agribusiness opportunities are expected. Tameness and reproductive ability are two important traits that underlie any successful animal domestication event. Androgen receptor gene has been reported to influence behaviour and fertility. The objective of this study was to identify genetic polymorphism in the androgen receptor gene that is likely to influence tameness and reproductive traits. Tissue samples were collected from a total of 116 including 81 domestic and 35 wild grasscutters in Accra and Mankessim, respectively, and genotyped at the androgen receptor glutamine repeat (ARQ) locus. Results of this study showed that ARQ ranges from six to 19 repeats and heterozygosities (*H*) were 0.769 and 0.778 for domestic and wild females respectively, indicating that this locus is highly polymorphic in grasscutters. As this locus influences transcriptional activity of the androgen receptor gene, these polymorphisms could influence tameness and reproductive traits in grasscutter. However, further studies are required to test the association of these polymorphisms with phenotypes.

Key Words: Androgen receptor; Glutamine repeat; Grasscutter; Domestication; Tameness.

INTRODUCTION

Grasscutter is a non-ruminant herbivore that is distributed throughout the humid regions of sub-Saharan Africa (Annor et al., 2009). This fairly large rodent has been the subject of domestication efforts in West and Central Africa since the 1970s. Livestock and other domestic animals have been domesticated over several centuries (Bruford et al., 2003; Mignon-Grasteau et al., 2005). Domestication of grasscutter therefore provides a unique model of livestock domestication processes in the present times.

It is an absolute fact that the grasscutter meat is enjoyed by many people in West and Central Africa, creating huge agribusiness opportunities in these subregions if the grasscutter is successfully domesticated. However, since the grass-

cutter is still at its infancy stage of domestication, several challenges limit the progress of domestication. These challenges including high mortality due to aggression, scarcity of feed resources in the dry season, diseases, long gestation period and relatively low average litter size, make grasscutter production less attractive as an agribusiness venture. Nevertheless, many successful farms exist in Ghana and other West and Central African countries. In order to overcome these challenges, intensive research into these areas of grasscutter production is paramount. Selective breeding programs would have to be implemented to breed grasscutters that are docile, fast growing and able to produce higher litter sizes with corresponding litter survival rates (Annor et al., 2009).

Kyoto University in partnership with University of Ghana with sponsorship package from Japan International Co-operation Agency (JICA), launched a project in 2010 in the Upper West Region of Ghana to promote the domestication of grasscutter. In that project, farmers were provided with cages and grasscutters after thorough training. At the time that this manuscript was going to press, there were about 300 grasscutters distributed across over 50 farms from an initial number of about 100 distributed grasscutters. The project has been very successful despite the challenges and can therefore be regarded as one of the flagship agricultural and livelihood support projects in Ghana (Grasscutter Initiative for Rural Transformation, 2017).

One of the approaches in breeding programs is to use genetic markers to select individuals with higher genetic potentials for breeding; the so-called marker assisted selection (MAS). In our effort to promote the grasscutter domestication, we aimed at developing genetic markers that could complement population management and selective breeding (Adenyo et al., 2012; Adenyo et al., 2017). For selective breeding, our aim is to identify polymorphisms in candidate genes that influence traits of economic importance especially docility in the grasscutter since this trait underlies animal domestication events. In this study, we report the polymorphism of androgen receptor gene, one of such genes controlling behaviour and reproductive ability in mammals.

The role of androgen receptor in masculine phenotype including behaviour and morphological appearance has long been recognized (Quigley et al., 1995; Zitzmann & Nieschlag, 2003; Fuxjager et al., 2018). Androgen receptor gene (*AR*) is known to influence aggressive behaviour in humans (*Homo sapiens*). Exon 1 of this gene has a tri-nucleotide (CAG) repeat leading to a glutamine (Q) repeat in the N-terminal region of the polypeptide which is the transcription activation or regulation domain (Quigley et al., 1995). Shorter alleles were shown to be associated with aggression, fertility and depression whereas longer alleles (more than 40 Q repeats) were associated with Kennedy disease in humans (Albertelli et al., 2006; Palazzolo et al., 2008). In non-human mammals, polymorphisms have been associated with personality and fertility (Konno et al., 2011; Lai et al., 2008; Lyons et al., 2014; Revay et al., 2012; Ramadan et al., 2018). For instance, glutamine repeat polymorphisms have been found to be associated with aggression in Japanese Akita Inu (Konno et al., 2011). In rodents, however, no study has reported *AR* polymorphism and its association with behaviour. In this study, we report the polymorphism of the tri-nucleotide repeat (CAG or Q) in the exon 1 of the *AR*

in grasscutters and discuss its potential as a marker for tameness and reproductive ability.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

Pieces of muscle and liver tissues were taken from wild grasscutters (n = 35) at Mankessim from hunters' kills and ear snips were taken from semi-domesticated grasscutters (n = 81) at various grasscutter farms in Accra (Fig. 1) and stored in 70% ethanol. The study was approved by the ethical committee at the Wildlife Research Center, Kyoto University (WRC-2017-003A) and samples were collected in strict accordance with ethical guidelines regarding animal experimentation.

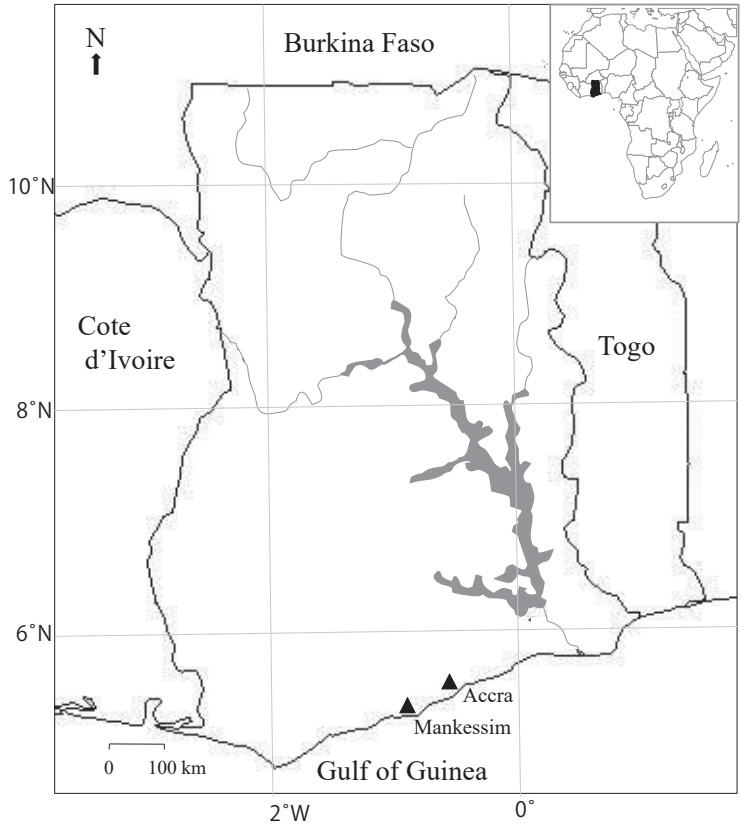

Fig. 1. The map of Ghana showing sampling locations indicated by triangle

Genomic DNA was extracted from tissue samples using QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, Germany) according to the manufacturer's protocol. The DNA was stored at –20°C until use.

Primer Design and PCR

To design polymerase chain reaction (PCR) primers, androgen receptor exon one sequences of rat, mouse and guinea pig containing the CAG repeats with accession numbers J05454 (Yarbrough et al., 1990), X59592 (Faber et al., 1991) and AJ893531 (Poux et al., 2005) respectively were aligned with ClustalW (Thompson et al., 1994) implemented in MEGA 6 (Tamura et al., 2013). Forward primer 5'-CCCCAAGCTCACATCAGAGG-3' and reverse primer 5'-CCAAGCCCATGGACACAGAT-3' were then designed at the conserved regions flanking the CAG repeat with expected product size of 598 bp. PCR mixture contained 0.5 U of LA Taq DNA polymerase (TaKaRa, Japan), 400 µM of each dNTP, 0.4 µM of each forward and reverse primers, PCR buffer and 20 ng of genomic DNA in a total volume of 10 µl. PCR cycling conditions consisted of 95°C of initial denaturation for 2 mins, 35 cycles of 95°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 1 min and a final extension of 72°C for 10 min. PCR products were purified with High Pure PCR Product Purification kit (Roche, Germany) and sequenced on Applied Biosystems 3130xl Genetic Analyzer using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequences were checked using Bioedit version 7.0.5. A new set of primers ARgf 5'-GCCCCACTTTCTCCAGTTTA-3' and ARgr 5'-CATTGCTGCTGCTTTCTGAG-3' were designed using sequences obtained. The forward primer was then labelled with 6-FAM for genotyping after successful amplification.

Genotype Analysis

Genotyping PCR was conducted on GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, USA) with cycling conditions as above. PCR products were then diluted (1:100) and electrophoresed on ABI 3130xl DNA Analyzer (Applied Biosystems, USA). The fragments sizes were scored using 400 HD Rox size standard with GeneMapper version 4 (Applied Biosystems, USA). Allele frequencies and distributions were computed using GenAlEx version 6.4 (Peakall & Smouse, 2006). Since androgen receptor gene is located on the X chromosome and males are hemizygotes, the data were analysed separately for males and females.

RESULTS AND DISCUSSION

Alleles (amplified PCR length, base pair) ranged from 261–300 corresponding to 6 to 19 CAG or Q repeats in the domestic population whilst 264–294 corresponding to 7 to 17 Q repeats were found in the wild population. Allele 285 (14 Q repeats) had the highest frequency in the wild population whereas the most frequent allele in the domestic population was 282 (13 Q repeats; Table 1 and

Table 1. Androgen receptor glutamine repeat (Q) alleles in wild and domestic grasscutters of Ghana. Heterozygosity (H) was calculated according to the following equation: $H = 2n(1 - \Sigma q_i^2)/(2n - 1)$

			Allele frequency (q_i)											
			6Q	**7Q**	**9Q**	**10Q**	**11Q**	**12Q**	**13Q**	**14Q**	**15Q**	**16Q**	**17Q**	**19Q**	
Population	**Sex**	***n***	**261**	**264**	**270**	**273**	**276**	**279**	**282**	**285**	**288**	**291**	**294**	**300**	***H***
Domestic	Male	29	0.034	0.069	0.034	0.069	0.069	0.103	0.207	0.103	0.172	0.103	0	0.034	
	Female	52	0.01	0.087	0.058	0.038	0.019	0.125	0.298	0.144	0.135	0.067	0	0.019	0.769
Wild	Male	17	0	0.059	0	0	0	0	0.176	0.412	0.235	0	0.118	0	
	Female	18	0	0.111	0.028	0	0	0.111	0.25	0.139	0.167	0.056	0.139	0	0.778

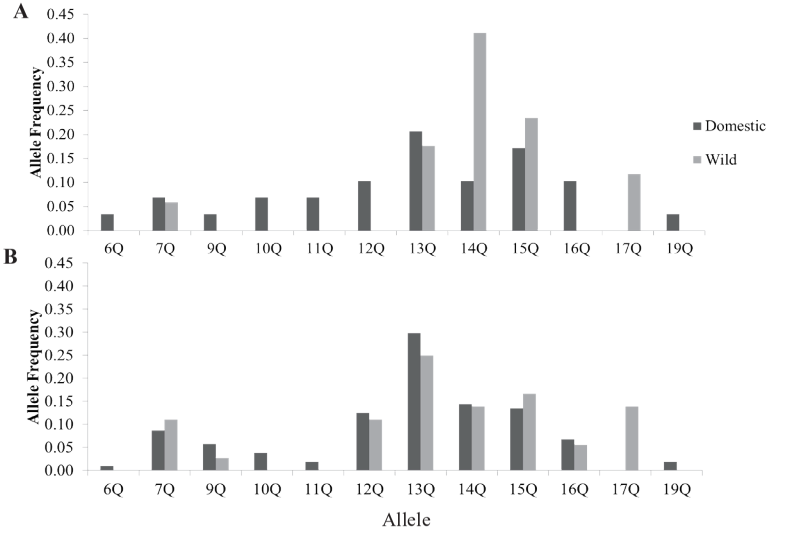

Fig. 2. Allele frequencies of androgen receptor repeat region of domestic and wild populations of grasscutters in (A) males and (B) females

Fig. 2). The sequence of allele 291 is shown as accession number LC422290 in the DDBJ database. Alleles 261 (6 Q repeats), 273 (10 Q repeats), 276 (11 Q repeats) and 300 (19 Q repeats) were not found in the wild population whereas 294 (17 Q repeats) was only found in the wild population. These results indicate that CAG repeat translating to glutamine (Q) repeat in exon 1 of the androgen receptor gene in grasscutter varies considerably in both wild and domestic grasscutters. Except for more variation in the number of alleles between wild and domestic grasscutters which could be due to the sample numbers, there was no clear difference between wild and domestic populations.

It has been amply demonstrated that the repeat length influences the transcriptional activity of the *AR* (Tut et al., 1997). Shorter alleles enhance transcriptional activity by increased interactions between the receptor and coactivators (Chamberlain et al., 1994). In Japanese Akita Inu breed of dogs, individuals with shorter alleles tended to be more aggressive (Konno et al., 2011). In grasscutter, the wild ones tended to have longer repeats than the domestic individuals regardless of sex. However, allele frequency distribution did not differ between domestic and wild populations ($X^2 = 1.32$, $p > 0.05$). Also, observed allele frequencies did not differ significantly from Hardy-Weinberg Equilibrium in both domestic ($X^2 = 57.05$, $p = 0.399$) and wild ($X^2 = 25.34$, $p = 0.609$) populations.

Longer Q tract leads to decreased AR activation both in-vitro and in-vivo whereas shorter alleles are associated with increased transcriptional activity and aggression in humans (Albertelli et al., 2006). Significant allele frequency difference between species were reported in chimpanzees and bonobos (Garai et al., 2014), New World monkeys (Hiramatsu et al., 2017), elephants (Yasui et al., 2013) and zebras (Ito et al., 2015). This might reflect species differences in aggressiveness. In rodents, no ARQ polymorphism has been reported in literature. The fact that ARQ allele is highly polymorphic in grasscutters shows the possibility of this allele influencing transcriptional activity of AR and its effect on androgen metabolism and consequently on behaviour and reproductive ability. This creates an opportunity for further investigations to be conducted on the role of ARQ polymorphism in the grasscutter and its influence on tameness and domestication.

Given the many alleles found in this study, there is a lot of variation for successful selective breeding for these traits if significant association is established. Several studies on *AR* knockout mouse models show complete infertility in males and reduced fertility in females leading to lower litter size (Walters et al., 2010). More so, it has been shown that exon 1 floxed knockout mice exhibited complete absence of the *AR* protein in the brain of both sexes (Kato, 2002; Holdcraft & Braun, 2004; Shiina et al., 2006). Glutamine repeat polymorphisms in the exon 1 have been associated with androgen insensitivity in humans (Zitzmann & Nieschlag, 2003). These strongly support the hypothesis that polymorphisms in the exon 1 might affect the function of *AR* in grasscutter with respect to behaviour and reproductive ability. Although our study does not permit us to conclude on genotype-phenotype association, it does point to the possibility of differential transcription activation of *AR* which is essential for the androgenic system. This warrants further studies to elucidate the function of ARQ polymorphism in the grasscutter.

The results of this study suggest that domesticated population of grasscutters is not genetically isolated enough from the wild population. Domestication of grasscutter started in the 1970s but the number of generations till date is quite difficult to estimate. One reason for the lack of difference between domestic and wild populations is that domestication is quite recent and there has not been any grasscutter selective breeding programme in Ghana. Coupled with that, grasscutter farmers sometimes introduce new stock from the wild due to lack of domesticated breeding stock. In the near future, if significant associations are found between individual ARQ genotypes and reproductive ability and/or tameness, this locus could be used in MAS to breed highly prolific and tame grasscutters.

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REFERENCES

Adenyo, C., R. Ogden, B.B. Kayang, M. Onuma, N. Nakajima & M. Inoue-Murayama 2017. Genome-wide DNA markers to support genetic management for domestication and commercial production in a large rodent, the Ghanaian grasscutter (*Thryonomys swinderianus*). *Animal Genetics*, 48: 113–115.

Adenyo, C., A. Hayano, E. Inoue, B.B. Kayang & M. Inoue-Murayama 2012. Development of microsatellite markers for grasscutter (*Thryonomys swinderianus*, RODENTIA) using next-generation sequencing technology. *Conservation Genetic Resources*, 4: 1011–1014.

Albertelli, M.A, A. Sheller, M. Brogley & D.M. Robins 2006. Replacing the mouse androgen receptor with human alleles demonstrates glutamine tract length dependent effects on physiology and tumorigenesis in mice. *Molecular Endocrinology*, 20: 1248–1260.

Annor, S.K., E.K. Adu, J. Donkor, H.R. Otsyina & J. Ahiaba 2009. *Grasscutter Production: A Handbook*. GTZ/MOAP, Accra.

Bruford, M.W., D.G. Bradley & G. Luikart 2003. DNA markers reveal the complexity of livestock domestication. *Nature Reviews Genetics*, 4(11): 900.

Chamberlain, N.L., E.D. Driver & R.L. Miesfeld 1994. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Research*, 22(15): 3181–3186.

Faber, P.W., A. King, H.C. van Rooij, A.O. Brinkmann, N.J. de Both & J. Trapman 1991. The mouse androgen receptor: Functional analysis of the protein and characterization of the gene. *Biochemical Journal*, 278: 269–278.

Fuxjager, M. J., M. C. Miles & B. A. Schlinger 2018. Evolution of the androgen-induced male phenotype. *Journal of Comparative Physiology A*, 204: 81–92.

Garai, C., T. Furuichi, Y. Kawamoto, H. Ryu & M. Inoue-Murayama 2014. Androgen receptor and monoamine oxidase polymorphism in wild bonobos. *Meta Gene*, 2: 831–843.

Grasscutter Initiative for Rural Transformation 2017. *Grasscutter Keeping Project in Ghana*, Newsletter 3. Online. http://grasscutter.sakuraweb.com/wp-content/uploads/2018/05/

Newsletter-Vol-3.pdf (Accessed December 1, 2018).
Hiramatsu, C., A. Paukner, H. Kuroshima, K. Fujita, S.J. Suomi & M. Inoue-Murayama 2017. Short poly-glutamine repeat in the androgen receptor in New World monkeys. *Meta Gene*, 14: 105–113.
Holdcraft, R.W. & R.E. Braun 2004. Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. *Development*, 131: 459–467.
Ito, H., T. Langenhorst, R. Ogden & M. Inoue-Murayama 2015. Androgen receptor gene polymorphism in zebra species. *Meta Gene*, 5: 120–123.
Kato, S. 2002. Androgen receptor structure and function from knock-out mouse. *Clinical Pediatric Endocrinology*, 11: 1–7.
Konno, A., M. Inoue-Murayama & T. Hasegawa 2011. Androgen receptor gene polymorphisms are associated with aggression in Japanese Akita Inu. *Biology Letters*, 7(5): 658 –660.
Lai, C.L., H. L'Eplattenier, R. van den Ham, F. Verseijden, A. Jagtenberg, J.A. Mol, & E. Teske 2008. Androgen receptor CAG repeat polymorphisms in canine prostate cancer. *Journal of Veterinary Internal Medicine*, 22(6): 1380–1384.
Lyons, R.E., N.T. Loan, L. Dierens, M.R. Fortes, M. Kelly, S.S. McWilliam, Y. Li, R.J. Bunch, B.E. Harrison, W. Barendse, S.A. Lehnert & S.S. Moore 2014. Evidence for positive selection of taurine genes within a QTL region on chromosome X associated with testicular size in Australian Brahman cattle. *BMC Genetics*, 15: 6.
Mignon-Grasteau, S., A. Boissy, J. Bouix, J.M. Faure, A.D. Fisher, G.N. Hinch, P. Jensen, P. Le Neindre, P. Mormède, P. Prunet & M. Vandeputte 2005. Genetics of adaptation and domestication in livestock. *Livestock Production Science*, 93(1): 3–14.
Palazzolo, I., A. Gliozzi, P. Rusmini, D. Sau, V. Crippa, F. Simonini, E. Onesto, E. Bolzoni, & A. Poletti 2008. The role of the polyglutamine tract in androgen receptor. *The Journal of Steroid Biochemistry and Molecular Biology*, 108: 245–253.
Peakall, R.O.D. & P.E. Smouse 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6(1): 288–295.
Poux, C., O. Madsen, E. Marquard, D.R., Vieites, W.W. de Jong & M. Vences 2005. Asynchronous colonization of Madagascar by the four endemic clades of primates, tenrecs, carnivores, and rodents as inferred from nuclear genes. *Systematic Biology*, 54(5): 719–730.
Quigley, C.A., A. De Bellis, K.B. Marschke, M.K. El-Awady, E.M. Wilson & F.S. French 1995. Androgen receptor defects: Historical, clinical, and molecular perspectives. *Endocrine Reviews*, 16(3): 271–321.
Ramadan, S., A.M. Nowier, Y. Hori & M. Inoue-Murayama 2018. The association between glutamine repeats in the androgen receptor gene and personality traits in the dromedary camel (*Camelus dromedarius*). *PLoS ONE*, 13(2): e0191119.
Revay, T., D.A. Villagomez, D. Brewer, T. Chenier & W.A. King 2012. GTG mutation in the start codon of the androgen receptor gene in a family of horses with 64, XY disorder of sex development. *Sexual Development*, 6(1–3): 108–116.
Shiina, H., T. Matsumoto, T. Sato, K. Igarashi, J. Miyamoto, S. Takemasa, M. Sakari, I. Takada, T. Nakamura, D. Metzger, P. Chambon, J. Kanno, H. Yoshikawa & S. Kato 2006. Premature ovarian failure in androgen receptor-deficient mice. *Proceedings of the National Academy of Sciences*, 103: 224–229.
Tamura, K., G. Stecher, D. Peterson, A. Filipski & S. Kumar 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12): 2725–2729.
Thompson, J.D., D.G. Higgins & T.J. Gibson 1994. CLUSTALW: improving the sensitivity of progressive weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22: 4673–4680.

Tut, T.G., F.J. Ghadessy, M.A. Trifiro, L. Pinsky & E.L. Yong 1997. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. *Journal of Clinical Endocrinology and Metabolism*, 82(11): 3777–3782.

Walters, K.A., U. Simanainen & D.J. Handelsman 2010. Molecular insights into androgen actions in male and female reproductive function from androgen receptor knockout models. *Human Reproduction Update*, 16(5): 543–558.

Yarbrough, W.G., V.E. Quarmby, J.A. Simental, D.R. Joseph, M. Sar, D.B. Lubahn, K.L. Olsen, F.S. French & E.M. Wilson 1990. A single base mutation in the androgen receptor gene causes androgen insensitivity in the testicular feminized rat. *The Journal of Biological Chemistry*, 265(15): 8893–8900.

Yasui, S., A. Konno, M. Tanaka, G. Idani, A. Ludwig, D. Lieckfeldt & M. Inoue-Murayama 2013. Personality assessment and its association with genetic factors in captive Asian and African elephants. *Zoo Biology*, 32(1): 70–78.

Zitzmann, M. & E. Nieschlag 2003. The CAG repeat polymorphism within the androgen receptor gene and maleness. *International Journal of Andrology*, 26(2): 76–83.

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