Biomarkers, 2012; 17(5): 402–406

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2012.677066

informa
healthcare

RESEARCH ARTICLE

Relationship of SNP of H2BFWT gene to male infertility in a Chinese population with idiopathic spermatogenesis impairment

Hou-qun Ying[1,2], Matthew B. Scott[1,3], and Zhou-cun A.[1]

[1]*Department of Genetics, College of Agriculture and Biology, Dali University, Dali, China,* [2]*College of Basic Medicine, Dali University, Dali, China, and* [3]*Institute of Eastern Himalaya Biodiversity Research, Dali University, Dali, China*

Abstract

The H2B family, member W, testis specific (H2BFWT) gene encodes a testis specific histone that plays a crucial role in reorganization and remodeling of chromatin and epigenetic regulation during spermatogenesis, suggesting that the gene may be involved in spermatogenesis impairment. To test the speculation, the allele and haplotype frequencies of two single-nucleotide polymorphism loci in this gene, −9C>T and 368A>G, were investigated in 409 infertile patients with idiopathic azoospermia or oligozoospermia and 209 fertile men as controls using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. As the results, the frequencies of −9T (52.8% vs. 41.6%, $p = 0.009$) and 368G (43.0% vs. 32.5%, $p = 0.012$) were significantly higher in patients than those in controls; after stratifying patients, the significant higher frequencies were still detected in allele −9T for azoospermia (57.4% vs. 41.6%, $p = 0.001$) and allele 368G for oligozoospermia (45.4% vs. 32.5%, $p = 0.007$). The haplotype CA was significantly decreased (22.8% vs. 33.0%, $p = 0.006$) whereas TG was significantly increased (18.3% vs. 7.2%, $p < 0.001$) in infertile patients compared with controls. These results indicated that the polymorphism −9C>T and 368A>G in H2BFWT gene are associated with male infertility with idiopathic azoospermia or oligozoospermia, suggesting that H2BFWT gene might be contribute to susceptibility to spermatogenesis impairment in Chinese population.

Keywords: H2BFWT, single-nucleotide polymorphism, male infertility, spermatogenesis impairment

Introduction

Infertility is a pervasive affliction that affects between one in six and one in ten childbearing couples worldwide (De Kretser and Baker, 1999); issues with the male account for half of those affected couples (Stouffs et al., 2009). The most common clinical disorder seen in infertile men is impaired spermatogenesis, a condition caused by heat, toxins, injury, endocrine or genitourinary system disorders, or underlying genetic disorders, which result in low sperm concentration or poor sperm motility. A significant proportion of male infertility involves genetic defects (Ferlin et al., 2006, 2007; O'Flynn, O'Brien et al., 2010). Spermatogenesis is a complex process including spermatogonial stem cell proliferation, meiosis and spermatid differentiation. Genetic variation of those genes involved in this process may play an important role in impaired spermatogenesis.

Histones are the major nuclear proteins in eukaryotic cell nuclei that are responsible for the nucleosome structure of chromatin. During spermatogenesis, the chromatin is profoundly reorganized and remodeled into a unique nucleosomal structure. Testis-specific histone variants are exclusively expressed and accumulated in both spermatogenic cells and testis tissue (Cole et al., 1986; van Roijen et al., 1998). These histone variants function not only in the replacement of conservational histones and epigenetic regulation of gene transcription (Steger et al., 1998; Kimmins and Sassone-Corsi, 2005; Pradeepa and Rao, 2007), but also play a key role in the histone mediated regulation of chromatin metabolism, differentiation

Address for Correspondence: Prof. Zhou-cun A, Department of Genetics, College of Agriculture and Biology, Dali University, Dali, Yunnan, 671003, China. Tel. and Fax: +86-0872–2219030. E-mail: azhoucun@163.com

(Received 29 December 2011; revised 06 March 2012; accepted 14 March 2012)

and histone post translational modification during spermatogenesis (Henikoff and Ahmad, 2005; Ausio, 2006). The disruption of testis specific histone variants is related to abnormal formation of spermatid nuclei (Tanaka et al., 2005), suggesting that testis specific histone variants may be essential for normal spermatogenesis.

The H2B family, member W, testis specific (H2BFWT) gene is the one of the testis specific histone variant genes related to spermatogenesis (Churikov et al., 2004). This gene is an X-linked gene located at Xq22.2, and consists of three exons and two introns that encodes a member variant protein of the H2B histone family. H2BFWT is exclusively synthesized and accumulated in human testis and sperm nuclei in a specific stage of spermatogenesis (van Roijen et al., 1998; Churikov et al., 2004). Although H2BFWT is only 45% homologous with the conventional H2B, it can efficiently displace the conventional H2B and exhibits the same stability as conventional H2B histone in nucleosome (Boulard et al., 2006). Spermatozoa from infertile men have more nuclear H2B histone than fertile men, and an abnormally higher proportions of histone H2B can lead to sperm DNA damage (Zhang et al., 2006; Zini et al., 2008), suggesting that low amounts of H2BFWT may be related to abnormal spermatogenesis. It has been revealed that H2BFWT is an indispensable component of the sperm telomere-binding protein complex necessary for the transmission of the telomeric chromatin through generations (Gineitis et al., 2000; Churikov et al., 2004; Boulard et al., 2006). Also, H2BFWT is essential for specific functions in meiosis during chromatin reorganization and the regulation of spermatogenesis (Gineitis et al., 2000). Thus, it is reasonable to hypothesize that H2BFWT gene may be involved in the impairment of male spermatogenesis.

In the present study, we chose two single-nucleotide polymorphism (SNP) loci that may affect the function of H2BFWT gene, –9C>T and 368A>G, and investigated allele and haplotype frequencies distribution of them in 409 infertile patients with idiopathic azoospermia or oligozoospermia and 209 fertile controls using a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay to explore the possible association between the H2BFWT gene and Chinese male infertility.

Materials and methods

Study population and inclusion criteria

The patient group composed 409 infertile patients, including 204 idiopathic azoospermia and 205 oligozoosepemia (sperm count less than 15×10^6/ml) aged from 25 to 42 years. Patients with history of orchitis, maldescensus of testis, varicocele, and obstruction of the vas deferens were excluded from survey; patients with chromosomal abnormalities and deletions of AZF region on Y chromosome were also excluded by running chromosome analysis and molecular analysis on individuals (Simoni et al., 2004). All patients underwent at least two semen analyses according to WHO guidelines (World Health Organization, 2010). The control group includes 209 men proven fertile aged from 26 to 45 years. All participants of the study were of Han nationality, which makes up more than 90% of the Chinese population. The present study was approved by the Institution Ethics Commission of Dali University and informed approval was obtained from participants. The semen parameters of infertile patient and control groups were shown in Table 1.

Choice of SNPs

Exonic SNPs were selected to study based on the genomic sequence of the H2BFWT gene according to National Center for Biotechnology Information (NCBI) SNP database (dbSNP). Based on this information, two SNPs in exon 1 of H2BFWT gene were selected for present study, including SNP –9C>T (rs7885967) located in 5′ untranslated region (5′UTR) and SNP 368A>G (rs553509) with a missense mutation.

PCR amplification

Genomic DNA was extracted from the peripheral blood leucocytes of patients and controls using a TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). A mismatch forward primer 5′-CAT CCA ATC AGA CGT GAA GCT GGC CCG *T*GA-3′ in which an underlined base indicates a mismatch to create the restriction site for enzyme Tsp451 and reverse primer 5′-TGC TTC TGG GAC GTA GTG GA-3′ were used to amplify 212 bp fragment including –9C>T site. Primers 5′-GTC TGG TCG TGC CAT CTA AT-3′ (forward strand) and 5′-TAC CTG AGG ACA GCC TTC GT-3′ (reverse strand) were used to amplify the 446 bp fragment with 368A>G site. PCR amplifications were carried out in a total volume of 25 µl containing about 100 ng of genomic DNA, 200 µmol/L dNTPs, 10 pmol of each primer, 2.5 µl 10 × PCR buffer, 1.5 mmol/l $MgCl_2$ and 1U Taq polymerase (Takara, Shiga, Japan). The reaction profile was predenaturation at 95°C for 5 min followed by denaturation at 94°C for 30 s, annealing at 54°C (–9C>T) and 56°C (368A>G), respectively, for 30 s and extension at 72°C for 30 s for 35 cycles, with a final extra extension at 72°C for 5 min.

Table 1. The parameters of semen in fertile and infertile group.

	Fertile men	Infertile patients	
Semen parameters	Total ($n = 209$)	Azoospermia ($n = 204$)	Oligozoospermia ($n = 205$)
Concentration (10^6/ml)	60.68 ± 36.10	—	10.69 ± 2.85*
Motility (PR + NP, %)	55.42 ± 13.70	—	53.02 ± 15.48
Morphology (normal forms, %)	73.51 ± 6.48	—	72.12 ± 7.60

*$p < 0.05$ compared with controls by T test.

Table 2. The distributions of allele frequencies of SNP –9C>T and 368A>G in H2BFWT gene in infertile patients with azoospermia or oligozoospermia and fertile men.

		Fertile men	Infertile patients			*p* Value*		
Locus	Allele	Total (*n* = 209)	Total (*n* = 409)	Azoospermia (*n* = 204)	Oligozoospermia (*n* = 205)	[1]	[2]	[3]
–9C>T	C	0.584 (122)	0.472 (193)	0.426 (87)	0.517 (106)			
	T	0.416 (87)	0.528 (216)	0.574 (117)	0.483 (99)	0.009	0.001	0.173
368A>G	A	0.675 (141)	0.570 (233)	0.593 (121)	0.546 (112)			
	G	0.325 (68)	0.430 (176)	0.407 (83)	0.454 (93)	0.012	0.086	0.007

*Controls vs. [1] Total infertile patients, [2] Azoospermia, [3] Oligozoospermia.

Table 3. The distributions of haplotype frequencies of SNP –9C>T and 368A>G in H2BFWT gene in infertile patients with azoospermia or oligozoospermia and fertile men.

	Fertile men	Infertile patients			*p* Value*		
Haplotype	Total (*n* = 209)	Total (*n* = 409)	Azoospermia (*n* = 204)	Oligozoospermia (*n* = 205)	[1]	[2]	[3]
CA	0.330 (69)	0.228 (93)	0.225 (46)	0.229 (47)	0.006	0.018	0.022
TA	0.344 (72)	0.342 (140)	0.368 (75)	0.317 (65)	0.957	0.623	0.553
CG	0.254 (53)	0.247 (101)	0.205 (42)	0.288 (59)	0.857	0.249	0.433
TG	0.072 (15)	0.183 (75)	0.202 (41)	0.166 (34)	<0.001	<0.001	0.003

*Controls vs. [1] Total infertile patients, [2] Azoospermia, [3] Oligzoospermia.

The allele (genotype) analysis

PCR products were digested with restriction enzymes Tsp451 for –9C>T and Eco911 (Fermentas, Vilnius, Lithuania) for 368A>G overnight according to the manufacturer protocols. Digestion products were then analyzed by electrophoresis on 3% agarose gel. At the –9C>T locus, allele T showed one 212 bp band and allele C showed two bands including 182 bp and 30 bp. Allele A and G of 368A>G locus showed two bands (320 bp and 126 bp) and one band (446 bp), respectively. The alleles were later confirmed by direct DNA sequencing of PCR products of some samples.

Statistical analysis

The allele and haplotype frequencies of –9C>T and 368A>G loci studied in patients and controls were calculated by counting. The frequency differences in allele and haplotype between patients and controls were evaluated using the Pearson χ^2-test. The level of significance was set at $p < 0.05$. A multiple logistic regression analysis was carried out to assess the risk of male idiopathic infertility. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated as a measure of the association of alleles and haplotypes of two SNPs with male idiopathic infertility. All statistical calculations were performed using SPSS 11.0 software (SPSS Inc., Chicago, IL).

Results

This study investigated the polymorphic distributions of SNPs –9C>T and 368A>G in the H2BFWT gene in 409 infertile patients with idiopathic azoospermia or oligozoospermia and 209 fertile men using a PCR-RFLP assay. The allele frequency distributions of the two SNP loci in patient and control groups are listed in Table 2. As shown in Table 2, the frequencies of allele –9T of SNP –9C>T (52.8% vs. 41.6%, $p = 0.009$, OR = 1.569, 95% CI = 1.121–2.198) and allele 368G of SNP 368A>G (43.0% vs. 32.5%, $p = 0.012$, OR = 1.566, 95% CI = 1.104–2.221) were significantly higher in infertile patients than those in controls. After classifying infertile patients into azoospermia and oligozoospermia subgroups, the frequency of allele –9T in azoospermia patients (57.4% vs. 41.6%, $p = 0.001$, OR = 1.886, 95% CI = 1.276–2.787) and allele 368G in oligozoospermia patients (45.4% vs. 32.5%, $p = 0.007$, OR = 1.722, 95% CI = 1.155–2.566) were still significantly higher compared with controls.

Four kinds of haplotypes of the two SNPs were observed in both infertile patients and controls. Haploype TG in total patients (18.3% vs. 7.2%, $p < 0.001$, OR = 2.904, 95% CI = 1.623–5.197), azoospermia (20.2% vs. 7.2%, $p < 0.001$, OR = 3.253, 95% CI = 1.738–6.090), and oligozoospermia (16.6% vs. 7.2%, $p = 0.003$, OR = 2.572, 95% CI = 1.354–4.884) were significantly increased, whereas Haploype CA in patients (22.8% vs. 33.0%, $p = 0.006$, OR = 0.597, 95% CI = 0.413–0.864), azoospermia (22.5% vs. 33.0%, $p = 0.018$, OR = 0.591, 95%CI = 0.382–0.914) and oligozoospermia (22.9% vs. 33.0%, $p = 0.022$, OR = 0.604, 95% CI = 0.391–0.932) was decreased compared with controls. The distribution of haplotypes was summarized in Table 3.

The representative results of allele analysis for 368A>G and –9C>T loci in H2BFWT gene by electrophoresis was shown in Figure 1.

Discussion

Spermatogenesis is a complex developmental process that requires the accurate expression of thousands of various related genes. Underlying genetic defects play an important role in impaired spermatogenesis. Genetic defects, such as abnormal chromosomes, micro-deletion of Y chromosome, and mutation of particular genes have been confirmed to be associated with impaired spermatogenesis (Ferlin et al., 2006, 2007; O'Flynn O'Brien et al., 2010). However, these known genetic

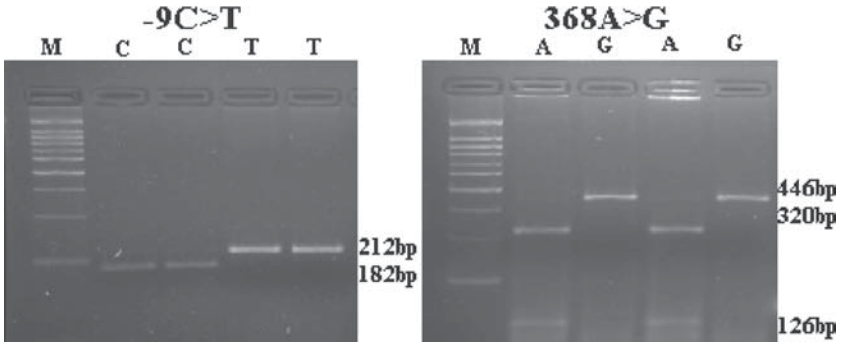

Figure 1. The results of allele analysis for SNP-9C>T and 368A>G by electrophoresis (30 bp band for –9C allele not shown in Figure). M: DNA size marker (100 bp ladder).

defects have been proven to only account for a small proportion of male infertility cases, and the underlying genetic etiology of large number of male infertility cases remains elusive. In recent years, numerous genes related to spermatogenesis have been successfully identified (Grootegoed et al., 1998), some of which may potentially be related to impaired spermatogenesis. However, for most of these genes tested confirmation is still required. In particular, genetic variation of these genes is considered to be a potential risk factor to failed human spermatogenesis, and may be responsible for the majority of idiopathic male infertility cases. Thus, studying SNPs of genes related to spermatogenesis is one of the most important ways to explore the genetic mechanism of idiopathic male infertility (Krausz and Giachini, 2007; Carrell and Aston, 2008; Nuti and Krausz, 2008).

As a new candidate gene for human spermatogenesis impairment, H2BFWT has been proven to be essential for human spermatogenesis (Gineitis et al., 2000; Churikov et al., 2004; Boulard et al., 2006). It was predicted that variation of the gene may be involved in impaired spermatogenesis. To verify the prediction, a case-controlled study of two SNPs, –9C>T and 368A>G, in the H2BFWT gene was conducted on a population of Chinese men afflicted with idiopathic impaired spermatogenesis. At the –9C>T locus, the frequency of –9T was significantly higher in infertile and azoospermia patients than that in fertile controls, suggesting that allele –9T may increase the risk of impaired spermatogenesis and male infertility, especially for azoospermia in Chinese population. SNP –9C>T is located in 5′UTR of exon 1 of the H2BFWT gene. The C to T change affects the expression efficiency of H2BFWT gene, and allele T reduces the translational level in comparison to allele C (Lee et al., 2009). Since H2BFWT plays a key role in spermatogenesis, the reduced translation of H2BFWT caused by allele –9T may lead to impaired spermatogenesis (Zhang et al., 2006; Zini et al., 2008).

In the present study, a significant difference in allele frequency distribution at the 368A>G locus was detected. The frequency of 368G was significantly higher in total infertile patients and oligozoospermia patients than that in control patients, indicating that polymorphism of 368A>G is associated with impaired spermatogenesis, and allele G may be a risk factor of oligozoospermia. 368A>G is a missense mutation at exon 1 and the allelic change of A to G contribute amino acid of 123 position His to Arg. We speculate that the exchange from His to Arg may affect the function of H2BFWT protein, subsequently impairing normal spermatogenesis. Of course, this speculation needs to be confirmed by further functional studies. Since it is not clear whether this missense mutation affects the function of H2BFWT, the polymorphism may be simply a genetic marker of impaired spermatogenesis that is linked with other locus susceptible to impaired spermatogenesis.

A recent study of a South Korean population reported that allele –9T was associated with impaired spermatogenesis and male infertility, but not with azoospermia and that SNP 368G was not associated with male infertility (Lee et al., 2009), which is somewhat contradictory to the findings of the present study. The contradictory results might be explained by difference in ethnic genetic background or different inclusion criteria for patients.

To further investigate the effects of the two SNPs on impaired spermatogenesis and male infertility, haplotype analysis of patient and control groups was performed. The results of haplotype analysis showed that haplotype CA significantly decreased and TG increased in each patient group in comparison to the control group, suggesting that haplotype TG may increase the susceptibility to azoospemia or oligozoospermia in males, and haplotype CA may be a protection factor from impaired spermatogenesis. It is interesting that haplotype TG (OR = 2.904) increased the risk of male infertility approximately one fold in comparison to allele T (OR = 1.569) or G (OR = 1.566) alone in the present study, demonstrating a combined effect of the two SNPs on susceptibility to spermatogenesis impairment. These findings again suggest that the two SNPs of H2BFWT gene may contribute the predisposition of males to impaired spermatogenesis and infertility.

Conclusions

In summary, the present study has demonstrated an association of SNPs –9C>T and 368A>G in H2BFWT

gene with male infertility with idiopathic azoospermia or oligospermia, and allele T and G as well as haplotype TG may increase the risk of impaired spermatogenesis in Chinese men. This provides evidence that H2BFWT gene may be involved in with impaired spermatogenesis and male infertility. Further studies in different ethnic populations, as well as in larger size samples and functional research are still needed to confirm our findings, and elucidate the role of H2BFWT gene in spermatogenesis impairment.

Declaration of interest

This work was supported by National Natural Science Foundation of China (Grant number: 30770809). The authors have declared no conflicts of interest with respect to the authorship and/or publication of this article.

References

Ausió J. (2006). Histone variants–the structure behind the function. *Brief Funct Genomic Proteomic* 5:228–243.

Boulard M, Gautier T, Mbele GO, Gerson V, Hamiche A, Angelov D, Bouvet P, Dimitrov S. (2006). The NH2 tail of the novel histone variant H2BFWT exhibits properties distinct from conventional H2B with respect to the assembly of mitotic chromosomes. *Mol Cell Biol* 26:1518–1526.

Carrell DT, Aston KI. (2011). The search for SNPs, CNVs, and epigenetic variants associated with the complex disease of male infertility. *Syst Biol Reprod Med* 57:17–26.

Churikov D, Siino J, Svetlova M, Zhang K, Gineitis A, Morton Bradbury E, Zalensky A. (2004). Novel human testis-specific histone H2B encoded by the interrupted gene on the X chromosome. *Genomics* 84:745–756.

De Kretser DM, Baker HW. (1999). Infertility in men: recent advances and continuing controversies. *J Clin Endocrinol Metab* 84:3443–3450.

Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, Foresta C. (2007). Male infertility: role of genetic background. *Reprod Biomed Online* 14:734–745.

Ferlin A, Arredi B, Foresta C. (2006). Genetic causes of male infertility. *Reprod Toxicol* 22:133–141.

Gineitis AA, Zalenskaya IA, Yau PM, Bradbury EM, Zalensky AO. (2000). Human sperm telomere-binding complex involves histone H2B and secures telomere membrane attachment. *J Cell Biol* 151:1591–1598.

Grootegoed JA, Baarends WM, Roest HP, Hoeijmakers JH. (1998). Knockout mouse model and gametogenic failure. *Mol Cell Endocrinol* 145:161–166.

Henikoff S, Ahmad K. (2005). Assembly of variant histones into chromatin. *Annu Rev Cell Dev Biol* 21:133–153.

Krausz C, Giachini C. (2007). Genetic risk factors in male infertility. *Arch Androl* 53:125–133.

Cole KD, Kandala JC, Kistler WS. (1986). Isolation of the gene for the testis-specific H1 histone variant H1t. *J Biol Chem* 261:7178–7183.

Kimmins S, Sassone-Corsi P. (2005). Chromatin remodelling and epigenetic features of germ cells. *Nature* 434:583–589.

Lee J, Park HS, Kim HH, Yun YJ, Lee DR, Lee S. (2009). Functional polymorphism in H2BFWT-5'UTR is associated with susceptibility to male infertility. *J Cell Mol Med* 13:1942–1951.

Nuti F, Krausz C. (2008). Gene polymorphisms/mutations relevant to abnormal spermatogenesis. *Reprod Biomed Online* 16:504–513.

O'Flynn O'Brien KL, Varghese AC, Agarwal A. (2010). The genetic causes of male factor infertility: a review. *Fertil Steril* 93:1–12.

Pradeepa MM, Rao MR. (2007). Chromatin remodeling during mammalian spermatogenesis: role of testis specific histone variants and transition proteins. *Soc Reprod Fertil Suppl* 63:1–10.

Stouffs K, Tournaye H, Liebaers I, Lissens W. (2009). Male infertility and the involvement of the X chromosome. *Hum Reprod Update* 15:623–637.

Steger K, Klonisch T, Gavenis K, Drabent B, Doenecke D, Bergmann M. (1998). Expression of mRNA and protein of nucleoproteins during human spermiogenesis. *Mol Hum Reprod* 4:939–945.

Simoni M, Bakker E, Krausz C. (2004). EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. *Int J Androl* 27:240–249.

Tanaka H, Iguchi N, Isotani A, Kitamura K, Toyama Y, Matsuoka Y, Onishi M, Masai K, Maekawa M, Toshimori K, Okabe M, Nishimune Y. (2005). HANP1/H1T2, a novel histone H1-like protein involved in nuclear formation and sperm fertility. *Mol Cell Biol* 25:7107–7119.

van Roijen HJ, Ooms MP, Spaargaren MC, Baarends WM, Weber RF, Grootegoed JA, Vreeburg JT. (1998). Immunoexpression of testis-specific histone 2B in human spermatozoa and testis tissue. *Hum Reprod* 13:1559–1566.

World Health Organization. (2010). WHO laboratory manual for the examination and processing of human semen, 5th edition. Geneva:World Health Organization.

Zhang X, San Gabriel M, Zini A. (2006). Sperm nuclear histone to protamine ratio in fertile and infertile men: evidence of heterogeneous subpopulations of spermatozoa in the ejaculate. *J Androl* 27:414–420.

Zini A, Zhang X, San Gabriel M. (2008). Sperm nuclear histone H2B: correlation with sperm DNA denaturation and DNA stainability. *Asian J Androl* 10:865–871.