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Research Article

In silco Prediction of Target Genes for Up-Regulated MicroRNAs in Male Infertile Patients

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ABSTRACT

MicroRNAs are small molecules that control expression post transcriptionally. Due to diversity in microRNAs and difficulty in experimental identification of target genes, the specific function of most microRNAs are not known. It is necessary to find their target mRNAs. Computational target prediction helps MiRNA-mRNA interactions for experimental studies. Infertility is an important health related problem. Approximately 10-15% of couples face infertility problem worldwide. About 50% of infertility issues are attributable to male factors. A large number of infertility cases are idiopathic, since their molecular mechanism is unknown. MicroRNAs are differentially expressed in male patients with complications in fertility. They are involved in regulating various reproductive processes such as embryogenesis and spermatogenesis. The current study was carried out to find out the important genes that are targets for up-regulated microRNAs in infertile patients. Up-regulated microRNAs reported by previous studies were searched and a list of up-regulated microRNAs in male infertile patients was obtained. Total 24 microRNAs were enlisted from various studies. To find out the target genes three online tools were used including microRNA Data Base (mir-DB), Targetscan (TS) and DIANA tools. In the final list those microRNAs-targets were included which were predicted by all the three target prediction tools (TPTs) and were present on the top 20 list of the respective TPTs. Also those microRNAs were included which were predicted by at least two software and were targets for more than one up-regulated microRNAs in infertile patients. In the result we created a list of 36 genes predicted by all the three TPTs and another list of 15 genes that are predicted targets of more than one microRNA. On the basis of our results it is stated that exploring the role of these genes might provide an important insight into the mechanism and reasons behind idiopathic male infertility.

Keywords: Idiopathic male infertility; Dysregulated microRNAs; Target genes prediction

INTRODUCTION

Infertility is the incapability of a sexually active, non-contracepting couple to achieve natural pregnancy within the specified time [1]. Approximately 10-15% of couples face infertility problem worldwide [2,3]. About 50% of infertility issues are contributed by male factors [4,5]. There has been a concerning decrease in male reproductive health including male infertility, decrease sperm production and function [5]. The molecular mechanism of more than half of male infertility cases are unknown termed as idiopathic [6,7]. Male infertility is a syndrome associated with a number of disorders. Recent studies have disclosed the molecular basis of male infertility but most of them are still a clinical hurdle.

MicroRNAs (miRNAs) are small, highly conserved noncoding short (19–24 nt) RNA molecules that control gene expression post-transcriptionally, either by degradation of target mRNAs or by inhibition of protein translation [8]. It is already known that miRNAs have significant roles in key biological pathways [9-11]. More than 50% of all genes in the human genome are known to be regulated by miRNA. Recently it is shown that abnormal expression of miRNA is related to a broad range of human diseases [9]. A large number of miRNAs have been discovered by cloning and size-fractionated RNA techniques [12-14]. Our previous study have shown altered expression profile of selected miRNA in caloric metabolism and gastric cancer by using whole genome miRNA profiling studies using blood samples from human and related knock-out mouse models [15]. Targets genes of the majority of the miRNA are not known, but the predicted limit is from a single to hundreds genes for a certain microRNA, which is based on target estimation by applying an array of bioinformatics methods. Previously we have investigated the target sequences for microRNAs within the Tffgene cluster [16]. The biogenesis of miRNAs is a highly regulated process, and alteration in their regulation is linked with many diseases in human. Expression of miRNAs is tissue specific and also influenced by different developmental stages [17-19]. Hence, microRNAs exhibiting abnormal expression

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levels in the situation of certain diseases are of great importance as therapeutic targets.

Cellular and molecular integrity of sperm cells are imperative for fertilization [20,21]. Spermatogenesis is not only controlled by protein coding part of the genome but also by non-coding regions, including loci that produce small RNAs [22-25]. A significant number of miRNAs are involved in normal spermatozoa development in mammals. It is feasible that any down-regulation or up-regulation in the expression levels of miRNAs considerably influence spermatogenesis pathways and results in several kinds of reproductive defects [26-29]. Alterations in the regulatory function of these small RNA leads to malfunctioning of spermatozoa. Different expression profiles of miRNA have been reported in the reproductive cells or testicular tissues of infertile people. Through inhibition of the expression of target genes miRNAs are involved in the meiotic, mitotic and post-meiotic stages of spermatogenesis [30]. During spermatogenesis, each mitotic and meiotic division is controlled by a dense network of proteins. Modification in any of these phases may lead to hypo-spermatogenesis and consequently male infertility [31]. Several miRNAs are preferentially expressed in the mouse testis and retardation of spermatogenesis has been observed in the mouse testis upon deletion of dicer. Supporting the view that miRNAs may play important roles in spermatogenesis; miR-122a has been reported to control expression of transition protein 2 (TNP2) gene, a testis-specific gene in post-meiotic germ cells which is controlled post-transcriptionally. Correspondingly it has been observed that with the progress in differentiation, the genome is firmly packed and is not accessible to be transcribed. Consequently, transcripts have to be collected in advance on behalf of possible utilization in the subsequent steps like sperm differentiation, fertilization and further development. Thus, it is rational to consider that miRNAs may play a role in meiotic gene repression, and that change in the expression pattern of miRNA might be a key factor in male infertility. Due to large number of microRNAs and the ability of a single to target more than one mRNA it is very difficult to experimentally identify miRNA target genes. Therefor computationally identifying miRNA targets is a very important initial step for finding out miRNA-mRNA interactions [32].

MATERIALS AND METHODS

Data collection

Google search was used for finding relevant literature. Data mining was carried out from internet using combination of various words such as; microRNAs and infertility, de-regulated microRNAs in infertile patients, Up-regulated microRNAs - male infertile patients, microRNAs-biomarker- male infertility. All the available data published (up to 2016) about dysregulation (up and down regulation) of microRNAs in male infertile patients was downloaded and collected. The published papers were studied and list of microRNAs that were reported to be dysregulated was created. To avoid complexity of the data due to many targets of each microRNA; only the up-regulated microRNAs were further processed for identification of target mRNAs.

Target prediction

Prediction of target genes for each individual microRNA was carried out using three online tools such as (1) Mir-D (2) Target-scan Human and (3) DIANA Tool.

Selection of target genes

These online bioinformatics tools provided a large number of target genes for each microRNA investigated. We selected only the top 20 targets for an individual microRNA from each software list. The top 20 target genes were selected on the basis of score provided by the respective target scanning software. After selection of top 20 microRNA targets we further selected those microRNAs target genes which were:

1) Predicted by all the three software tools

2) Predicted by at least two software tools

3) Predicted by at least 2 tools and were targets for more than one microRNA.

Target scan human

The TPT examines conserved sites on mRNAs for matches to the seed region of microRNAs. The predicted targets are sorted on the basis of collective weighted context scores of the sites. The software recognizes matches in the 3-untranslated region and their orthologs, as demarcated by UCSC whole-genome alignments and uses updated miRNA families curated from [33-41].

DIANA-microT-CDS

The bioinformatics TPT recognizes negative and positive set of MREs (microRNA recognition elements) existing in both the 3-UTR and coding regions. It gives information about the species having the conserved binding site and filter the results. DIANA-microT-CDS was accessed using the link: http://www.microrna.gr/microT-CDS [32,35].

MicroRNA data base (MiRDB)

This is an online database for prediction of target genes for miRNAs, using MirTarget. The database is established by investigating thousands of interactions between microRNA and target genes obtained from sequencing tests. Common features related with miRNA target binding have been recognized and applied to predict miRNA targets with machine learning procedures. MiRDB contain predicted miRNA targets in five species: human, mouse, rat, dog and chicken [36].

RESULTS

We studied various published literature on dysregulated microRNAs in male infertile patients, and tried our best to cover all published data. The dysregulated microRNAs reported were enlisted and then only up-regulated microRNAs were selected. As a result we obtained 24 up-regulated miRNAs from different studies. The data is shown below in Table 1.

By using three target prediction tools a list of those common predicted target genes was created that were reported to be targets for the up-regulated microRNAs by all the three TPTs used in the study. The list of genes with their abbreviated and full names are shown in the Table 2. There are a total of 36 genes located on different chromosomes. We found that some genes are targets for more than one microRNA. Hence giving them importance because of increased regulation. There are 15 genes which are targets for more than one microRNA (Table 3).

Table 1: List of Up-regulated microRNAs in male infertile patients.

MicroRNA	Sample Source	Patient condition
hsa-mir-574-5p, hsa-mir-1275, hsa-mir-297, hsa-mir-122, hsa-mir-193b, hsa-mir-185, hsa-mir-373	Semen	asthenozoospermic
hsa-mir-491-3p, hsa-mir-302a	Testes	NOA
hsa-let-7a, has-miR-19b	Seminal plasma	Oligospermic
has-mir-196b, has-mir-99a	Semens	Oligospermic
hsa-miR-141, has-miR-429 and has-miR-7-1-3p	seminal plasma	NOA
hsa-miR-429	Seminal plasma	NOA
hsa-miR-34c-5p, has-miR-122, has-miR146b-5p, has-miR-181a, hsa-miR-374b, has-miR-509–5p, and has-miR-513a-5p	Seminal plasma	Asthenozoospermia

Table 2: Target genes predicted by all the three databases.

Gene	Name	Gene	Name
ZSWIM1	zinc finger SWIM-type containing 1	HOXA7	homeobox A7
VAMP2	vesicle associated membrane protein 2	HOXC8	homeobox C8
RAB35	RAB35, member RAS oncogene family	KCNA4	potassium voltage-gated channel subfamily A member 4
MAT2A	methionine adenosyltransferase 2A	SLC9A6	solute carrier family 9 member A6
UBE2B	ubiquitin conjugating enzyme E2 B	ZNF367	zinc finger protein 367
SLC10A6	solute carrier family 10 member 6	HOXB7	homeobox B7
DCAF7	DDB1 and CUL4 associated factor 7	KBTBD8	kelch repeat and BTB domain containing 8
PRAF2	PRA1 domain family member 2	IGDCC3	immunoglobulin superfamily DCC subclass member 3
LIN28B	lin-28 homolog B	HS3ST3B1	heparan sulfate-glucosamine 3-sulfotransferase 3B1
SMARCA5	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	ZMYND11	zinc finger MYND-type containing 1
HOXA5	homeobox A5	HS3ST2	heparan sulfate-glucosamine 3-sulfotransferase 2
BAZ2A	bromodomain adjacent to zinc finger domain 2A	FGFR3	fibroblast growth factor receptor 3
MCTP1	multiple C2 and transmembrane domain containing 1	AP1AR	adaptor related protein complex 1 associated regulatory protein
HIPK3	homeodomain interacting protein kinase 3	ZEB2	zinc finger E-box binding homeobox 2
VAMP2	vesicle associated membrane protein 2	TRAF6	TNF receptor associated factor 6
GALC	Galactosylceramidase	GTF2B	general transcription factor IIB
SYT1	synaptotagmin 1	MTOR	mechanistic target of rapamycin
RAVER2	ribonucleoprotein, PTB binding 2	ZBTB2	zinc finger and BTB domain containing 2

From the Table 3 it is clear that NR6A1 is target for three up-regulated microRNAs. Being targets for more dysregulated microRNAs, the expression of these genes is expected to be more down-regulated.

Table 4 shows number of targets genes for up-regulated microRNAs on each chromosome. Maximum target genes are present on chromosome 17 (5 target genes) followed by ch.1, 4 and ch.12. From these results it is concluded that chromosome 17 might play a significant role in male infertility.

Table 5 shows gene, gene location and the number of target genes located on it. Chromosome 1 contains 16 target genes followed by ch.10 (12 target genes), Ch. 2 (10TG) Ch. 3, Ch. 4, 5, 17 (9 each). Based on these predictions Ch. 1, X and 2 are supposed to play important role in male fertility (Figures1-3).

DISCUSSION

About half of infertility issues are contributed by male factors [37]. Spermatogenesis is not only controlled by protein coding part of the genome but also by non-coding regions, including loci that produce small RNAs [38]. A significant number of miRNAs are involved in normal spermatozoa development in mammals [39-42]. It is feasible that any down-regulation or up-regulation in the expression levels of miRNAs considerably influence spermatogenesis pathways and results in several kinds of reproductive defects [43,44]. Different expression profiles of miRNA have been reported in the reproductive cells or testicular tissues of infertile people [45,46]. Through inhibition of the expression of target genes miRNAs are involved in the meiotic, mitotic and post-meiotic stages of spermatogenesis [47]. Recently up-regulated mir-210 has been shown to be associated with spermatogenesis. This microRNA is

Table 3: List of genes which are predicted targets for more than one microRNA.

GENE NAME	Corresponding microRNAs	Gene location
VAMP2	Has-mir-1275, Has-mir-34c-5p	17p13.1
UBE2B	Has-mir-302a-3p, Has-mir-373-3p	5q31.1
ZNF367	Has-mir-373-3p, Has-mir-302a-3p	(9q22.32)
ZMYND11	Has-mir-19b-3p, Has-mir-196b-5p	(10p15.3)
KBTBD8	Has-mir-99a-5p, Has-mir-19b-3p	(3p14.1)
HS3ST2	Has-mir-99a-5p, Has-mir-99a-3p	16p12.2 (4)
ZEB2	Has-mir-141-3p, Has-mir-429	2q22.3 (15)
HIPK3	Has-mir-429, Has-mir-146b-5p	(11p13)
SYT1	Has-mir-19b-3p, Has-mir-34c-5p	(12q21.2)
GNG13	Has-mir-513a-5p, Has-mir-185-39	(16p13.3)
CROT	Has-mir-373-3p, Has-mir-320a-3p	(7q21.12)
ZEB1	Has-mir-141-3p, Has-mir-429	
NR6A1	Has-mir-196b-5p, Has-mir-let-7a-5p, Has-mir-99a-5p	(9q33.3)
LCOR	Has-mir-509-5p, Has-mir-302a-3p	(10q24.1)
FIGN	Has-mir-509-5p, Has-mir-let-7a-5p	(2q24.3)

Table 4: Chromosomal location of Target genes predicted by all the three target prediction tools.

Ch. No.	Gene names	Ch. Arm		Ch. No	Gene Names	Ch. Arm	
		P	Q			P	Q
1	ZSWIM1, GTF2B, RAVER2, MTOR	4	0	13		0	0
2	MAT2A, ZEB2	1	1	14	GALC	0	1
3	KBTBD8	1	0	15	IGDCC3	0	1
4	SLC10A6, SMARCA5, AP1AR, FGFR3	1	3	16	HS3ST2	1	0
5	UBE2B, MCTP1	0	2	17	VAMP2, DCAF7, HOXB7, HS3ST3B1, VAMP2	3	2
6	LIN28B, ZBTB2	0	2	18		0	0
7	HOXA7	1	0	19		0	0
8		0	0	20		0	0
9	ZNF367	0	1	21		0	0
10	ZMYND11, HOXA5	2	0	22		0	0
11	KCNA4, HIPK3, TRAF6	3	0	x	PRAF2, SLC9A6	1	1
12	RAB35, HOXC8, BAZ2A, SYT1	0	4	Y		0	0

shown to regulate spermatogenesis in by targeting IGF2 gene [42]. Due to large number of microRNAs and the ability of a single to target more than one mRNA it is very difficult to experimentally identify miRNA target genes. Therefore computationally identifying miRNA targets is a very important initial step for finding out miRNA-mRNA interactions [48].

The present study composed of a review of previous studies for finding dysregulated microRNAs in male infertile patients, compilation of the available data and then finding out possible target genes regulated by these abnormally expressed microRNAs [49]. For this purpose three online target prediction tools were used such as MIRANDA, Target Scan and DIANA tool. Data was reduced to 25 target genes for each microRNA on the basis of the score assigned by the respective TPT. Further the data was filtered as target genes predicted by at least two/ three TPTs and genes which are targets for more than one dysregulated microRNA. As a result of data mining, we created a list of 24 up regulated microRNAs reported by various authors in different infertile patients. The studies were carried out in male infertile patients including, Asthenozoospermic, Non-obstructive azoospermia (NOA) and Oligospermia. The data is shown in Table 1. Mostly these microRNAs analysis were carried out in the reproductive system related tissues, such as testes, semen and seminal plasma of the patients. The review of previously published literature concluded in a list of microRNAs that can be used for further research for the formation of non-invasive biomarkers for the infertile patients. On the basis of predicted targets for these microRNAs a list of genes has been created by the collection of those important genes that have been declared to be a target for an up-regulated microRNA in infertile male patients. Similarly a list of those genes has been created which are targets for more than one up-regulated microRNA making them more important for studying their role in male infertile patients. Further studies on these microRNAs either genetic or expression can help in development of biomarkers for male infertile patients. Further, we summarized the genes (Table 2) which are have been picked as targets for up-regulated microRNAs by three online target prediction tools [50-52]. Data mining resulted in 24 up-regulated microRNAs in infertile male patients. In the result we created a list of 36 genes (Table 2) predicted by all the three target prediction tools and another list of 15 genes (Table 3) that are predicted targets of more than one microRNA. Hence

Table 5: Chromosomal location of Target genes predicted by at least two target prediction tools.

Ch. No.	Genes	Ch. No	Genes
1	GATAD2B, TC39A, TGFB2, OLFM3, ANP32E, RNF11, BAI2, ANKRD13C, 1CLIC4, SOX13, SMG7, DR1, C1orf54, FAM76A, CLK2	2	MAT2A, KANSL1L, LRP2, FKBP1B, FIGN, PUM2, VSNL1, MYCN, FIGN, AGFG1
3	DGKG, TRIM71, SPTSSB, KBTBD8, AP2M1, IL17RD, TGFBR2, GMPS, ATP13A4	4	SLC25A31, ARFIP1, CDS1, FHDC1, UBE2D3, FIP1L1, GABRA2, CDKN2AIP, GPM6A
5	UBE2B, BDP1, MEF2C, BDP1, HAND1, FAM169A, ZFR, CREBRF, TNPO1	6	PBX2, BVES, TMEM170B, MAP3K5, DLL1
7	CROT, LRRC17, LRRC17, HBP1, HOXA9, ZNF138, CROT, C1GALT1	8	FBXO25, SLC52A2, TCEB1, FAM167A
9	ZNF367, NR6A1, NR6A1, NR6A1, ELAVL2, ZNF367, RANBP6, PTPN21, ZEB2, ZEB1 RAB14	10	FOXI2, P4HA1, ZMYND11, SGMS1, PCGF5, LCOR, CCSER2, LCOR, CREM, FZD8, ZEB1, RECK
11	FLI1, MPPED2, SF1, DYNLT3, NOX4, HIPK3	12	CALCOCO1, RFX4, SYT1, SBNO1, RIMKLB, NAV3, ZNF268
13	SLC16A2, LATS2, KLF12, LHFP	14	AREL1, SOCS4, NOVA1, NUMB, SEC23A, FBXO34, NPAS3
15	LRRC28, ARID3B	16	UBE2I, GNPTG, GNG13, HS3ST2, GNG13
17	CX3CL1, CA10, IGF2BP1, FAM222B, SLC16A6, FAM222B, ZZEF1, DUSP3, RHOT1	18	PTPRM
19	KCNC3, KIR3DX1, TSHZ3, ZSWIM4, RRAS, ZNF788	20	PMEPA1
21	C21orf88	22	HORMAD2
x	RLIM, AP1S2, IRAK1, IGSF1, FMR1, CMC4		

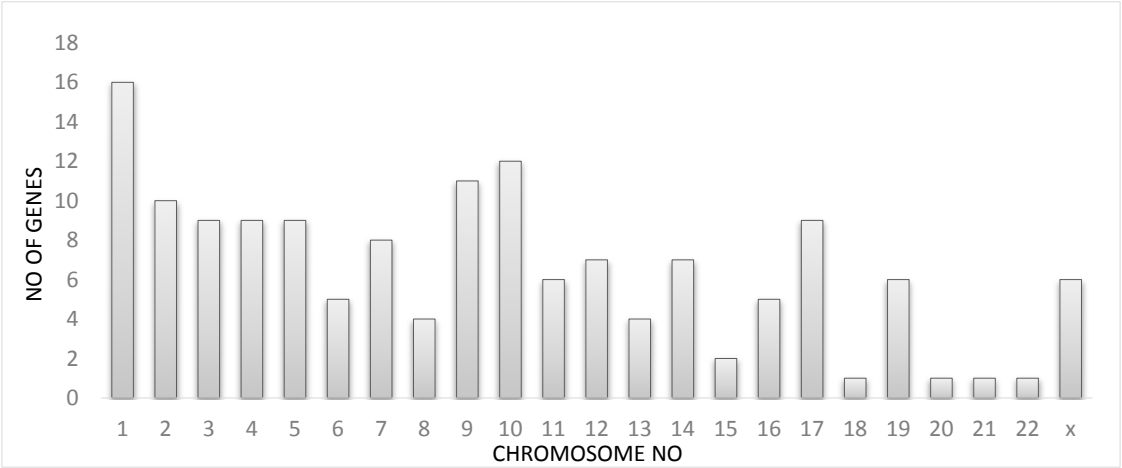

Figure 1: Number of Target genes on each chromosome based on three TPT.

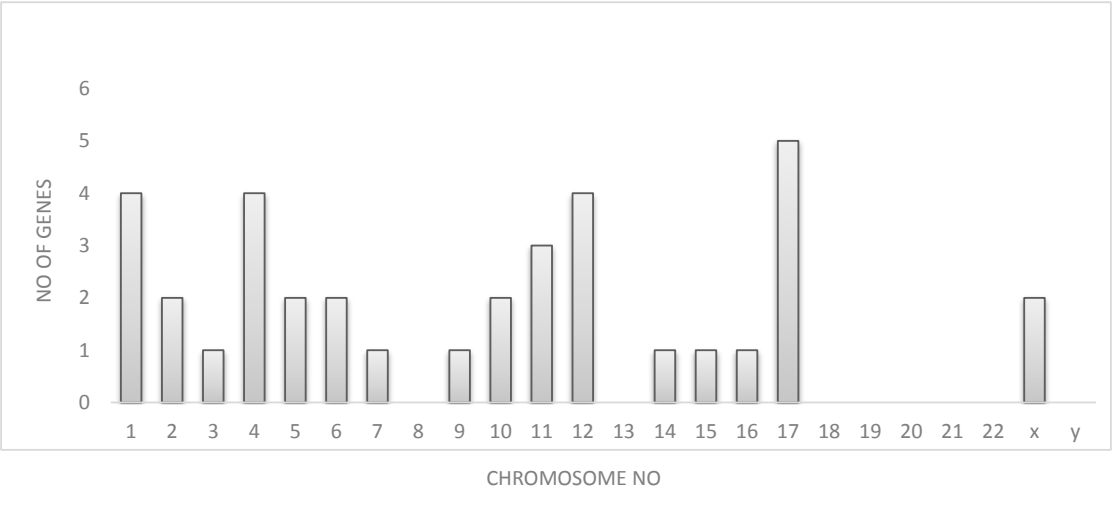

Figure 2: No of target genes on each chromosome based on data from two TPTs.

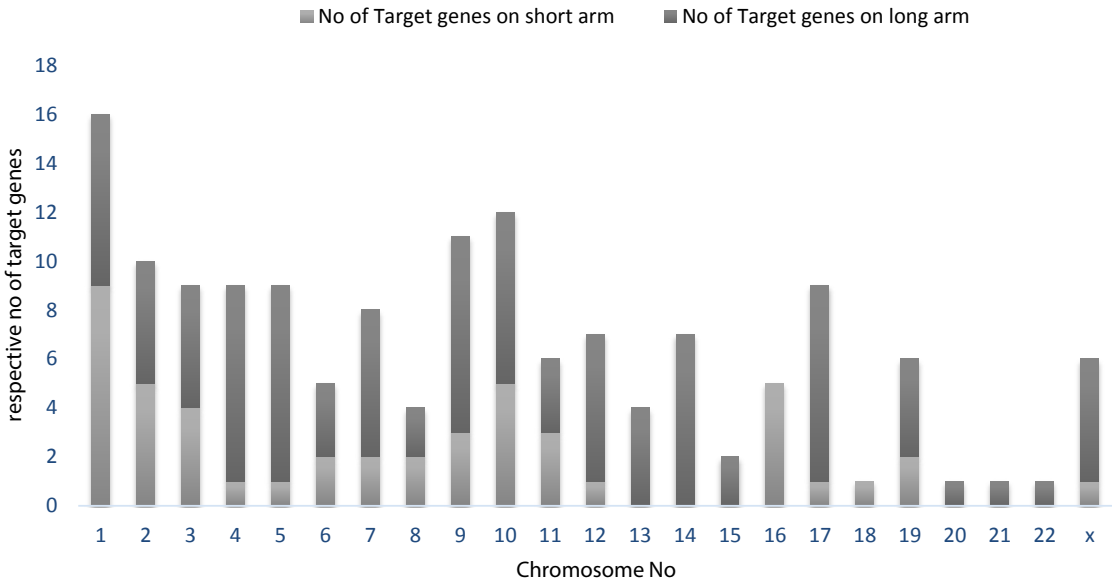

Figure 3: Distribution of target genes on short (p) and long (q) arm of chromosome.

making their regulation more effected in infertile patients. NR6A1 is target for three up-regulated microRNAs. Being targets for more dysregulated microRNAs, the expression of these genes is expected to be more down-regulated. Maximum target genes are present on chromosome 17 (5 target genes) followed by ch.1, 4 and ch.12 [53]. From these results it is concluded that chromosome 17 might play a significant role in male infertility. On the basis of our results it is stated that exploring the role of these genes might provide an important insight into the mechanism and reasons behind idiopathic male infertility [54].

CONCLUSION

With the help of Bioinformatics tools we created a list of 36 genes predicted by all the three software and another list of 15 genes that are predicted targets of more than one microRNA. Exploring the role of these genes might provide an important insight into the mechanism and reasons behind idiopathic male infertility.

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