Indian J.Sci.Res. 18(2): 01-08, 2018

ISSN: 0976-2876 (Print)
ISSN: 2250-0138(Online)

YOGA BASED LIFESTYLE INTERVENTION IN THE MANAGEMENT OF RECURRENT IMPLANTATION FAILURE

VIDHU DHAWAN[a], RAJIV KUMAR[b], NEENA MALHOTRA[c], NEETA SINGH[d] AND RIMA DADA[e1]

[ae]Laboratory for Molecular Reproduction & Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India
[b]Department of Urology, All India Institute of Medical Sciences, New Delhi, India
[cd]Department of Obstetrics & Gynaecology, All India Institute of Medical Sciences, New Delhi, India

ABSTRACT

Recurrent implantation failure (RIF), the failure to achieve clinical pregnancy in minimum three fresh or frozen cycles in ART is a stressful condition for the couples undergoing treatment for infertility. The management of paternal factors for RIF should not be ignored. Studies have quoted the impact of yoga based lifestyle interventions (YBLI) on oxidative stress and DNA damage in sperm but its impact on the dysregulation of selective paternal transcripts has not been evaluated. This prospective ongoing exploratory study was designed with an aim to assess the impact of YBLI on sperm gene expression, oxidative stress and DNA damage. It was done on 30 male partners of females who experienced RIF from August 2016 to July 2017 in YBLI program. Semen samples were obtained both pre- and post- YBLI (21 days). Gene expression analysis was done on spermatozoal *OGG1, PARP1, FOXG1*, *SOX3,RPS6, RBM9, RPS17,* and *RPL29* by q-PCR. The extent of DNA damage and oxidative stress was also assessed at baseline and end of intervention to measure DNA fragmentation index (DFI) and reactive oxygen species (ROS) levels respectively. An upregulation in expression of *OGG1, PARP1 SOX3, RPS17* and dowregulation of *FOXG1*, *RBM9,* and *RPL29* was seen. There was a significant reduction in the ROS levels, increase in sperm progressive motility, sperm count (done twice) and minimal non significant decrease in DFI was also seen. DNA damage shows significant reduction after 6 months of yoga practice. YBLI may aid in the regulation of sperm transcript levels along with correction of oxidative DNA damage which may help in improving implantation rates and pregnancy outcomes.

KEY WORDS: Sperm DNA Damage, Dysregulation, Meditation, Oxidative Stress, Sperm Transcript, Yoga

There is a growing body of evidence relating to the dramatic impact of urbanization and various lifestyle factors and poor social habits on health and well-being of people. Lifestyle factors in this unregulated western society play a pivotal role in both positively and negatively affecting the reproductive health. Infertility, a complex lifestyle-related disorder has become a major medical and social preoccupation. With almost half of the cases being contributed by male infertility, 8%-10% men are affected in the reproductive age (Dada and Tolahunase, 2018). Male infertility is the cause of more than 40% of patients reverting to assisted reproductive techniques as a resort to the management of the same. Though the pregnancy rate following ART can be as high as 60% but despite best of management, couples do fail repeatedly (Margalioth et al., 2006). Recurrent implantation failure (RIF) is the failure to achieve pregnancy in minimum three fresh or frozen cycles after transfer of at least four good quality embryos was done (Simon & Laufer, 2012; Coughlan et al., 2014). This is a stressful condition not only from the medical perspective but also carries immense psychological burden on the couples. Various psychological interventions to address this issue have been adopted over several years. However, motivating the patients to adopt lifestyle modifications is particularly challenging. Though an allopathic approach for the management had been the norm but the knowledge about holistic, complementary and alternative medicine (CAM) approaches has picked up the pace. CAM is popularizing in the rapidly growing field of regenerative medicine in the current era of genomics. Integrative medicine (IM) integrating both the allopathic and complementary medicine approaches is the recent normality being propagated (Kanherkar et al., 2017).

Apart from various embryonic, endometrial and immunological factors playing a role in the implantation failures, the paternal contributions in the same are largely ignored. Historically, less attention has been paid to paternal contributions in early embryonic development. Impaired embryonic development results from both ‘‘early’’ and ‘‘late’’ paternal effects transcription gets initiated at 4-8 cell stage of embryonic genome. The early paternal effects are not associated with sperm DNA damage and are marked by poor zygote and early embryo morphology and low cleavage speed. On the other hand, the late paternal effects arise due to marked sperm DNA damage and are manifested by poor developmental competence, implantation failure and pregnancy loss (Barroso et al., 2009). These are associated with abnormailities at the level of sperm chromatin, mitochondrial dysfunctions and abnormal delivery of paternal mRNA to the oocyte at fertilization. Both early and late paternal effects can be caused by genomic

[1]**Corresponding Author**

imprinting anomalies (Barroso et al. 2009). Various genetic and epigenetic factors contribute to the biological makeup of the embryo but the regulation of lifestyle factors is imperative as these cause further alteration of the same and may negatively impact sperm epigenome.

Oxidative stress (OS) and associated DNA damage is the main cause of defective sperm function. Sperm is particularly vulnerable to oxidative attack on account of being rich in polyunsaturated fatty acids in plasma membrane, deficient in cytosolic antioxidants and limited DNA damage detetion and repair mechanism. The induction of lipid peroxidation cascade by excessive production of reactive oxygen species (ROS) predisposes to the clustering of different lipid aldehydes (Aitken et al., 2013; Bisht et al., 2017). The DNA damage in the spermatozoa is mainly oxidative and is characterized by single and double strand breaks, abasic sites, DNA fragmentation, intrastrand DNA cross linking, accelerated telomeric shorteneing and epigenetic alterations. Oxidative DNA damage (ODD) and its repair is crucial for the viability of cleavage and blastocyst stage embryos. Sperm being deficient in repair mechanisms undergoes chromatin rearrangements in order to avoid the damage. Sperm chromation undergoes compaction characterized by replacement of histones with arginine-rich protamines. 5-15% of chromatin still remains associated with histones in the peripheral nucleohistones which is vulnerable to oxidative attack. Compaction of chromatin is accompanied by a shutdown of nuclear transcription because of inability of polymerase machinery to access the DNA (Krawetz et al. 2005; Ostermeier et al. 2002; Dhawan et al. 2017). Terminally differentiated sperm is dependant on residual or stored mRNA which are transferred to oocyte at fertilization and support implantation and early embryogenesis (Krawetz et al., 2005; Boerke et al. 2007; Kumar et al., 2012; Kumar et al., 2016). Thus this dynamic process of preimplantation embryo development is characterized by changes in gene expression profile along with various histone modifications, chromatin organization (Kumar et al., 2012; Kumar et al., 2016; Dhawan et al., 2016).

The current prospective exploratory study was designed with an aim to analyze the expression pattern of the genes selected based on previous studies which are crucial for early embryonic development (Zhao et al., 2006; Ferlin et al., 2012). The genes selected for the current study were *FOXG1* (Forkhead Box G1), *SOX3* (SRY-related HMG-box 3), *RPS6* (Ribosomal Protein S6), *RBM9* (RNA Binding Motif Protein 9), *RPS17* (Ribosomal Protein S17), and *RPL29* (Ribosomal Protein L29). As the spermatozoa is deficient in DNA damage detection and repair mechanisms, the genes of base excision repair pathway i.e. *OGG1* (8-Oxoguanine DNA Glycosylase) and *PARP1* (Poly (ADP-Ribose) Polymerase 1) were also selected. The dysregulation of these transcripts is one of the causal factors for implantation failures and pregnancy loss. The present study aims to assess the changes in the gene expression profile with the adoption of yoga based lifestyle intervention (YBLI). It is imperative to minimize the damage caused by overwhelming OS and ODD which may cause such dysregulation and epigenetic alteration in the sperm genome (Bisht et al., 2017). YBLI normalizes transcripts and reduces OS and ODD and thus should be adopted as an adjunct in management of male factor infertility associated with OS. This simple lifestyle intervention not only improves reproductive potential and carries home birth rate, it also improves quality of life and incidence of genetic and epigenetic disorders.

MATERIAL AND METHODS

The current study was done to assess the impact of yoga based lifestyle intervention (YBLI) on male partners of infertile couples who had a history of RIF from August 2016 to July 2017. All couples had normal cytogenetic analysis and the female partners had no evidence of any risk factors for RIF. There was no history of any uternine anatomical abnormalities, endocrine disorders, also tested negative for antiphospholipid antibodies, lupus antibodies and had normal coagulogram and ovarian function. The patients were referred to the laboratory for Molecular Reproduction & Genetics in the department of Anatomy and recruited in YBLI program after informed written consent. Out of a total of 40 men selected for the study, 2 didn't meet the inclusion criteria, 3 declined to take part and 5 men were excluded due to non-compliance with the intervention, and finally 30 men were recruited for the study. The semen samples were also obtained for the control group from 30 men of proven fertility without the use of ART and with no history of fertility problems. The relative gene expression at the end of active intervention was compared with that of healthy fertile controls. The study was initiated after ethical clearance (IECPG-325/29-06-2016) from the institutional ethical review board of AIIMS, New Delhi, India.

Yoga Based Lifestyle Intervention (YBLI)

The predesigned YBLI program was conducted for an average 2 hours per day under the direct supervision of qualified, registered yoga instructors. This 21-day

structured program consisting of various yoga asanas, meditation and interactive lectures was designed with an aim to alleviate the stress and anxiety associated with RIF (Table 1). The patients were followed up after the completion of YBLI and advised to adhere with the yoga practice at home.

Laboratory Procedures

Semen samples were obtained from the participants at the start (day 0) and at the end of YBLI (day 21). The semen analysis was done as per WHO guidelines (2010). TRIZOL method was used for the extraction of RNA from 1×10^7 spermatozoa and quantified by spectrophotometry. 1000ng of RNA was reverse transcribed into complementary DNA using iScript c-DNA synthesis kit (Biorad) CFX96 real-time system (Bio-Rad, California, USA) was used for quantitative analysis and relative quantification was done by $2^{-\Delta\Delta Ct}$ method after normalization of expressed mRNA using 2 internal housekeeping genes β-actin and GAPDH. The levels of reactive oxidative species (ROS) were assessed by luminal dependant chemiluminiscence and levels were expressed as relative light units (RLU)/sec/million sperm. The extent of DNA damage in the sperm was ascertained by sperm chromatin structure assay (SCSA) and was expressed in percentage as DNA Fragmentation Index (DFI).

Statistical Analysis

Analysis of the data was done using statistical software, Stata 14.0. The quantitative data was expressed as mean±S.D and median (min-max) and followed normal and skewed distribution respectively. Paired t-test and Wilcoxon Signed Rank test used to compare continuous variable both pre- and post- YBLI. Significance was considered at $p<0.05$.

RESULTS

The mean age of male partners of couples with RPL who participated in the YMLI intervention was 36.3 ± 7.6 years. The changes in the seminal parameters after YBLI witnessed a significant increase in sperm count according to the 2 reading taken 4 days apart ($p=0.038$). An increase in sperm progressive motility ($p<0001$) was seen with a concominant 74.3% decrease in seminal ROS levels ($p<0.001$) (Table 2). The cut-off value of ROS levels was set as 27.8 RLU/sec/million sperm. A minimal insignificant decrease in the DFI was also observed ($p>0.05$). The cut-off for DFI was set at 30.7% Fig. 1, Table 2). The validity of differential expression of 8 selected target genes was done by qPCR analysis. The relative expression (average ΔCt) of the genes at baseline (day 0) and at the end of active intervention (day 21) as well as in normal healthy fertile males is shown in Table 3. was seen to normalize towards that of the control values. The spermatozoal *SOX3, OGG1* and *PARP1,* were seen to be upregulated, while *FOXG1*, *RPS6, RBM9, RPS17* and *RPL29* were downregulated ($p >0.05$) (Table 3).

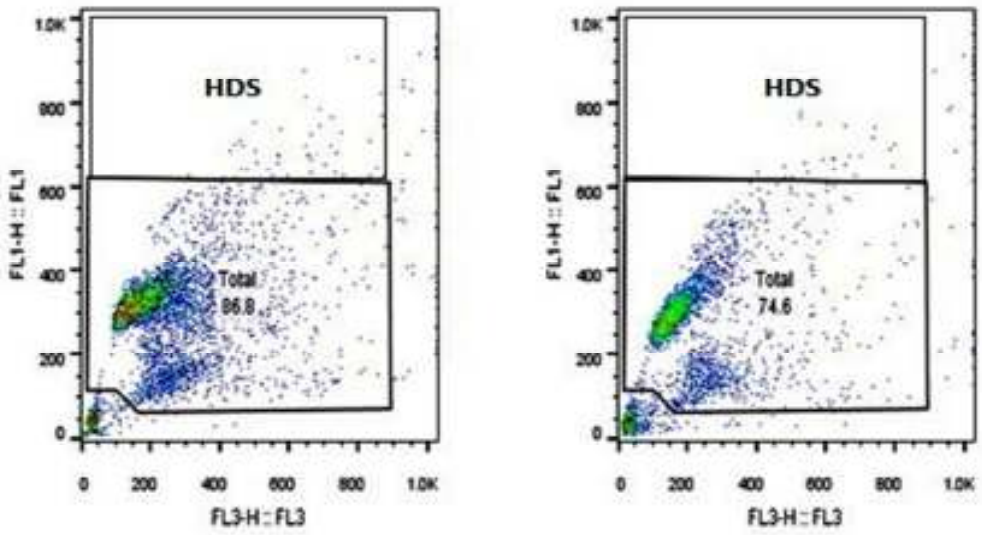

Figure 1: Dot plot cytograms of semen samples at both baseline (day 0) and at the end of YBLI (day 21) by SCSA. FL3 on X-axis represents fragmented DNA and FL1 on Y-axis represents native DNA showing percentage of

spermatozoa with high levels of DNA fragmentation. Each dot in the cytogram represents a single sperm with red and green fluorescence values. The debris at the bottom left corner was excluded from analysis

Table 1: Details of practices done in a session of Yoga and Meditation based Lifestyle Intervention (YMLI) program

Sl. No.	Practices do be done			Duration
1	Session preparation			5 min
2	Starting prayer			3 min
3	Loosening practices (Sukshma Vyayama)			5 min
4	Sun Salutations (Surya Namaskar) with mool bandha			10 min
5	Asanas (Postures)	Standing	Tadasana, Ardha chakrasana, Padahastasana, Vrikshasana	20 min
		Sitting	Paschimattanasana, Janu Sirasana, Badha Kanasana, Vakrasana	
		Prone	Bhujangasana, Salabhasana, Naukayasana, Makrasana	
		Supine	Uttanapadasana, Malasana, Pavana mukhtasana, Matsyasana	
6	Relaxation			5 min
7	Pranayama (Breathing exercises)			20 min
8	Dhayana			5 min
9	Nada Anusandhana (A-U-M chanting)			5 min
10	Closing prayer			7 min
11	Interactive Session			15 min
12	Total			120 min

Table 2: Impact of lifestyle intervention on quantitative levels of various experimental parameters at baseline (Day 0) and the end of active intervention (Day 21)

Experimental Parameters	Baseline (Day 0)	End of active intervention (Day 21)	p-Value
Sperm Count (million/ml) [Median (min-max)]	34.3 (0.3-58.3)	43.4 (1.1-66.7)	0.038**
Progressive Motility (%) (mean±S.D)	32.3±13.23	39.6±18.1	<0.001***
ROS (RLU/sec/million sperm) [Median (min-max)]	45.3 (20.1-1186.9)	15.6 (3.57-113.4)	<0.001***
DFI (%) (mean±S.D)	43.2±6.28	42.1±6.36	>0.05

p-value <0.05 was considered to be significant

Table 3: Relative expression (average ΔCt) of the genes of interest (GOI) with respect to β-actin and GAPDH in semen samples of patient as well as controls and axis fold change (AFC) in the gene expression post-yoga with respect to pre-yoga

GOI	ΔCt Pre-Yoga	ΔCt Post-Yoga	ΔCt Controls	Axis fold change
FOXG1	5.3 ± 2.1	4.2 ± 0.75	3.6 ± 1.2	0.32
SOX3	7.1 ± 2.68	6.6 ± 2.6	3.6.7 ± 1.4	1.33
OGG1	4.8 ± 1.18	3.9 ± 1.34	3.4 ± 1.8	1.67
PARP1	6.2 ± 2.3	5.1 ± 2.3	3.8 ± 1.5	1.79
RPS6	4.3 ± 1.9	3.86 ± 1.23	2.13 ± 0.98	0.22
RBM9	4.1 ± 2.08	3.4 ± 0.44	2.1 ± 1.1	0.11
RPS17	3.13 ± 1.2	2.86 ± 1.34	1.8 ± 1.1	0.82
RPL29	1.53 ± 1.3	1.01 ± 1.0	0.34 ± 1.1	0.73

p-value >0.05 (ns) (P-value <0.05 was considered to be significant)

DISCUSSION

Yoga and meditation based lifestyle interventions have been observed to decrease OS, ODD as well as mutagenic load in the sperm DNA and thus prove to be therapeutic for the same. A reduction in ROS levels accompanied by an increase in progressive motility and sperm count was seen in the current study. There was a minimal non significant improvement in ODD following this brief YBLI. Significant improvement in the DNA

integrity and seminal oxidative stress in 6 months duration in the previous studies reported from our laboratory. Though the changes in the semen parameters are reported for a period of 21days but patients were encouraged to continue the yoga practice and were followed up to a period of 90-180days (Dada et al., 2015; Kumar et al., 2015; Rima D et al., 2016). The present study quoted the changes in gene expression profile with brief YBLI for 21 days. YBLI can also reduce the rate of cellular aging and also reduce the rate of testicular aging by decreasing OS, ODD and increases telomerase activity (Tolahunase et al., 2017).

Sperm motility which is one of the first functions to be affected by OS is a vital for both natural and assisted conceptions (except ICSI) for sperm's transit to oocyte as well as oocyte penetration. The oxidative damage to the mitochondrial genome and impaired ATP production along with axonemal damage play a pivotal role (Aitken et al., 2014). It is observed to be dependent on various regulatory mechanisms and metabolic pathways. The important metabolic pathways involved are calcium (Ca^{2+}) pathway and the cAMP-dependent protein kinase pathway (Darzon et al., 2006; Pereira et al., 2017). These pathways involve calcium ions, adenyl cyclases, bicarbonate ions, along with various phosphorylation events catered by NADPH pathway and different membrane channels. The regulated functioning of these pathways is fundamental for acquisition of functional competency of the spermatozoa to fertilize the oocyte, namely capacitation, hyperactivity, and acrosome reaction. Increased ROS levels have shown to be correlated with decreased sperm motility and increased proportion of various sperm head and tail anomalies (Pereira et al., 2017). The correction of elevated ROS levels and impending ODD may aid in improving cleavage and blastocyst quality and improving implantation rates, pregnancy outcomes. This may further decrease the incidence of congenital malformations, various childhood carcinomas and decreasing childhood morbidity and thus exert a positive impact on lifelong health of the offspring.

The ODD in both sperm and oocyte at fertilization should be repaired prior to S-phase of first mitotic division at the time of fertilization in order to reduce the risk of mutations in the zygote. The damage occurs primarily at the guanine bases by virtue of their low oxidative potential. The accumulation of 8-OHdG is mutagenic and has a propensity to form a stable base pair with adenine, resulting in G:C to T:A transversion mutations (Aitken et al., 2013; Aitken et al., 2014). It also has a propensity to affect the sperm epigenome, cause genomic instability and cause sperm transcript dysregulation. Sperm harbouring inefficient and incomplete repair mechanisms is dependent on the oocyte's repair mechanisms for the same (Mishra et al., 2013). The sperm cells only possess 8-oxoguanine glycosylase 1(OGG1) which is the first enzyme responsible for the detection of DNA damage in the base excision repair pathway, cleaves 8-OHdG to the extracellular space, while they lack the downstream enzymes APE1 and XRCC1 (Aitken et al., 2013, Mishra et al., 2013). Lower expression levels of *PARP1* for the repair of DNA damage have been reported in idiopathic male infertility patients indicating the persistence of DNA damage from a previous study in our laboratory (Mishra et al., 2014). Both *OGG1* and *PARP1* genes showed a positive change in gene expression with an upregulation in the expression after YBLI and thus shows that increase in expression of transcripts to maintain genomic integrity.

YBLI has previously reported a significant improvement in ODD and decline in 8-OHdG levels over a period of 6 months (Dada et al. 2015; Kumar et al., 2015; Bisht et al. 2017). A nonsignificant improvement in DFI was witnessed within 21 days of YBLI in the current study and thus mandatory for people with ODD to follow YBLI for 6months and integrate into their lifestyle. ODD also impacts the sperm telomere length as it is negatively affected by OS causing testicular aging and genomic instability. Telomere length is made and maintained by telomerase. An upregulation of telomerase activity along with improvement in seminal OS and ODD has been reported with YBLI (Dada et al., 2015; Kumar et al. 2015; Rima D. et al., 2016). Optimal ROS level is central for maintenance of telomere length. Antioxidants being prescribed for the alleviation of deranged OS parameters may certainly reduce the ROS levels and ODD but may cause reductive stress causing premature nuclear decondensation and impaired pronuclear formation if taken indiscriminately.

The preimplantation embryo development is associated with marked chromatin reorganization and histone modifications and changes in gene expression profile. Dysregulation of these selective paternal transcripts cited in the current study may be caused by OS induced genome wide hypomethylation. The relative gene expression of *FOXG1* and *SOX3* were found to normalize towards that of the levels seen in the healthy fertile controls at the end of active YBLI for a period of 21 days. *FOXG1,* a regulator of neurogenesis is a transcriptional

repressor is responsible for development of ventral telencephalon. Downregulation in *FOXG1* expression as seen in the current study is essential of pyramidal cell migration in the cortex to maintain the cortical neuron density (Hanashima et al., 2002, Miyoshi et al., 2012). *SOX3* gene functions in determining cell fate for male sex determination in the developing fetus and craniofacial morphogenesis. This transcriptional factor is required for the formation of hypothalamo-pituitary axis, suppresses neuronal differentiation and a downregulation is seen during differentiation. *SOX3* dosage has been associated with X-linked hypopituitarism and neural tube defects. Its expression in the developing urogenital ridge is associated with seminiferous tubule functioning and decreased expression suppresses spermatogenesis (Raverot et al. 2005).

RPS6 phosphorylation is a marker for neuronal activation, and dephosphorylation is observed at phases of growth arrest. An altered phosphorylation predominantly hyperphosphorylation is thus responsible for being the causative factor for various neurodevelopmental disorders including Autism and Down syndrome (Blever et al., 2015). RBM9 an RNA binding protein is a member of RbFox family, is responsible for alternative exon splicing in the nervous system and has also been shown as an essential requirement for embryonic cell viability (Guallar et al., 2014). The relative gene expression of both RPS6 and RBM9 was also seen to normalize towards that of control levels and showed a downregulation with respect to the pre-intervention levels. The expression of RPS17 and RPL29 were seen to be downregulated in the current study and showed normalization to control levels.RCT from our own laboratory in the previous study in the primary open angle glaucoma patients showed a downregulation of pro-inflammatory genes and an upregulation of cellular repair genes (Dada et al., 2016).

Thus as evidence from the current study the advent of CAM therapy approach may aid in the regulation of the altered gene expression profile and also cause correction of deranged oxidative stress parameters. YBLI helps in the modification of sperm epigenome by causing histone modifications and changes in DNA methylation pattern. Ongoing studies in our laboratory are being done to determine the impact of YBLI on pattern of sperm methylation. YBLI invigorates both the mind and the body by introducing changes at the psychological level and overall improves quality of life. Delay in testicular aging and normalization of sperm transcripts may improve reproductive potential and reduce incidence of infertility, number of couples requiring assisted conceptions and also incidence of RIF.

CONCLUSION

Deranged oxidative stress parameters as well as sperm transcript dysregulation have been thus shown to be one of the chief causes of pre and post implantation losses as well as pregnancy loss. YBLI is being opted as the modalities of choice in this current era of advances in medical field. The integration of these not only aid in maintainnce of genomic integrity and decrease in accumulation of mutagenic load in the germline thus positively impacting the epigenome. It not only helps in improving the reproductive health and reduction in incidence of genetic and epigenetic disorders in the next generation, but is also essential for the overall physical, psychological health of the individual.

ACKNOWLEDGEMENTS

The authors acknowledge the authorities of 27th Swadeshi Science Congress for giving the opportunity to publish the manuscript. We are also thankful to Sudhir Chaudhary and Sneha Chandna for all the yoga instructions and the patients for participating in yoga program.

CONFLICT OF INTEREST

The authors declare no potential conflict of interests.

REFERENCES

Dada R. and Tolahunase M., 2018. Chapter 9 - Yoga Meditation Lifestyle Intervention: Impact on Male Reproductive Health A2 - Sikka, Suresh C. In: Hellstrom WJG, editor. Bioenvironmental Issues Affecting Men's Reproductive and Sexual Health. Boston: Academic Press, 135-156.

Margaolith E.J., Ben-Chetrit A., Gal A. and Eldar-Geva T., 2006. Investigation and treatment of repeated implantation failure following IVF-ET. Hum Reprod, **12**: 3036-3043.

Simon A. and Laufer N., 2012. Assessment and treatment of repeated implantation failure (RIF). J. Assisst Reprod Genet, **29**: 1227-1239.

Coughlan C. et al., 2014. Recurrent Implantation failure: definition and management. Reprod Biomed Online, **28**: 14-38.

Kanherkar R.R., Stair S.E., Dey N.B., Mills P.J., Chopra D. and Csoka A.B., 2017. Epigenetic mechanisms

of integrative medicine. Evid Based Complement Alternat Med, Article ID 4365429: 19.

Barroso G., Valdespin C., Vega E., Kershenovich R., Avila R. and Avenda C., 2009. Oehninger S. Developmental sperm contributions: fertilization and beyond. Fertil Steril, **92**(3): 835-848.

Aitken R.J., Smith T.B., Lord T., Kuczera L., Koppers A.J., Naumovski N., Connaughton H., Baker M.A. and De Iuliis G.N., 2013. On methods for the detection of reactive oxygen species generation by human spermatozoa: analysis of the cellular responses to catechol oestrogen, lipid aldehyde, menadione and arachidonic acid. Andrology, **1**: 192–205.

Bisht S., Faiq M., Tolahunase M. and Dada R., 2017. Oxidative stress and male infertility. Nat. Rev. Urol., **14**(8): 470-485.

Krawetz S.A., 2005. Paternal contribution: new insights and future challenges. Nat. Rev. Genet, **6**: 633-42.

Ostermeier G.C., Dix D.J., Miller D., Khatri P. and Krawetz S.A., 2002. Spermatozoal RNA profiles of normal fertile men. Lancet, **360**: 772–777.

Dhawan V., Kumar M. and Dada R., 2017. Effect of sperm molecular factors, oxidative damage and transcripts in childhood disorders. J. Child Dev. Disord., **3**(1): 6.

Boerke A., Dieleman S.J. and Gadella B.M., 2007. A possible role for sperm RNA in early embryonic development. Theriogenol, S147-S155.

Kumar K., Deka D., Singh A., Chattopadhay P. and Dada R., 2012. Expression pattern of *PRM2, HSP90* and *WNT5A* in male partners of couples experiencing idiopathic recurrent miscarriages. J. Genet, **91**(3): 363-366.

Kumar M., Dhawan V., Kranthi V. and Dada R., 2016. Paternal factors- Role in idiopathic recurrent pregnancy losses. Int. J. Reprod Fertil Sex Health, S2(001): 1-6.

Dhawan V., Kumar M., Deka D., Dadhwal V. and Dada R., 2016. Expression pattern of RPS6, RBM9 and RPL10A in recurrent pregnancy loss. Andrology, **4** (Suppl 1): 110.

Zhao Y., Li Q., Yao C., Wang Z., Zhou Y. and Wang Y., 2006. Characterization and quantification of mRNA transcripts in ejaculated spermatozoa of fertile men by serial analysis of gene expression. Hum Reprod, **21**(6): 1583-1590.

Ferlin A., 2012. New genetic markers of male infertility. Asian J. Androl, **14**: 807-808.

Dada R., Kumar S.B., Tolahunase M., Mishra S., Mohanty K. and Mukesh T., 2015. Yoga and meditation as a therapeutic intervention in oxidative stress and oxidative DNA damage to paternal genome. J. Yoga Phys. Ther., **5**:4.

Kumar S.B., Gautam S., Tolahunase M., Chawla B., Yadav R.K., Kumar P., Mitra D.K. and Dada R., 2015. Improvement in sperm DNA quality following simple lifestyle intervention: A study in fathers of children with non-familial sporadic heritable retinoblastoma. J. Clin. Case Rep., **5**(3).

Rima D., Shiv B.K., Bhawna Ch., Shilpa B. and Saima Kh., 2016. Oxidative stress induced damage to paternal genome and impact of meditation and yoga- can it reduce incidence of childhood cancer? Asian Pac J Cancer Prev., **17**(9): 4517-4525.

Tolahunase M., Sagar R. and Dada R., 2017. Impact of Yoga and Meditation on Cellular Aging in Apparently Healthy Individuals: A Prospective, Open-Label Single-Arm Exploratory Study. Oxid Med Cell Longev, 9.

Aitken R.J., Smith T.B., Jobling M.S., Baker M.A. and Iuliis GNDe., 2014. Oxidatice stress and male reproductive health. Asian J. Androl., **16**(1): 31-38.

Darszon A., LópezMartínez P., Acevedo J.J., HernándezCruz A. and Treviño C.L., 2006. T□type Ca2+ channels in sperm function. Cell Calcium, **40**: 241–252.

Pereira R., Sa Rosalia., Barros A. and Sousa M., 2017. Major regulatory mechanisms involved in sperm motility. Asian J. Androl, **19**: 5-14.

Mishra S., Kumar R., Malhotra N. and Dada R., 2013. Expression of PARP1 in primary infertility patients and correlation with DNA fragmentation index a pilot study. J. Anat. Soc. India, **62**: 98–104.

Bisht S. and Dada R., 2017. Oxidative stress: Major executioner in disease pathology, role in sperm

DNA damage and preventive strategies. Front Biosc., **9**: 420-447.

Kumar S.B., Yadav R., Yadav R.K., Tolahunase M. and Dada R., 2015. Telomerase activity and cellular aging might be positively modified by a yoga-based lifestyle intervention. J. Alt. Complement Med., **21**(6): 370-372.

Hanashima C., Shen L., Li S.C. and Lai E., 2002. Brain factor-1 controls the proliferation and differentiation of neocortical progenitor cells through independent mechanisms. J. Neurosci., **22**: 6526-6536.

Miyoshi G. and Fishell G., 2002. Dynamic *FoxG1* expression coordinates the integration of multipolar pyramidal neuron precursors into the cortical plate. Neuron, **74**(6): 1045-1058.

Raverot G., Weiss J., Park S.Y., Hurley L. and Jameson L., 2005. *Sox3* expression in undifferentiated spermatogonia is required for the progression of spermatogenesis. Dev. Biol., **283**: 215-225.

Blever A., Valjent E. and Pulghermanal E., 2015. Ribosomal Protein S6 phosphorylation in the nervous system: from regulation to fuction. Front Mol Neurosci, 8: Article 75.

Guallar D. and Wang J., 2014. RNA-binding proteins in pluripotency, differentiation and reprogramming. Front Biol., **9**(5); 389-409.

Dada T., Faiq M.A., Mohanty K., Mittal D., Bhat M., Yadav R.K., Sihota R. and Dada R., 2016. Effect of Yoga and Meditation Based Intervention on Intraocular Pressure, Quality of Life, Oxidative Stress and Gene Expression Pattern in Primary Open Angle Glaucoma: A Randomized Controlled Trial. ARVO, Seattle, Wash, USA.