ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

Exploring the Pathogenesis of Cryptorchidism Complicated with Azoospermia via Microarray Data Analysis

Xiaodong Zhao[1,a,*], Meng Zhao[2,b], Peng Luo[1,c], Zhenyu Wang[1,d], Huifang Liao[3,e], Jinchan Zhang[3,f], Xianliang Zeng[1,g]

[1]Pediatric Surgery, Heyuan Maternal and Child Health Hospital, Heyuan, China
[2]Country Obstetrics and Gynecology, Heyuan Maternal and Child Health Hospital, Heyuan, China
[3]Medical Department, Heyuan Maternal and Child Health Hospital, Heyuan, China
[a]xiaodong19951002@163.com, [b]13789520943@163.com, [c]13322698933@163.com, [d]13384725210@163.com, [e]@163.com, [f]13435309782@163.com, [g]13825381619@163.com
[*]Corresponding author: xiaodong19951002@163.com

Abstract: *Despite the growing evidence that the presence of cryptorchidism increases the risk of azoospermia, the common mechanisms via which it occurs remain incompletely elucidated. This research was conducted to gain a deeper understanding of the molecular mechanisms that contribute to the onset and progression of this complication. In total, 197 common DEGs (182 down-regulated and 15 up-regulated genes) were chosen for further investigation. The significance of cell cycle and metabolic regulation in the onset and progression of both diseases was highlighted by functional analysis. Eventually, nine important hub genes were identified using cystoHubba, including CCNB1, PCNA, AURKA, CCT5, CCT2, STIP1, UBE2N, RUVBL1, and HSF1. Our research elucidates a common pathogenesis between cryptorchidism and azoospermia. These common pathways and hub genes might inspire new avenues of investigation into underlying mechanisms.*

Keywords: *cryptorchidism; azoospermia; bioinformatics; differentially expressed genes; hub genes*

1. Introduction

Mounting research evidence indicates that males with cryptorchidism have an elevated relative risk of infertility, which occurs in 10%-30% of individuals with unilateral undescended testis. This increases to 35% to 65% or more for bilateral disease. If patients with bilateral cryptorchid testes are not treated, the probability of infertility rises to >90 % [1]. There is a correlation between cryptorchidism and varied degrees of endocrinopathy, which affects the hypothalamus-pituitary-gonadal axis. Several factors may lead to decreased fertility in men who have cryptorchidism. One of these factors is hyperthermia, which occurs when the temperature inside the scrotum is much higher than that of the ectopic position and can impair the spermatogenesis process [1]. It impairs Sertoli cell function and possibly leydig cell function as well[2]. The presence of an undescended testis is linked to a variety of anatomical anomalies, including testis-epididymal disjunction. During the orchiopexy procedure, there is a chance that the vas, epididymis, or testis may sustain damage. In infertile individuals who have a previous medical record of cryptorchidism, the prevalence of anti-sperm antibodies is much greater [3]. Deficient luteinizing hormone production is triggered by insufficient PROK2, CHD7, FGFR1, and SPRY4 gene expression in the high-infertility risk group of cryptorchid boys, leading to impaired mini-puberty and infertility[4].

Although cryptorchidism is recognized to be a risk factor for the development of azoospermia, the specific processes that underlie the coexistence of these two conditions continue to be a mystery. It's conceivable that gonocytes transforming and differentiating into AD spermatogonia is the most basic pathway[5]. The sex-specific development of the male germline initiates approximately 6–7 weeks post-

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

conception with the expression of sex-determining region Y and SRY-box transcription factor 9 in the gonadal somatic cells, triggering the formation of seminiferous cords with Sertoli cells[6]. Existing studies using differential gene expression profiling of gonocyte and spermatogonia show that telomerase reverse transcriptase, T-box transcription factor Brachyury, PAX7, and DMRTC2 all perform crucial roles in the development of Ad spermatogonia [7].

This common transcriptional characteristic between cryptorchidism and infertility may lead to a deeper understanding of its common pathogenic mechanism. This study seeks to detect hub genes involved in the pathogenetic mechanism of cryptorchidism compounded by infertility. We investigated two sets of GEO-downloaded gene expression data, GSE 149084 and GSE190752. Common DEGs and their activities in cryptorchidism and azoospermia were found using in-depth bioinformatics and enrichment analyses. In addition, we used the STRING database and the Cytoscape software to generate a PPI network for analyzing gene modules and locating hub genes. Eventually, we zeroed down on 9 key hub genes and evaluated their corresponding transcription factors. It is expected that the newly identified hub genes between cryptorchidism and infertility will shed light on the molecular underpinnings of both conditions.

2. Materials and Methods

2.1 Data Source

GEO (http://www.ncbi.nlm.nih.gov/geo)[8] is a public resource that contains numerous high-throughput sequencing and microarray data sets submitted by research institutions all around the globe. Using the terms cryptorchidism and azoospermia as search terms, we looked for associated gene expression datasets. Two independent expression profiles generated using the same sequencing technology with the largest sample size are required for inclusion. Furthermore, human test subjects should be used in the samples provided. Consequently, two microarray datasets, designated GSE149084 and GSE190752, were obtained. The GSE149084 dataset includes one case of incarcerated inguinal hernias, six children with cryptorchidism, and two children with testicular trauma. There are 3 cases of non-obstructive azoospermia and three patients with obstructive azoospermia in the GSE190752 dataset.

2.2 Ethical issues and informed consent

The project received approval from the local research ethics committee. Additionally, the Heyuan Maternal and Child Health Hospital Ethical Review Board granted its approval to the study protocol before it was carried out.

2.3 Identification of DEGs

R is an integrated suite of software facilities for data manipulation, calculation, and graphical display. The differential expression multiple was calculated utilizing the Limma package, and the data were read utilizing the GEOquery package. The threshold value of $P < 0.05$ was interpreted as significant, and the | log2 fold change (FC) | absolute value was adjusted to > 2. All DEGs were obtained after screening for FDR and P-values. R software can also regroup, integrate, and compare DEGs according to experimental requirements. In this particular experiment, the volcano diagrams of the DEGs were plotted using the ggplot2 package. The common DEGs were established with the use of a web-based Venn diagram generator. Accurate data were acquired by first pre-processing and standardizing the raw dataset.

2.4 Enrichment Analyses of DEGs

The Gene Ontology (GO)(http://geneontology.org/)[9] database was created by the Gene Ontology Federation, which provides simple annotations of gene products from functions, biological pathways

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

involved, and location in cells. The Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway database is specifically designed to store data on the gene pathways in a range of distinct species. KEGG Orthology Based Annotation System (KOBAS) (http://kobas.cbi.pku.edu.cn) [10] is a Web server that gathers data on the functional annotation of 4325 different species of organisms and is used for gene and protein functional annotation/enrichment. The KOBAS 3.0 database was used to compile the GO and KEGG Pathway enrichment study findings. The significance level was established at an adjusted P-value <0.05.

2.5 PPI Network Construction and Module Analysis

Constructing a PPI network with intricate regulatory linkages requires searching for links between proteins of interest, like direct binding associations or concurrent downstream and upstream regulatory pathways using the Search Tool for the Retrieval of Interacting Genes (STRING; http://string-db.org) (version 11.0) [11]. Statistical significance was assumed for interactions with a combined score >0.4. This PPI network was mapped out and visually represented with Cytoscape (http://www.cytoscape.org) (version 3.7.2) [12]. Key functional modules were analyzed utilizing the molecular complex detection technology plug-in (MCODE) in Cytoscape. The criteria for selection were as follows: node score cutoff = 0.2, max depth = 100, degree cutoff = 2, and K-core = 2. After that, KOBAS 3.0 was used to perform the GO and KEGG analyses of the relevant modular genes.

2.6 Selection and Analysis of Hub Genes

The Cytoscape's cytoHubba plugin was employed to identify the hub genes. In this work, hub genes were evaluated and chosen using seven widely recognized techniques (EPC, MNC, Stress, Degree, Radial Distance, Closeness, and MCC). We next used GeneMANIA (http://www.genemania.org/) [13], a robust method for finding internal associations among gene sets, to establish a co-expression network based on these hub genes.

2.7 Prediction of Transcription Factors (TFs)

To predict transcriptional regulatory networks, the Transcriptional Regulatory Relationships Unraveled by Sentence-based Text-mining (TRRUST) [14] database stores information corresponding to TFs, including the target genes for each TF and the regulatory associations between them. There are presently two species represented in TRRUST: humans and mice, containing 8,444 and 6,552 TFs targeting regulatory relationships of 800 human TFs and 828 mouse TFs, respectively. Additionally, the TRRUST database was searched for the TFs that regulate the hub genes, and the significance level was established at an adjusted P-value of < 0.05.

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

3. Results and Discussion

3.1 DEG Detection

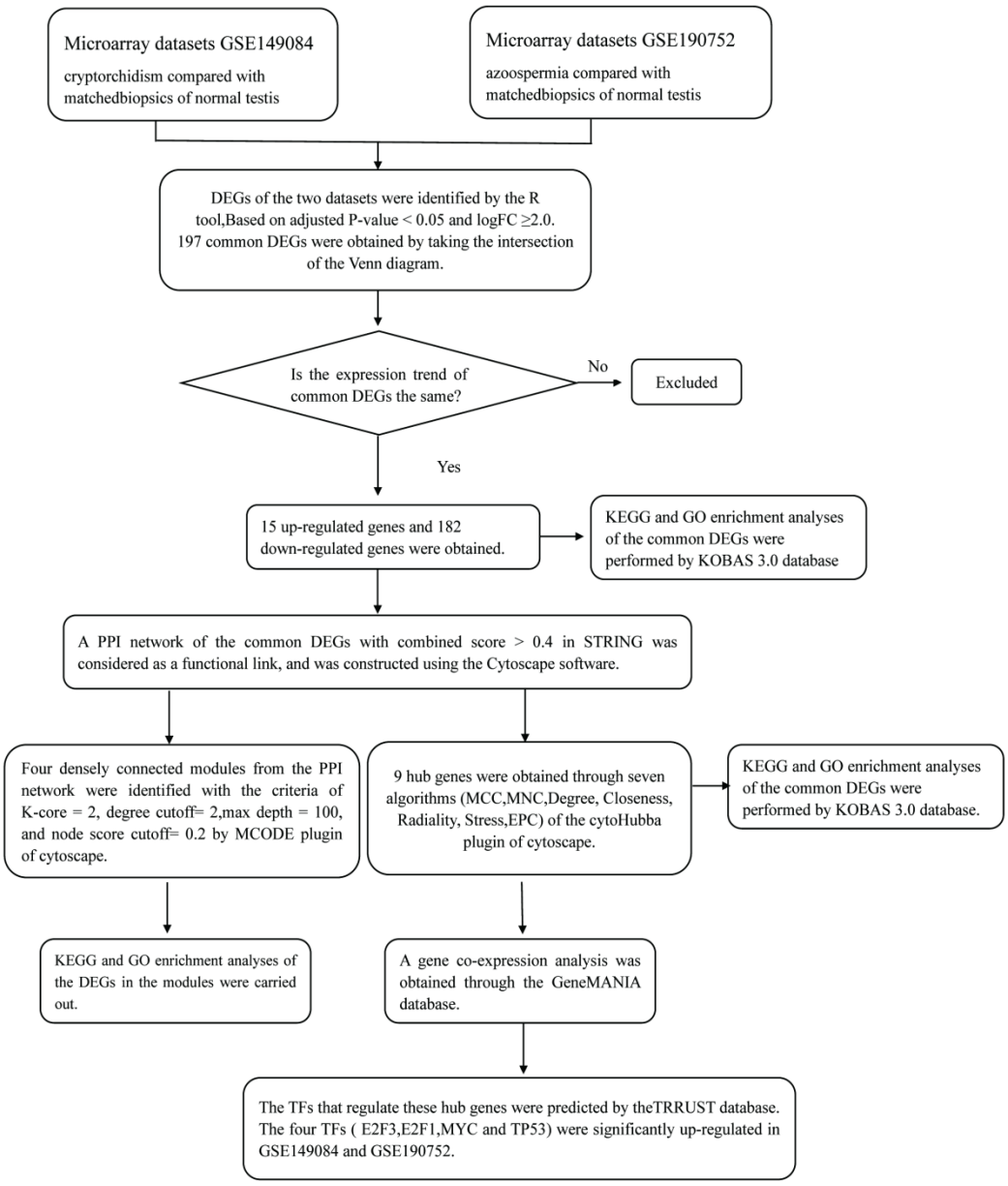

Figure 1: Research design flowchart.

Figure 1 displays the research process flow for the study. Following the standardization of the microarray findings, 2031 DEGs in GSE149084 and 4248 in GSE190752 were detected (Figures 2A, B). Following the completion of the Venn diagram's intersection, an aggregate of 253 common DEGs was found (Figure 2C). After removing genes from GSE30999 and GSE28829 whose expression levels were heading in the opposite direction, we were left with 197 DEGs, which are presented in Table S1.

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

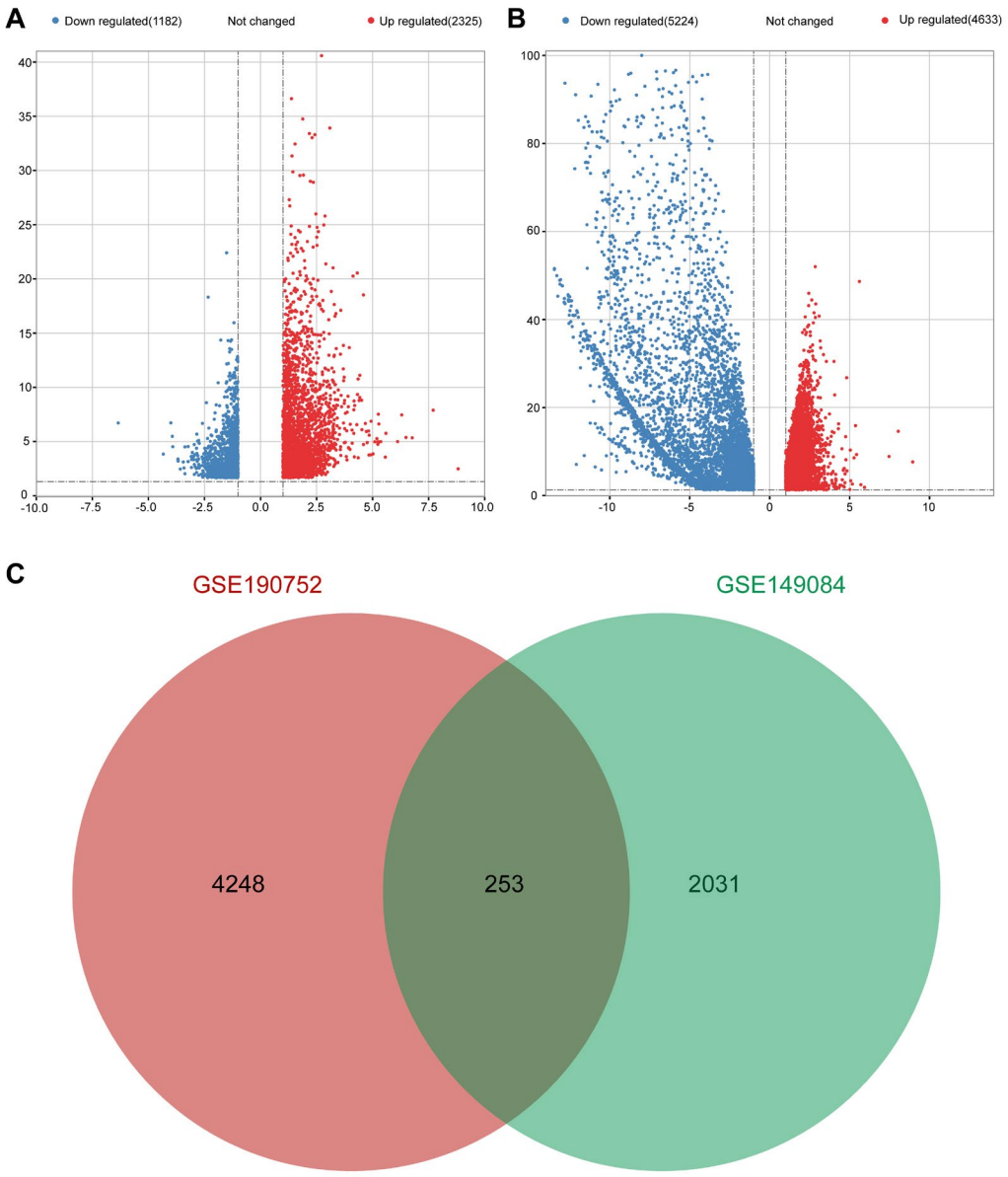

Figure 2: Volcano and Venn diagrams. (A) The GSE149084 volcano map. (B) The GSE190752 volcano map. Genes that have been up- or down-regulated are denoted by light red or blue colors, respectively. (C) In all, 253 DEGs were overlapped between the two datasets.

3.2 Functional Characteristic Analysis of Common DEGs

The 197 common DEGs were subjected to GO and KEGG Pathway enrichment analyses to determine their associated biological activities and pathways. Enrichment of these genes based on GO analysis was primarily observed in the nuclear part (P = 1.07E-07), nuclear lumen(P = 6.10E-07), nucleoplasm (P = 4.62E-08), cell cycle (P = 4.22E-07) and protein modification by small protein conjugation or removal (P = 1.05E-07) (Figure 3B). The three most markedly enriched KEGG pathways were progesterone-mediated oocyte maturation (P = 0.0103), ribosome biogenesis in eukaryotes (P = 0.0130), and Oocyte meiosis (P = 0.0245) (Figure 3A). Together, these findings point to a shared role for the cell cycle and progesterone-mediated oocyte maturation in the onset and progression of cryptorchidism and azoospermia.

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

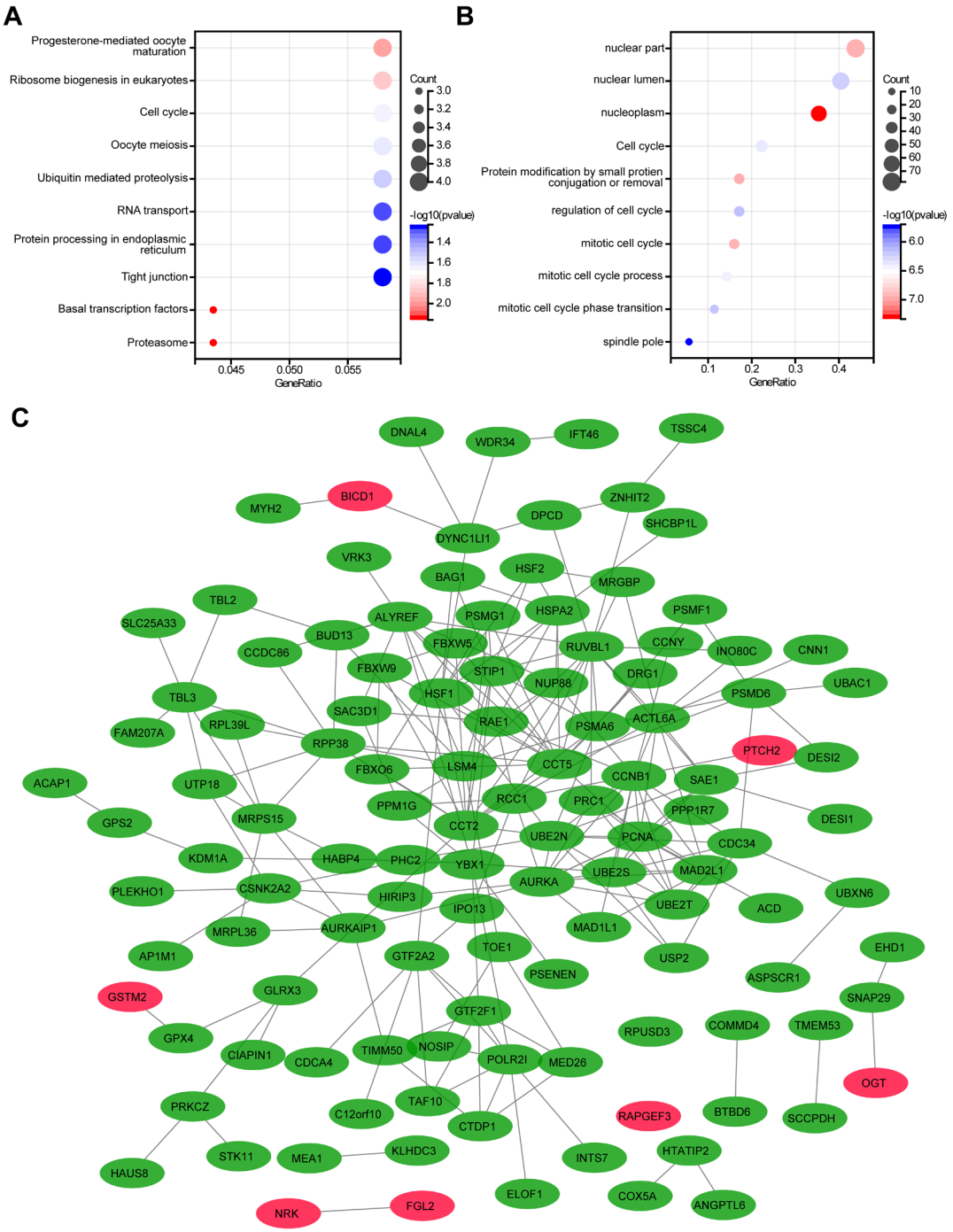

Figure 3: Enrichment analysis results of PPI network and common DEGs enrichment study. (A, B) Enrichment analysis results of GO and KEGG pathways. Significance was established at an adjusted P-value of < 0.05. (C) A representation of the PPI network. Genes that have been up- or down-regulated are denoted by red and green colors, respectively.

3.3 Development and Analysis of PPI Network Modules

The Cytoscape-built PPI network of common DEGs with combined scores > 0.4 consisted of 119 nodes and 226 interaction pairings (Figure 3C). In total, 20 common DEGs and 42 interaction pairings were found via Cytoscape's MCODE plug-in to produce four strongly related gene modules (Figures 4A–D). According to the GO analysis, these genes are implicated in metabolic processes and cellular synthesis (Figure 4E). Analysis of KEGG pathways revealed their primary roles in progesterone-

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

mediated oocyte maturation, eukaryotic ribosome biogenesis, oocyte meiosis, and the cell cycle (Figure 4F).

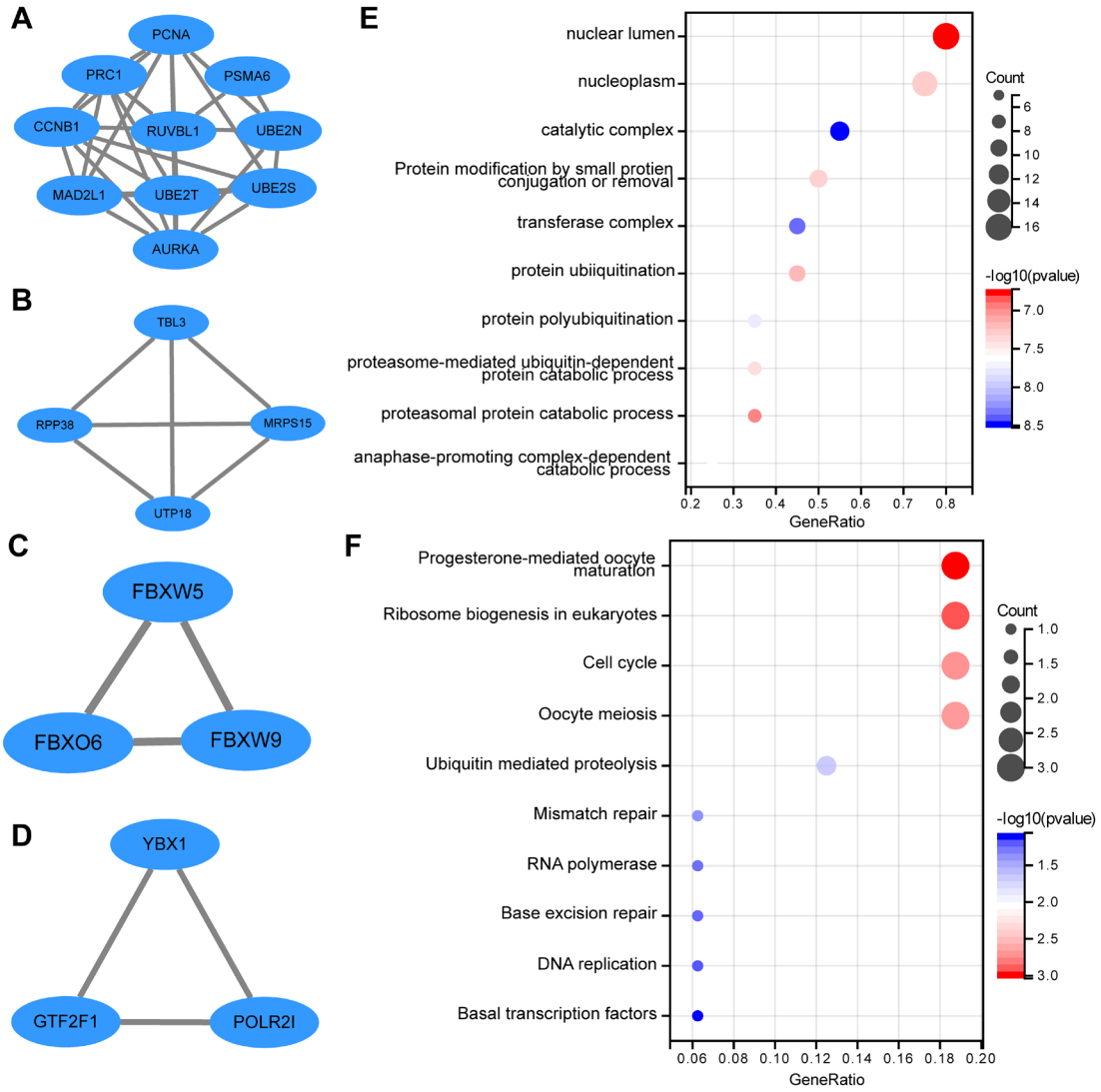

Figure 4: Analysis of significant gene modules and enrichment of the modular genes. (A–D) Three significant gene clustering modules. (E, F) The modular genes were analyzed for GO and KEGG enrichment. The number of involved genes is represented by the size of the circle, and their relative frequency in the term total genes is displayed on the abscissa.

3.4 Selection and Analysis of Hub Genes and Prediction of TFs

We determined the top twenty hub genes by using the seven algorithms that are included in the plug-in cytoHubba (Table 1). We identified 9 common hub genes by analyzing the intersection of the Venn diagrams: CCNB1, PCNA, AURKA, CCT5, CCT2, STIP1, UBE2N, RUVBL1, and HSF1 (Figure 5A). Their full names and respective roles are included in Table 2. The co-expression network as well as associated functions of these genes were examined using data from the GeneMANIA database. With co-expression at 41.23 %, co-localization at 0.22 %, physical interactions at 34.77 %, predicted at 18.13 %, and pathways at 2.54 %, these genes displayed a very intricate PPI network (Figure 5B). As per the GO analysis, these genes play a substantial role in the chaperone complex, microtubule cytoskeleton, microtubule organizing center, positive regulation of DNA metabolic process, and modulation of localization of telomerase RNA to the Cajal body (Figure 6A). These findings highlighted the significance of the cell cycle and telomerase in cryptorchidism and azoospermia. The genes are also

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

predominantly engaged in Progesterone-induced oocyte maturation, mismatch repair, oocyte meiosis, and Base excision repair, as revealed by KEGG Pathway analysis (Figure 6B). The expression of these genes may be regulated by 4 TFs based on the TRRUST database (Figure 6C and Table 3).

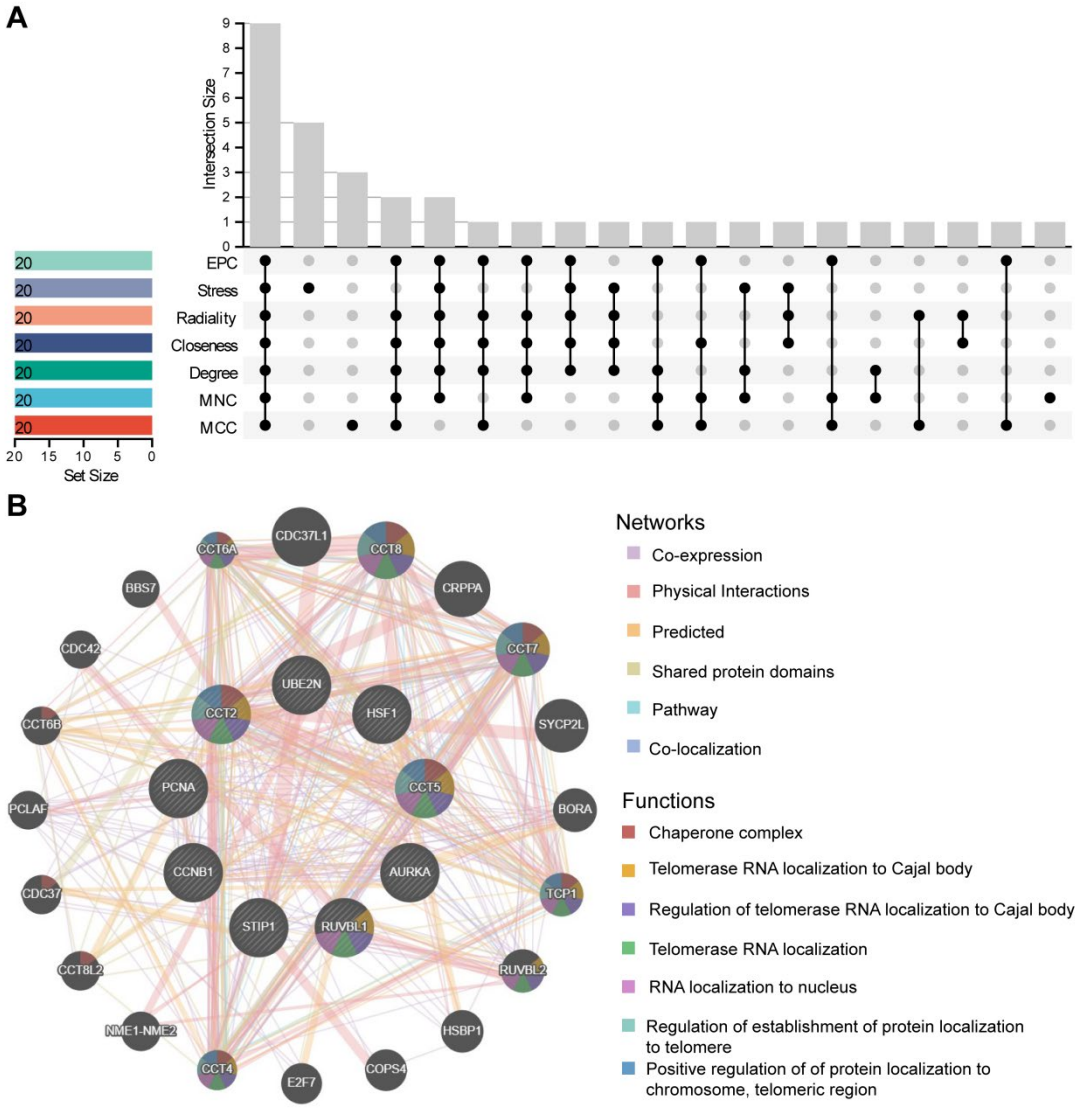

Figure 5: Venn diagram and co-expression network of hub genes. (A)The Venn diagram showed that seven algorithms have screened out 9 overlapping hub genes. (B) GeneMANIA evaluated hub genes and the genes that were co-expressed with them.

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

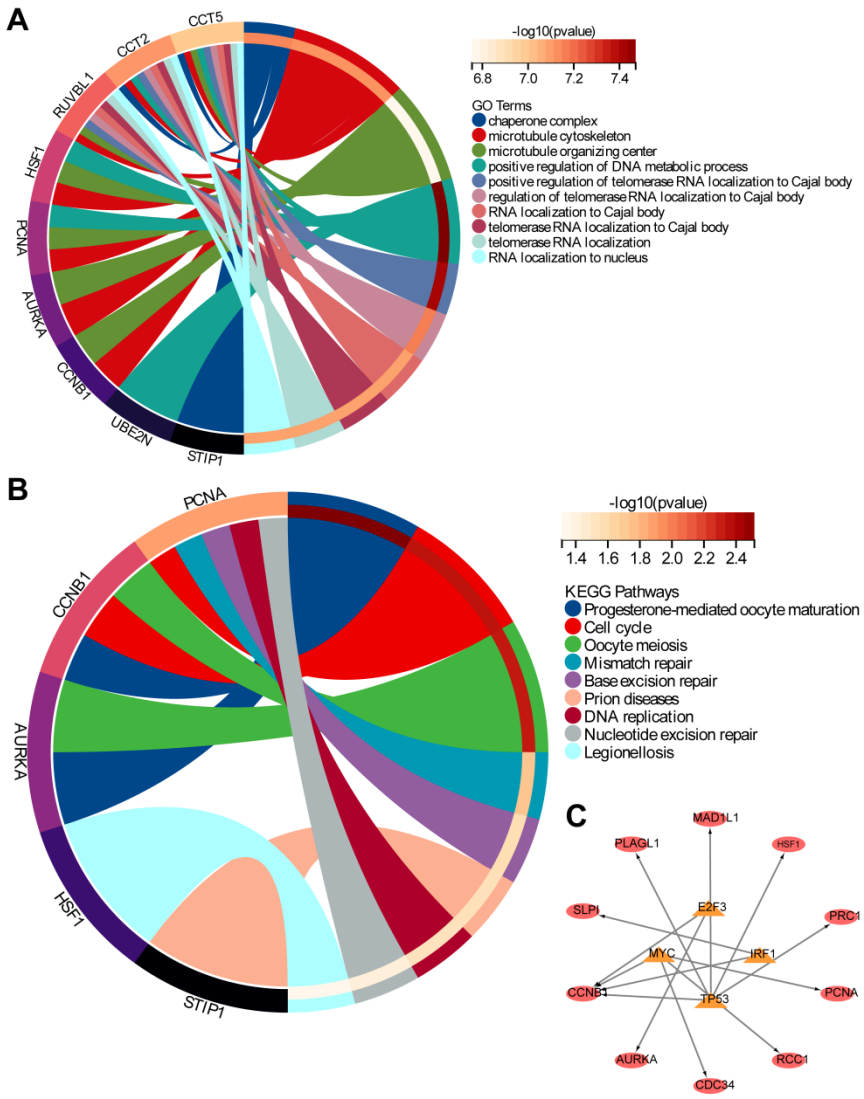

Figure 6: Hub gene enrichment analysis. (A, B) The hub genes were analyzed for GO and KEGG enrichment. Gene pathways are shown as concentric circles, with the outermost circle representing the associated term on the right and the inner circle representing the accompanying significant P-value. (C) Network of regulatory TFs. TFs were marked in yellow, and the hub genes were marked in red.

Table 1: The cytoHubba ranking shows the top twenty hub genes.

MCC	MNC	Degree	Closeness	Radiality	Stress	EPC
CCNB1	CCT5	CCNB1	CCT5	CCT2	CCT2	RUVBL1
PCNA	CCNB1	CCT5	CCT2	CCT5	UBE2N	CCT2
AURKA	CCT2	RUVBL1	RUVBL1	UBE2N	RUVBL1	CCT5
MAD2L1	RUVBL1	CCT2	UBE2N	HSF1	CCT5	CCNB1
UBE2T	UBE2S	AURKA	HSF1	RUVBL1	HSF1	UBE2N
UBE2S	STIP1	PCNA	STIP1	STIP1	RPP38	PCNA
PRC1	PCNA	UBE2N	CCNB1	PSMA6	CCNB1	STIP1

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

CCT5	AURKA	STIP1	PSMA6	PCNA	ALYREF	UBE2S
CCT2	PSMA6	ALYREF	PCNA	LSM4	AURKA	AURKA
STIP1	ACTL6A	UBE2S	ALYREF	ALYREF	YBX1	HSF1
UBE2N	MAD2L1	HSF1	AURKA	RPP38	GTF2A2	ACTL6A
RUVBL1	UBE2N	POLR2I	LSM4	CCNB1	STIP1	PSMA6
HSF1	HSF1	ACTL6A	ACTL6A	ACTL6A	GLRX3	MAD2L1
PSMA6	GTF2F1	RPP38	RPP38	AURKA	PHC2	ALYREF
FBXW5	UBE2T	PSMA6	UBE2S	RCC1	LSM4	UBE2T
FBXO6	POLR2I	MAD2L1	RCC1	HSPA2	POLR2I	LSM4
FBXW9	RAE1	LSM4	HSPA2	UBE2S	ACTL6A	HSPA2
PSMG1	LSM4	HSPA2	YBX1	PSMG1	DYNC1LI1	PRC1
HSPA2	SAE1	GTF2F1	SAE1	SAE1	PCNA	SAE1
CDC34	PRC1	SAE1	PRC1	YBX1	CSNK2A2	CDC34

Table 2: Specifics about the hub genes.

No.	Gene symbol	Full name	Function
1	CCNB1	cyclin B1	The product of this gene's encoding is a mitosis-related regulatory protein.
2	PCNA	proliferating cell nuclear antigen	The protein that is produced as a result of this gene's encoding is localized to the nucleus and functions as a cofactor for DNA polymerase delta. In DNA replication, the encoded protein performs the function of a homotrimer and contributes to an enhancement in the processivity of leading strand synthesis.
3	AURKA	Aurora kinase A	The cell cycle-regulated kinase that is encoded by this gene's product is thought to have a role in the production of microtubules and/or their stability at the spindle pole during the process of chromosomal segregation.
4	CCT5	chaperonin containing TCP1 subunit 5	The molecular chaperone that is encoded by this gene is a component of the chaperonin-containing TCP1 complex (CCT), which is also referred to as the TCP1 ring complex (TRiC).
5	CCT2	chaperonin containing	This gene's product is a molecular chaperone that functions as a part of the CCT, also referred to as the

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

		TCP1 subunit 2	TRiC.
6	STIP1	stress-induced phosphoprotein 1	In protein folding, STIP1 acts as an adapter protein, coordinating the activities of heat shock proteins 70 (HSP70; MIM 140550) and 90 (HSP90AA1; MIM 140571).
7	UBE2N	ubiquitin-conjugating enzyme E2 N	The ubiquitination function performed by this gene is a crucial biological process for directing proteins with aberrant or short-lived toward disintegration.
8	RUVBL1	RuvB like AAA ATPase 1	The protein produced by this gene exhibits both DNA-dependent ATPase and DNA helicase capabilities, and it is classified within the ATPases associated with diverse cellular activities (AAA+) protein family.
9	HSF1	heat shock transcription factor 1	The protein that is produced by this gene is a TF that binds heat shock promoter elements (HSE)and is quickly activated in response to temperature stress.

Table 3: Key transcriptional factors (TFs) of hub genes.

Key TFs	Description	P-value	Genes
E2F3	E2F transcription factor 3	1.57E-05	CCNB1,AURKA
E2F1	E2F transcription factor 1	2.85E-05	CCNB1,AURKA,PCNA
MYC	v-myc myelocytomatosis viral oncogene homolog	0.000977	CCNB1,PCNA
TP53	tumor protein p53	0.0026	HSF1,CCNB1

4. Discussion

Congenital cryptorchidism is a prevalent congenital malformation that is strongly linked to oligospermia and male infertility in the future[15]. The pathophysiology of cryptorchidism is multifaceted, with significant data from numerous research encompassing multiple genetic, maternal, and environmental risk factors. In addition, there is evidence that smoking during pregnancy, drinking alcohol, and having gestational diabetes all enhance this risk[16]. The development of spermatozoa in mammals is a three-step process that begins with the mitotic proliferation of spermatogonia, continues through meiosis in spermatocytes, and culminates in the formation of mature spermatozoa[17]. Early spermatogonia can guarantee a continuous supply of spermatocytes, which are essential for fertility. Gene expression must be precisely controlled either transcriptionally or post-transcriptionally to maintain spermatocyte function[18]. Hypogonadotropic hypogonadism, as shown by the study conducted by Faruk Hadziselimovic et al., is the most prominent contributor to cryptorchidism. Molecular evidence suggests a crucial involvement of PROK2 in the pathophysiology of cryptorchidism, in which aberrant gene expression of PROK2, CHD7, FGFR1, SPRY4, regulated by EGR4 and PITX1, causes LH deficiency [19]. Modifications in the PI3K-AKT-mTOR signaling pathway have been linked to spermatogenesis abnormalities, as demonstrated by Hongshuai Jia et al. Additionally, hsa-miR-7-5p and hsa-miR-519d-3p, two miRNAs linked to the protein kinase AKT3, could be useful as indicators of cryptorchidism and cryptorchid-induced azoospermia[20]. Patients with cryptorchidism have defective micro-puberty, which leads to azoospermia and infertility, as shown by the work of Hadziselimovic F et

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

al. using RNA-seq analysis[4]. Both investigations of cryptorchid males (with defective tubercles and missing Ad spermatogonia) found a reduction in the reproducibility of the gene set, providing strong evidence for a function for the HPG axis in the onset and progression of azoospermia and infertility[4].

Cryptorchidism and azoospermia may have overlapping pathogenic pathways. This study primarily sought to find common DEGs in cryptorchidism and azoospermia and reveal potential targets to predict biological agents for the treatment of cryptorchidism combined with azoospermia. Germline genomic integrity is critical to ensure successful gametogenesis and reproduction, and the maintenance of genomic integrity is largely regulated epigenetically. Thus, specific gene mutations have emerged as potential causes of male infertility. Herein, we found 197 shared DEGs between the two sets; 9 of them were hub genes (HSF1, CCNB1, PCNA, AURKA, CCT5, CCT2, STIP1, UBE2N, RUVBL1, and UBE2N). GO and KEGG pathway enrichment analyses confirmed that these genes were significant in the chaperone complex, microtubule cytoskeleton, microtubule organizing center, positive regulation of DNA metabolic process, and modulation of Cajal body RNA telomerase localization. Furthermore, we discovered that 4 TFs could regulate the expression of these genes.

Cell cycle protein B1 (CCNB1) is a crucial component of the machinery that controls the cell cycle and is necessary for mouse embryonic development, ablation of CCNB1 in germ cells and spermatogonia, preventing normal proliferation as well as increasing the apoptotic rate, ultimately leading to germ cell depletion and male infertility[21]. Septin 4 may perform an instrumental function in the meiotic G2/M transition by indirectly regulating the stability of CCNB1 in mouse oocytes[22]. However, it is currently uncertain whether CCNB1 performs different functions in the mammalian cell division processes of mitosis and meiosis. PCNA, or proliferating cell nuclear antigen, is a factor that is necessary for both the replication and repair of DNA. Research indicates that the downregulation of the PCNA gene is linked to inadequate spermatogenesis[23]. For centrosomes to mature and be properly segregated, a well-known mitotic regulator called Aurora A kinase (AURKA) must be present[24]. By using immunohistochemical labeling, Yuanyuan Wang and colleagues detected positive signals for AURKA in the hypothalamus, pituitary, and testicular tissues of Tibetan sheep. AURKA is necessary for animal reproduction and is found mostly in spermatozoa and supporting cells (SC). AURKA performs a dual role in mitosis and meiosis during spermatogenesis and in ensuring healthy sperm growth and motility. Notably, AURKA is found in the flagellum's main and middle segments, making it a promising substrate for regulating sperm motility[25]. TStress-inducible protein 1 (Stip1) is a 60kDa protein that plays crucial roles under stress and non-stress conditions[26]. The Stip1 phenotype is overexpressed in a variety of human tissues, such as the epididymis and testis. In rats, stip1 is developmentally regulated in the testis and epididymis and is upregulated following heat shock[27].

Our investigation focuses primarily on the identification of hub genes and corresponding TFs that are shared by both cryptorchidism and azoospermia. Using the nodes' common DEGs, we developed a complicated interaction network to isolate the key nodes. The reliability of this comprehensive bioinformatics approach has been shown in different diseases. Related TFs were also examined in this study. Our findings may help researchers determine new avenues to explore in their quest to understand the molecular process behind azoospermia with cryptorchidism. The hub genes linked to cryptorchidism and azoospermia have been investigated independently in previous research. However, only a few studies have used advanced bioinformatics techniques to investigate the common molecular mechanisms between them. Since azoospermia and cryptorchidism have a high comorbidity rate, we sought to comprehend their common molecular mechanisms by identifying DEGs, hub genes, and TFs for the first time. Nevertheless, there are several drawbacks to our study. For starters, we need to verify our results externally since this study uses a retrospective approach. Second, it is necessary to conduct additional experiments using an in vitro model to validate the function of the hub gene, which will be the focus of our future work.

5. Conclusions

Overall, using enrichment and PPI network analysis, we identified the common DEGs between

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

cryptorchidism and azoospermia. According to our findings, several specific hub genes may serve as mediators of the shared pathogenic processes that underlie cryptorchidism and azoospermia. The findings from this research provide new perspectives for understanding the molecular mechanisms behind cryptorchidism complicated with azoospermia.

Acknowledgments

We appreciate the support of the Heyuan City Social Development Science and Technology Program.

Author Contributions

A major research plan was developed by XZ, MZ, and XZ. ZW and HL performed data analysis, graphical representation, and manuscript writing. Data and references were gathered by JZ and PL. Each author made significant contributions and read and approved the submitted version.

References

[1] Urry, R L et al. "The incidence of antisperm antibodies in infertility patients with a history of cryptorchidism." The Journal of urology vol. 151, 2 (1994): 381-3. doi:10.1016/s0022-5347(17)34954-6

[2] Rodprasert, Wiwat et al. "Hypogonadism and Cryptorchidism." Frontiers in endocrinology vol. 10 906. 15 Jan. 2020, doi:10.3389/fendo.2019.00906

[3] Sinisi A A, Pasquali D, Papparella A, et al. Antisperm antibodies in cryptorchidism before and after surgery[J]. The Journal of urology, 1998, 160(5): 1834-1837.

[4] Hadziselimovic, Faruk et al. "Gene Expression Changes Underlying Idiopathic Central Hypogonadism in Cryptorchidism with Defective Mini-Puberty." Sexual development: genetics, molecular biology, evolution, endocrinology, embryology, and pathology of sex determination and differentiation vol. 10,3 (2016): 136–146. doi:10.1159/000447762

[5] Hadziselimovic, F, and B Herzog. "The importance of both an early orchidopexy and germ cell maturation for fertility." Lancet (London, England) vol. 358, 9288 (2001): 1156-1157. doi:10.1016/S0140-6736(01)06274-2

[6] Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH.Larsen's Human Embryology. Philadelphia, PA: Elsevier; 2021.

[7] Gegenschatz-Schmid, Katharina et al. "DMRTC2, PAX7, BRACHYURY/T and TERT Are Implicated in Male Germ Cell Development Following Curative Hormone Treatment for Cryptorchidism-Induced Infertility." Genes vol. 8,10 267. 11 Oct. 2017, doi:10.3390/genes8100267

[8] Edgar R, Domrachev M, Lash AE.Gene Expression Omnibus: NCBI gene expression and hybridization array data repository Nucleic Acids Res. 2002 Jan 1; 30(1):207-210.

[9] The Gene Ontology resource: enriching a GOld mine. Nucleic Acids Res. Jan 2021;49(D1):D325-D334.

[10] Dechao Bu, Haitao Luo, et al., KOBAS-i: intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis, Nucleic Acids Research, Volume 49, Issue W1, 2 July 2021, Pages W317–W325.

[11] Franceschini, Andrea et al. "STRING v9.1: protein-protein interaction networks, with increased coverage and integration." Nucleic acids research vol. 41,Database issue (2013): D808-D815. doi:10.1093/nar/gks1094

[12] Smoot, Michael E et al. "Cytoscape 2.8: new features for data integration and network visualization." Bioinformatics (Oxford, England) vol. 27,3 (2011): 431-432. doi:10.1093/bioinformatics/btq675

[13] Warde-Farley, David et al. "The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function." Nucleic acids research vol. 38,Web Server issue (2010): W214-W220. doi:10.1093/nar/gkq537

ISSN 2618-1584 Vol. 5, Issue 12: 12-25, DOI: 10.25236/FMSR.2023.051202

[14] Han, Heonjong et al. "TRRUST v2: an expanded reference database of human and mouse transcriptional regulatory interactions." Nucleic acids research vol. 46,D1 (2018): D380-D386. doi:10.1093/nar/gkx1013

[15] Nassau, Daniel E et al. "The pediatric patient and future fertility: optimizing long-term male reproductive health outcomes." Fertility and sterility vol. 113,3 (2020): 489-499. doi:10.1016/j.fertnstert.2020.01.003

[16] Barthold, Julia Spencer et al. "Genetic, Maternal, and Environmental Risk Factors for Cryptorchidism: An Update." European journal of pediatric surgery : official journal of Austrian Association of Pediatric Surgery ... [et al] = Zeitschrift fur Kinderchirurgie vol. 26,5 (2016): 399-408. doi:10.1055/s-0036-1592416

[17] de Rooij, D G, and J A Grootegoed. "Spermatogonial stem cells." Current opinion in cell biology vol. 10,6 (1998): 694-701. doi:10.1016/s0955-0674(98)80109-9

[18] Papaioannou, Marilena D, and Serge Nef. "microRNAs in the testis: building up male fertility." Journal of andrology vol. 31,1 (2010): 26-33. doi:10.2164/jandrol.109.008128

[19] Hadziselimovic, Faruk. "On the descent of the epididymo-testicular unit, cryptorchidism, and prevention of infertility." Basic and clinical andrology vol. 27 21. 14 Nov. 2017, doi:10.1186/s12610-017-0065-8

[20] Jia, Hongshuai et al. "AKT3 and related molecules as potential biomarkers responsible for cryptorchidism and cryptorchidism-induced azoospermia." Translational pediatrics vol. 10,7 (2021): 1805-1817. doi:10.21037/tp-21-31

[21] Tang, Ji-Xin et al. "Requirement for CCNB1 in mouse spermatogenesis." Cell death & disease vol. 8,10 e3142. 26 Oct. 2017, doi:10.1038/cddis.2017.555

[22] Chen, Li et al. "Septin 4 controls CCNB1 stabilization via APC/CCDC20 during meiotic G2/M transition in mouse oocytes." Journal of cellular physiology vol. 237,1 (2022): 730-742. doi:10.1002/jcp.30498

[23] Ibis, Muhammed Arif et al. "Can PCNA and LIM15 gene expression levels predict sperm retrieval success in men with non-obstructive azoospermia?." Revista internacional de andrologia vol. 20 Suppl 1 (2022): S31-S38. doi:10.1016/j.androl.2021.04.002

[24] Nikonova, Anna S et al. "Aurora A kinase (AURKA) in normal and pathological cell division." Cellular and molecular life sciences : CMLS vol. 70,4 (2013): 661-687. doi:10.1007/s00018-012-1073-7

[25] Lester, William C et al. "Aurora A Kinase (AURKA) is required for male germline maintenance and regulates sperm motility in the mouse." Biology of reproduction vol. 105,6 (2021): 1603-1616. doi:10.1093/biolre/ioab168

[26] Odunuga, O O et al. "Hop: more than an Hsp70/Hsp90 adaptor protein." BioEssays : news and reviews in molecular, cellular and developmental biology vol. 26,10 (2004): 1058-1068.doi:10.1002/bies.20107

[27] Wang, Yanwei et al. "Systematic study of stress-inducible protein 1 (Stip1) in male reproductive system and its expression during stress response." Gene vol. 554,1 (2015): 58-63. doi:10.1016/j.gene.2014.10.023