Male infertility is not liked with HSF1, HSF2 and UBE2I gene polymorphisms among Indian subjects

Pravin Kumar Gangwar¹, Satya Narayan Sankhwar¹, Shriya Pant¹, Bhupendra Pal Singh¹, Abbas Ali Mahdi² & Rajender Singh³*

¹Department of Urology, King George’s Medical University, Lucknow, U.P., India; ²Department of Biochemistry, King George’s Medical University, Lucknow, U.P., India; ³Division of Endocrinology, Central Drug Research Institute, Lucknow, U. P., India.

*Corresponding authors - Prof. Satya Narayan Sankhwar, E-mail: sankhwarsn@yahoo.com; Dr. Rajender Singh, E-mail: nainrs@gmail.com.

Author contacts:
Pravin Kumar Gangwar - gangwar.pravin1986@gmail.com; Satya Narayan Sankhwar - sankhwarsn@yahoo.com; Shriya Pant - shreyapant.18sep@gmail.com; Bhupendra Pal Singh - bpsingh@kgmcindia.edu; Abbas Ali Mahdi - abbasalimahdi@kgmcindia.edu; Rajender Singh - nainrs@gmail.com.

Received July 21, 2021; Revised August 9, 2021; Accepted August 9, 2021, Published August 31, 2021

DOI: 10.6026/97320630017715

Declaration on Publication Ethics:
The author’s state that they adhere with COPE guidelines on publishing ethics as described elsewhere at https://publicationethics.org/. The authors also undertake that they are not associated with any other third party (governmental or non-governmental agencies) linking with any form of unethical issues connecting to this publication. The authors also declare that they are not withholding any information that is misleading to the publisher in regard to this article.

Author responsibility:
The authors are responsible for the content of this article. The editorial and the publisher have taken reasonable steps to check the content of the article in accordance to publishing ethics with adequate peer reviews deposited at PUBLONS.

Declaration on official E-mail:
The corresponding author declares that official e-mail from their institution is not available for all authors.

Abstract:
We analysed the polymorphisms at rs78202224 (C/A) for HSF1 gene, rs139496713 (C/T) and rs45504694 (C/A) for HSF2 gene and rs116868327 (G/A) for UBE2I gene in 547 infertile cases (non-obstructive azoospermia = 464, asthenozoospermia = 83) and 419 proven fertile controls of similar age group and ethnicity. SNP genotyping was done using AgenaMassARRY platform (Agena Bioscience, CA). Common, heterozygous, rare genotypes and allelic frequencies were analysed using dominant, recessive and codominant models. Data shows no significant association between HSF1, HSF2 polymorphisms and male infertility. However, under dominant (GG vs GA+AA) and co-dominant (GG vs GA) model, polymorphism at the rs116868327 (G/A) locus in UBE2I gene was found to be linked with asthenozoospermia in males with a significant odd-ratio of 6.91 (confidence interval at 95% was 1.52-31.46; p=0.017). Moreover, frequency of rare allele was higher (24.4%) compared to controls (0.4%). Thus, this data showed a significant risk of developing asthenozoospermic condition in males (Odds ratio= 6.75; Confidence interval at 95%= 1.50-30.49; P= 0.018). Hence, more number of genotyping studies along with the functional assay in multiple cohorts is needed to validate potential variants associated with male infertility.

Keywords: Heat shock factor genes; Ubiquitin conjugating enzyme E2I; Single nucleotide polymorphism; Male infertility.

Background:
Heat shock factor 1 (HSF1) is required for the response against cellular stress and known as the primary transcription factor, while HSF2 is also involved in the regulation of heat shock protein expression to support cells against environmental stresses as well as in cellular processes and spermatogenesis [1]. Active form of HSF1 is responsible for the disturbances in spermatogenesis may be due to the mutations/ polymorphisms in this gene. It affects the spermatogenesis and causes the death of spermatocytes at pachytene stage where HSF1 remain present in an active state which could be a sign of accretion of defected proteins and induced cell death [2, 3]. Whereas, HSF1 null mouse,
having a complete loss of its activity displayed normal fertility. It also plays an important task by protecting immature germ cells along with spermatogonia against testis hyperthermia [4]. Taken together, HSF1 plays two opposite roles in spermatogenesis and can be involved in quality control of male germ cells [2-4]. Interestingly, loss of HSF2 activity resulted in a phenotype with low testicular volume and sperm count with increased apoptosis. Further, complete loss of both HSF1 and HSF2 gene function promotes severe defects in spermatogenesis causing sterility [5]. These data indicate the importance of the transcriptional activity of both HSF1 and HSF2 for normal spermatogenesis. UBE2I (Ubiquitin Conjugating Enzyme E2I) gene encodes a SUMO-conjugating enzyme UBC9 in humans, mainly expressed in heart, pancreas, kidney, liver, lung, skeletal muscle, placenta, brain and in testis as well. It plays an important role in sumoylation and ubiquitination processes. Recently, sumoylation has emerged as a crucial regulator of proteins with significant roles in spermatogenesis [6], Rogers et al. (2004) initially identified SUMO proteins in XY body of pachytene spermatocytes in rat and suggested the crucial function of sumoylation in spermatogenesis [7]. Several experimental studies suggest the significance of SUMO modifications in meiosis and spermatid elongation [6, 8]. Above data indicates the role of different SUMO isoforms in protein modifications during germ cell development. On the other hand excessive sumoylation has been observed as a potential marker of defective sperms in human [6]. Therefore, we may suggest an important role of UBE2I gene in spermatogenesis via SUMO conjugation pathway. These genes were selected based on their known expression and function in spermatogenesis/ spermiogenesis. However, data on genetic variations in HSF1, HSF2 and UBE2I genes for infertility in males is scanty. A single study on HSF2 gene in this context is known [9]. Therefore, it is of interest to document data on the link between male infertility and HSF1, HSF2 and UBE2I gene polymorphisms.

Materials and Methods:
Study Population
The study was done on the patients of Non-obstructive azoospermia (NOA) and Asthenozoospermia excluding obstructive azoospermia. The study was approved from the Institutional Ethics Committee, K.G.M.U, Lucknow, U.P, India (Letter no.1163/R-Cell/12, Dated-14/04-2012). All the participants were enrolled from the Department of Urology, King George’s Medical University (K.G.M.U). Lucknow, U.P, India, after obtaining their informed written consent. All the study participants belonged to the Indo-European ethnicity. All the subjects underwent detailed medical and physical examinations before sample collection. The case group consists of infertile men aged 21 to 40 years, having infertility more than one year with a normal fertile female partner to avoid any possibility of involvement of female factors. Further, the subjects with endocrine abnormalities, acquired and congenital structural defects of the urogenital system (cystic fibrosis, Young’s syndrome, etc.), karyotype abnormalities showing chromosomal defects and patients with any history of surgery of genital tract obstruction/dysfunction (varicocele, obstructive azoospermia) were excluded. The infertile individuals with excessive alcoholism, smoking, drug abuse (ecstasy, marijuana and recreational substances) and having any history of radiotherapy and chemotherapy were also excluded. The patient group, after following the above inclusion and exclusion criteria, comprised of 547 infertile individuals. Semen sample of all the patients was collected by masturbation after an abstinence period of 3-5 days. Semen parameters were analysed following the criteria of the World Health Organization (WHO), (2010) [10]. The patients were distributed into azoospermia (absence of mature sperm in semen, n = 464) and asthenozoospermia (progressive sperm motility < 32% and sperm count ≥ 15 million/ml, n = 83). In control group, 419 proven fertile men were enrolled, belonged to the same age-group (21–40 years) and ethnicity as that of the cases, who had fathered a child during the last three years without having history of any sexual abnormality. All the control samples were collected from the individuals visiting the urology OPD for problems other than infertility. We also collected 5 ml blood sample from all the study participants for DNA isolation and further genotyping experiments.

SNP Genotyping:
SNP genotyping was performed using AgenaMassARRY platform (Agena Bioscience, CA). The MassARRY system is non-fluorescent detection uses mass spectrometry to accurately measure polymerase chain reaction (PCR)-derived amplicons. It has high capability of multiplexing up to around 40plex from a single well. In brief, genotyping perform in two steps of PCR reactions; In the first step, a locus-specific PCR was run to amplify the DNA stretch containing the polymorphic site. In the second step, a single base extension was performed using mass modified dideoxy nucleotide terminators of oligonucleotide primer, which annealed immediately upstream of the polymorphic site of interest. Further, the distinct mass of the extended primer was identified as the SNP allele using MALDI-TOF mass spectrometry.

Statistical Analysis:
Statistical analysis was performed using the bio statistical tools online (http://www.vassarstats.net) and using different models such as genotypic, allelic, dominant, recessive and co-dominant models. Comparison of genotypes was performed by Fisher exact probability test. Statistical significance at p-value < 0.05 was considered as a significant difference.

Results:
A total of 4 SNPs, a missense variant; rs78202224 (C/A) for HSF1 gene, a 3’ UTR variant; rs139496713 (C/T) and a 5’ UTR variant; rs45504694 (C/A) for HSF2 gene and one 3’ UTR variant; rs116868327 (G/A) for UBE2I gene were selected based on their SIFT, PolyPhen and GMAF scores. We did large scale SNP genotyping in 966 samples. This cohort of samples included 419 fertile control samples and 547 infertile samples (NOA, n=464 and asthenozoospermia; n= 83). Average genotype calling in our study cohort was more than 95%, which depicted that genotyping was successful in 958 samples (545 cases and 413 controls) for rs78202224 (C/A) for HSF1 gene, 921 samples (527 cases and 394 controls) for rs139496713 (C/T), 943 samples (538 cases and 405 controls) for rs45504694 (C/A) for HSF2 gene and 946 samples (539 cases and 407 controls) for rs116868327 (G/A) for UBE2I gene. We found that all SNPs in our study cohort had minor allele frequency (MAF) more than or equal to 1%. MAF was ranging from 1% (rs116868327 (G/A) locus in HSF2 gene) to 18% (rs45504694 (C/A) locus in HSF2 gene). (Table 1) The frequencies of genotypes CC, CA and AA for rs78202224 (C/A) for HSF1 gene in all infertile, azoospermic and asthenozoospermic cases were 71.4%, 26.6% and 2.0%; 71.7%, 26.5% and 2.0% and 0%; 62.8%, 37.2% and 0%; 62.3%, 37.7% and 0% and 100%, 0% and 0% while in controls it was 97.2%, 2.8% and 0%; 96.8%, 3.2% and 0%; 96.2%, 3.8% and 0% and 0% and 100%, 0% and 0% respectively. (Table 2) Whereas, the genotype frequencies of CC, CT and TT for rs139496713 (C/T) for HSF2 gene in all infertile, azoospermic and asthenozoospermic individuals were found to be 96.8%, 3.2% and 0%, 96.2%, 3.8% and 0% and 0% and 0% and 0% and 0% while in controls it was 97.2%, 2.8% and 0%, respectively. (Table 3) In addition, frequencies of CC, CA and AA genotypes of rs45504694 (C/A) for HSF2 gene in all infertile, azoospermic and asthenozoospermic patients were observed as 62.8%, 37.2% and 0%; 62.3%, 37.7% and 0% and 100%, 0% and 0%.

Bioinformation 17(8): 715-720 (2021) ISSN 0973-2063 (online) 0973-8894 (print) ©Biomedical Informatics (2021)
65.9%, 34.1% and 0%, while in controls it was 64.2%, 35.6% and 0.2%, respectively. (Table 4) No significant association was observed between case and control group for variations at rs78202224 C/A, rs139496713 C/T and rs45504694 (C/A) of HSF1 and HSF2 genes and susceptibility to infertility in males. Moreover, allelic frequencies of variations in HSF1 and HSF2 genes showed no significant differences between the two groups (p<0.05). On the other hand, genotypic distribution of GG, GA and AA genotypes for SNP (rs116868327, G/A) locus in UBE2I gene was 98.7%, 1.3% and 0% in all infertile patients; 99.3%, 0.7% and 0% in asthenozoospermic group but in control group it was 99.3%, 0.7% and 0%, respectively. The genotypic analysis under dominant model (GG vs GA+AA) and co-dominant/ additive model (GG vs GA) showed a significant association with asthenozoospermia [GG vs GA+AA: Odds ratio (95% Confidence interval) = 6.91 (1.52-31.46), P value = 0.017; GG vs GA: Odds ratio (95% Confidence interval) = 6.91 (1.52-31.46), P value = 0.017]. Moreover, rare allele frequency in asthenozoospermic group was higher than control group and it also showed a significant association with asthenozoospermia [Odds ratio (95%Confidence interval) = 6.75 (1.50-30.49), P value = 0.018]. (Table 5)

Table 1: SNPs, genotype distribution and allelic frequencies in the study cohort

| Gene      | Common Genotype | Heterozygote Genotypes | Rare Genotype | Total | % Genotype Calling | Minor allele freq. |
|-----------|-----------------|------------------------|---------------|-------|--------------------|--------------------|
| HSF1      | rs 7802224c     | 683                    | 252           | 23    | 958                | 99.17              |
|           |                 |                        |               |       | 0.84               | 0.16               |
| HSF2      | rs139496713b    | 893                    | 28            | 0     | 921                | 95.34              |
|           |                 |                        |               |       | 0.98               | 0.02               |
|           | rs45504694c     | 598                    | 344           | 1     | 943                | 97.62              |
|           |                 |                        |               |       | 0.82               | 0.18               |
| UBE2I     | rs 116868327d   | 936                    | 10            | 0     | 946                | 97.92              |
|           |                 |                        |               |       | 0.99               | 0.01               |

Table 2: Distribution of Genotypes, n (%) for HSF1 gene, rs78202224 in infertile men

| Genotype/Allele | Controls; n=413 (%) | All cases; n=545 (%) | OR (95% CI) | p-Value |
|-----------------|-----------------------|----------------------|-------------|---------|
| Genotype       | CC (71.2) | 389 (71.4)   | Ref.          |         |
| CA 107 (25.9) | 145 (26.6) | 1.02 (0.76-1.37) | 0.88   |
| AA 12 (2.9)   | 11 (2.0)   | 0.69 (0.30-1.59) | 0.40   |
| CA+AA 119     | 156       | 0.99 (0.75-1.31) | 1.0     |
| Allele         | C 495 (84.1) | 923 (84.7)   | Ref.          |         |
| A 131 (15.9)  | 167 (15.3)  | 0.96 (0.75-1.23) | 0.791   |

Table 3: Distribution of Genotypes, n (%) for HSF2 gene, rs139496713 in infertile men

| Genotype/Allele | Controls; n=394 (%) | All cases; n=527 (%) | OR (95% CI) | p-Value |
|-----------------|----------------------|----------------------|-------------|---------|
| Genotype       | CC (97.4) | 383 (96.8)   | Ref.          |         |
| CT 11 (2.8)  | 17 (3.2)    | 1.16 (0.54-2.51) | 0.850   |
| TT 0 (0.0)    | 0 (0.0)     | -                  | -           |
| CT+TT 11      | 17         | 1.16 (0.54-2.51) | 0.850   |
| Allele         | C 777 (98.6) | 1037 (98.4)  | Ref.          |         |
| T 11 (1.4)    | 17 (1.6)    | 1.16 (0.54-2.49) | 0.863   |

OR: Odds-ratio; CI: Confidence interval; Ref: Reference
Various experimental reports on mouse models have proven the important function of HSF1 and HSF2 genes in germ cell development in males while UBE2I gene in oocyte development in females [1-5, 12]. To our surprise, we could find only two studies in humans that have looked into this aspect till now. First study by Mou et al. (2013) highlighted the association of genetic variants of HSF2 gene with idiopathic azoospermia (IA) in males while another study tried to explore the potential role of SNPs in HSF1 gene with human diseases [9, 13]. Many genetic association studies have documented the potential impact of HSF1 and HSF2 on human health and diseases and tried to connect HSF1 gene variants and its altered levels, to schizophrenia, bipolar disorder, attention deficit hyperactivity disorder and breast cancer [14-17]. In the same way, low level of HSF2 mRNA was also observed in different types of malignancies in humans like invasive breast carcinoma, prostate carcinoma and various other carcinomas [16-18]. Moreover, UBE2I gene also plays an important role in progression of several cancers as lung, breast and bladder carcinoma [19, 20].

It is of interest to explore the biological and clinical significance of genetic variants, this study evaluated the genetic polymorphisms of HSF1, HSF2 and UBE2I genes in human and tried to find out their involvement in the pathogenesis of male infertility. This

| Table 4: Distribution of Genotypes, n (%) for HSF2 gene, rs45504694 in infertile men |
|----------------------------------|------------------------|------------------------|-------------------|------------------------|
| Genotype/Allele                  | Controls; n=405 (%)    | All cases; n=538 (%)   | OR (95% CI)        | p-Value                |
| Genotype                         |                        |                        |                   |                        |
| CC                               | 260 (64.2)             | 338 (62.8)             | Ref.              |                        |
| CA                               | 144 (35.6)             | 200 (37.2)             | 1.07 (0.82-1.4)   | 0.630                  |
| AA                               | 1 (0.2)                | 0 (0.0)                | -                 |                        |
| CA+AA                            | 145 (35.4)             | 200 (37.2)             | 1.06 (0.81-1.39)  | 0.680                  |
| Allele                           |                        |                        |                   |                        |
| C                                | 664 (82.0)             | 876 (81.4)             | Ref.              |                        |
| A                                | 146 (18.0)             | 200 (18.6)             | 1.04 (0.82-1.31)  | 0.807                  |
| Genotype/Allele                  | Controls; n=405 (%)    | Azoospermic; n=456 (%) | OR (95% CI)        | p-Value                |
| Genotype                         |                        |                        |                   |                        |
| CC                               | 260 (64.2)             | 284 (62.3)             | Ref.              |                        |
| CA                               | 144 (35.6)             | 172 (37.7)             | 1.09 (0.83-1.44)  | 0.571                  |
| AA                               | 1 (0.2)                | 0 (0.0)                | 0                 | 0.457                  |
| CA+AA                            | 145 (35.4)             | 172                  | 1.08 (0.82-1.43)  | 0.572                  |
| Allele                           |                        |                        |                   |                        |
| C                                | 664 (82.0)             | 740 (81.1)             | Ref.              |                        |
| A                                | 146 (18.0)             | 172 (18.9)             | 1.06 (0.83-1.35)  | 0.698                  |
| Genotype/Allele                  | Controls; n=405 (%)    | Asthenozoospermic; n=82 (%) | OR (95% CI)        | p-Value                |
| Genotype                         |                        |                        |                   |                        |
| CC                               | 260 (64.2)             | 54 (65.9)              | Ref.              |                        |
| CA                               | 144 (35.6)             | 28 (34.1)              | 0.93 (0.56-1.54)  | 0.802                  |
| AA                               | 1 (0.2)                | 0 (0.0)                | -                 | -                      |
| CA+AA                            | 145 (35.4)             | 28                  | 0.93 (0.56-1.54)  | 0.802                  |
| Allele                           |                        |                        |                   |                        |
| C                                | 664 (82.0)             | 136 (82.9)             | Ref.              |                        |
| A                                | 146 (18.0)             | 28 (17.1)              | 0.94 (0.60-1.46)  | 0.863                  |

| Table 5: Distribution of Genotypes, n (%) for UBE2I gene, rs116868327 in infertile men |
|----------------------------------|------------------------|------------------------|-------------------|------------------------|
| Genotype/Allele                  | Controls; n=407 (%)    | All cases; n=539 (%)   | OR (95% CI)        | p-Value                |
| Genotype                         |                        |                        |                   |                        |
| GG                               | 404 (99.3)             | 532 (98.7)             | Ref.              |                        |
| GA                               | 3 (0.7)                | 7 (1.3)                | 1.8 (0.46-6.9)    | 0.530                  |
| AA                               | 0 (0.0)                | 0 (0.0)                | -                 | -                      |
| GA+AA                            | 3                     | 7                     | 1.8 (0.46-6.9)    | 0.530                  |
| Allele                           |                        |                        |                   |                        |
| G                                | 811 (99.6)             | 1071 (99.4)            | Ref.              |                        |
| A                                | 3 (0.4)                | 7 (0.6)                | 1.77 (0.46-6.85)  | 6.10                   |
| Genotype/Allele                  | Controls; n=407 (%)    | Azoospermic; n=457 (%) | OR (95% CI)        | p-Value                |
| Genotype                         |                        |                        |                   |                        |
| GG                               | 404 (99.3)             | 454 (99.3)             | Ref.              |                        |
| GA                               | 3 (0.7)                | 3 (0.7)                | 0.88 (0.18-4.43)  | 1.0                    |
| AA                               | 0 (0.0)                | 0 (0.0)                | -                 | -                      |
| GA+AA                            | 3                     | 3                     | 0.88 (0.18-4.43)  | 1.0                    |
| Allele                           |                        |                        |                   |                        |
| G                                | 811 (99.6)             | 911 (99.7)             | Ref.              |                        |
| A                                | 3 (0.4)                | 3 (0.3)                | 0.89 (0.18-4.42)  | 1.91                   |
| Genotype/Allele                  | Controls; n=407 (%)    | Asthenozoospermic; n=82 (%) | OR (95% CI)        | p-Value                |
| Genotype                         |                        |                        |                   |                        |
| GG                               | 404 (99.3)             | 78 (95.1)              | Ref.              |                        |
| GA                               | 3 (0.7)                | 4 (4.9)                | 6.91 (1.52-31.46) | 0.017                  |
| AA                               | 0 (0.0)                | 0 (0.0)                | -                 | -                      |
| GA+AA                            | 3                     | 4                     | 6.91 (1.52-31.46) | 0.017                  |
| Allele                           |                        |                        |                   |                        |
| G                                | 811 (99.6)             | 160 (97.6)             | Ref.              |                        |
| A                                | 3 (0.4)                | 4 (2.4)                | 6.75 (1.50-30.49) | 0.018                  |
study depicted the presence of rs7820224 variant (rare) genotype of HSF1 gene exclusively in 23 DNA samples out of 958 subjects. It suggest that the rare genotype frequency of this variant might be somewhat lower in our study population. Similarly, Bridges et al. (2015) found this variant in only 3 of 84 samples of different ethnicity. All the three reported subjects were of African American ethnicity, which is quite higher than our study group. Interestingly, they reported a novel SNP C1220A in 3 of 48 subjects with Asian ethnicity with 6% minor allele frequency [13]. These findings are contradictory to our results. On the basis of the genotypic analysis of our findings, we could not find any relationship between rs7820224 (C>A), rs139496713 (C>T) and rs45504694 (C>A) SNPs in HSF1 and HSF2 genes and male infertility. However, a association of rs78202224 of HSF1 was observed by Almotwaa et al. (2018) with breast cancer in Saudi females. On the other hand, Bridges et al. (2015) described 34 variants in the exonic sequence of human HSF1 gene and tried to analyse their biological consequences in human diseases [13, 17]. Mou et al. (2013) identified three synonymous and five missense mutations of HSF2 gene in IA patients. Study demonstrated that the mutant genotype of HSF2 (R502H) suppressed the transcriptional regulatory function of the wild type allele through a dominant-negative effect and might be involved in human spermatogenesis failure. It suggested further implication of HSF2 as a potential therapeutic target [9]. These studies are in contrary to our observations.

Interestingly, both allelic and genotype association analysis revealed that the rs11686327 (G/A) variant in UBE2I gene is significantly associated with asthenozoospermia. Moreover, the genotype distribution between cases and controls also revealed that heterozygous condition at rs11686327 (G/A) locus is associated with an increased risk of male infertility [Odds ratio= 6.91, Confidence interval at 95% = 1.52-31.46, P value= 0.017]. Moreover, the allelic association analysis depicted that allelic distribution at rs11686327 locus in UBE2I gene differed significantly between cases and controls [Odds ratio= 6.75, Confidence interval at 95% = 1.50-30.49, P value= 0.018]. Similarly, some previous studies also depicted the role of UBE2I gene polymorphism in breast tumour progression. These studies concluded that women carrying the rare allele for rs7187167 in UBE2I gene showed an increased risk of grade 1 breast tumours [21, 22]. Selection of variants for large-scale cohort analysis was a big challenge. While common variants may not be the risk factor for the disease and rare variants may not be present in the population at all. Accepting all the common variants and ignoring the rare variants may not be the way ahead. Therefore, we used a filtration method to find out the variants of interest. SNPs based on SIFT and Polyphen scores provided the work for future perspective.

Conclusion:
Variation at rs11686327 locus in UBE2I gene increases the risk of asthenozoospermia. Allelic association analysis suggested that rare allele frequency was more in cases associated with the risk of asthenozoospermia in infertile males. However, none of the SNPs in HSF1 and HSF2 genes is linked with infertility in men in the study cohort. However, it is known earlier that HSF1, HSF2 and UBE2I genes are essential regulators for spermatogenesis. Thus, a representative analysis of variants/mutations of HSF1, HSF2 and UBE2I genes in multiple cohorts along with their functional assay will provide insights into the cause of male infertility.

Acknowledgement:
This study was financially supported by the Council of Science and Technology, U.P., Lucknow (letter no. CST/SERP/D-215, dated-11/05/2015). We would like to thank all the volunteers who participated in the study.

References:
[1] Ji Z et al. Asian Pacific journal of Reproduction. 2012 1:76.
[2] Nakai A et al. EMBO Journal. 2000 19:1545. [PMID: 10747023]
[3] De-Rooji DG & de-Boer P. Cytogenet Genome Res. 2003 103:267. [PMID: 15051947]
[4] Izu H, et al. Biology of reproduction. 2004 70:18. [PMID: 12954729]
[5] Eddy EM & O’Brien DA. Curr Top Dev Biol. 1997 37:141. [PMID: 9352186]
[6] Vigodner M, et al. Hum Reprod. 2013 28:210. [PMID: 23077236]
[7] Rogers RS et al. Chromosoma. 2004 113:233. [PMID: 15349788]
[8] Vigodner M & Morris PL. Dev Biol. 2005 282:480. [PMID: 15950612]
[9] Mou L et al. Human genetics. 2013 132:159. [PMID: 23064888]
[10] www.who.int
[11] Christians E et al. Nature. 2000 407:693. [PMID: 11048707]
[12] Rodriguez A et al. Development. 2019 146:1.
[13] Bridges TM et al. Cell Stress Chaperones. 2015 20:47.
[14] Lasky-Su J et al. Am J Med Genet B Neuropsychiatr Genet. 2008 147B:1355. [PMID: 18937294]
[15] Wang KS et al. Schizophr Res. 2010 124:192. [PMID: 20889512]
[16] Santagata S et al. PNAS. 2011 108:18378. [PMID: 22042860]
[17] Almotwaa S et al. PLoS ONE. 2018 13:e0193095. [PMID: 29494616]
[18] Bjork K et al. Oncogene. 2016 35:1770. [PMID: 26119944]
[19] Li H et al. Oncology reports. 2013 29:1588. [PMID: 23381475]
[20] Huang X et al. Scientific Reports. 2020 10:20670. [PMID: 33244139]
[21] Wozniak K et al. Pathol. Oncol. Res. 2014 20:67. [PMID: 23873416]
[22] Dunnebier T et al. Breast Cancer Res Treat. 2010 121:185. [PMID: 19760037]

License statement: This is an Open Access article which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. This is distributed under the terms of the Creative Commons Attribution License.

Edited by P Kanguane

Citation: Gangwar et al. Bioinformation 17(8): 715-220 (2021)
