Transcriptome Analysis of Spermatogenically Regressed, Recrudescent and Active Phase Testis of Seasonally Breeding Wall Lizards *Hemidactylus flaviviridis*

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**Abstract**

**Background:** Reptiles are phylogenetically important group of organisms as mammals have evolved from them. Wall lizard testis exhibits clearly distinct morphology during various phases of a reproductive cycle making them an interesting model to study regulation of spermatogenesis. Studies on reptile spermatogenesis are negligible hence this study will prove to be an important resource.

**Methodology/Principal Findings:** Histological analyses show complete regression of seminiferous tubules during regressed phase with retracted Sertoli cells and spermatogonia. In the recrudescent phase, regressed testis regain cellular activity showing presence of normal Sertoli cells and developing germ cells. In the active phase, testis reaches up to its maximum size with enlarged seminiferous tubules and presence of sperm in seminiferous lumen. Total RNA extracted from whole testis of regressed, recrudescent and active phase of wall lizard was hybridized on Mouse Whole Genome 8 x 60 K format gene chip. Microarray data from regressed phase was deemed as control group. Microarray data were validated by assessing the expression of some selected genes using Quantitative Real-Time PCR. The genes prominently expressed in recrudescent and active phase testis are cytoskeleton organization GO:0005856, cell growth GO:0045927, GTPase regulator activity GO:0030695, transcription GO:0006352, apoptosis GO:0006915 and many other biological processes. The genes showing higher expression in regressed phase belonged to functional categories such as negative regulation of macromolecule metabolic process GO:0010605, negative regulation of gene expression GO:0010629 and maintenance of stem cell niche GO:0045165.

**Conclusion/Significance:** This is the first exploratory study profiling transcriptome of three drastically different conditions of any reptilian testis. The genes expressed in the testis during regressed, recrudescent and active phase of reproductive cycle are in concordance with the testis morphology during these phases. This study will pave the way for deeper insight into regulation and evolution of gene regulatory mechanisms in spermatogenesis.

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**Introduction**

Reptiles hold a phylogenically crucial place in animal evolution as they are ancestor to both birds and mammals. Exhibition of amniotic membrane places reptiles in close proximity with birds and mammals [1]. Tubular form of spermatogenesis, where spermatogenesis takes place in seminiferous tubule and myoid peritubular cells appear for the first time in reptilian testes [2]. These are the features found in testis of higher order mammals also. These features are maintained in higher order mammals also. The wall lizard is a seasonally breeding animal exhibiting a prenuptial cycle of sperm development [3]. Prenuptial reptiles produce sperm prior to or during the mating season. Reproductive cycle of wall lizard is clearly divided in three phases, regressed from late May to August, recrudescent September–November, and spermatogenically active phase from December to early May [4]. In the recrudescent phase, proliferating spermatogonia and meiotic germ cells are present; in active phase advanced germ cells undergoing spermiogenesis and fully mature sperm supported by mature Sertoli cells are present. While in regressed phase, only quiescent spermatogonia and retracted Sertoli cells are present for long time retaining of their ability to repopulate & redifferentiate. Since, any defect in onset and/or reinitiation of spermatogenesis may lead to testicular condition similar to certain forms of infertility. This periodic subtle transition between spermatogenically active and inactive state of testis in this reptile model offer an appropriate situation to understand over and under expression of genes involved in initiation of mitosis, meiosis, maintenance of sperm production, and finally, seizure of spermatogenic process, which is otherwise not possible in mice or rat.
In mice, microarray has been used as a powerful technique to construct gene regulatory networks for a global view of varied states of testes in different age groups [5]. With the advent of microarray technology, the comparison of mRNA expression by germ cells and hormone-induced gene expression in testes has been addressed [6]. Microarray studies have helped in the identification of novel candidate genes (previously non-documented) that play crucial role in the regulation of spermatogenesis [7]. In light of this, an attempt was made to profile genes expressed during regressed, recrudescent, and active state of testis of wall lizard by employing microarray technique. Although South American green lizard anole genome has been sequenced recently [8], microarray chips for the same are not yet commercially available. Because of this constrain and to study genes conserved in lizard and mice, we have used mouse whole genome gene array chips. The present investigation in wall lizard, *H. flaviviridis* is the first exploratory study documenting gene expression across all the phases of its reproductive cycle. Further functional studies based on this microarray data will provide deeper insight in to the specific role of these genes in regulation of spermatogenesis. Although there are several forms of idiopathic male infertility which are untreatable, men do not show distinct demarcation in ability of testis and its recrudescence, hence do not provide opportunity to identify genes important for switching on or off during spermatogenesis.

**Materials and Methods**

**Ethics Statement**

The guidelines of the “Committee for the Purpose of Control and Supervision of Experiment on Animals CPCSEA,” Government of India, were followed in handling, maintenance, and sacrifice of animals with Institutional Animal Ethics Committee IAEC approval DUZOO/IAEC-R/2011/17.

**Animals Maintenance and Tissue Collection**

The wall lizards *Hemidactylus flaviviridis* were procured from in and around the city of Delhi, India. Male animals were housed in wooden cages and acclimatized to laboratory conditions 12L:12D at least for 8 days before the commencement of the experiments. They were provided food and water *ad libitum*. On an average, 4–6 animals were sacrificed in spermatogenically active phase, 6–8 in recrudescent and 50–60 in regressed phase. The decapsulated testes were snap frozen in liquid nitrogen and crushed to powder with sterile mortar and pestle. The powdered tissue was resuspended in 2 ml of RNA-Save, Biological Industries, Israel, with sterile mortar and pestle. The powdered tissue was resuspended in 2 ml of RNA-Save, Biological Industries, Israel, and purity of extracted RNA were assessed using standard instructions of the manufacturer. Equal amount of crushed tissue from each phase of testis was used for RNA isolation. The yield and purity of extracted RNA were assessed using standard protocols of taking absorbance ratio between 260 nm and 280 nm, and in 1% agarose gel electrophoresis. RNA integrity of individual samples was also assessed by Bioanalyzer 2100, using RNA 6000 Nano Lab Chip, Agilent Technologies Inc., Palo Alto, CA. The algorithm of Agilent 2100 Expert Software automatically calculates RNA Integrity Number RIN for the assessment of total RNA quality based on electrophrogram output. RIN score provided a quantitative value for RNA integrity that facilitated the standardization of quality interpretation. Total RNA was considered to be of good quality when the rRNA 28S/18S ratios were greater than or equal to 1.5, with the rRNA contribution being 30% or more and an RNA integrity number RIN was ≥8.0 out of maximum scoring of 10 [10].

**cRNA Synthesis, In vitro Transcription, Labelling and Microarray Hybridization**

Individual RNA samples from each sub-groups and conditions having RIN scores >8.0 were subjected to whole transcriptome array experiment using the Agilent Whole genome Mouse 3×60 K format Array chip, AMADID: 26986, according to the manufacturer’s recommendations. See table S1 for the subject details of the selected samples. The probe synthesis, hybridization, and post-hybridization stringency wash were performed as described by manufacturer’s protocol Agilent Technologies http://www.genomics.agilent.com. Briefly, both first and second strand cDNA were synthesized by incubating 500 ng of total RNA with 1.2 μl of oligo dT-T7 Promoter Primer in nuclease-free water at 65°C for 10 min followed by incubation with 4.0 μl of 5× First strand buffer, 2 μl of 0.1 M DTT, 1 μl of 10 mM dNTP mix, 1 μl of 200 U/μl MMLV-RT, and 0.5 μl of 40 U/μl RNaseOUT, at 40°C for 2 hour. Immediately following cDNA synthesis, the reaction mixture was incubated with 2.4 μl of 10 mM Cyamine-3-CTP (Perkin-Elmer, Boston, MA), 20 μl of 4X Transcription buffer, 8 μl of NTP mixture, 6 μl of 0.1 M DTT, 0.5 μl of RNaseOUT, 0.6 μl of Inorganic pyrophosphatase, 0.8 μl of T7 RNA polymerase, and 15.3 μl of nuclease-free water at 40°C for 2 hour. Qiagen’s RNeasy mini spin columns were used for purifying amplified cRNA samples. The quantity and specific activity of cRNA was determined by using NanoDrop ND-1000 UV-VIS Spectrophotometer version 3.2.1. Samples with specific activity >8 were used for hybridization. 825 ng of each Cyanine 3 labeled cRNA in a volume of 41.8 μl were combined with 11 μl of 10× Blocking agent and 2.2 μl of 25X Fragmentation buffer Agilent, and incubated at 60°C for 30 minutes in dark. The fragmented cRNA was mixed with 55 μl of 2× Hybridization Buffer Agilent. About 110 μl of the resulting mixture was applied to the Agilent Mouse Whole Genome 60 k Array chip Agilent Technologies, and hybridized at 65°C for 17 hours in an Agilent Microarray Hybridization Chamber SureHyb: G2534A in rotating Hybridization Oven. After hybridization, array slides were washed with Agilent Gene expression Wash Buffer I for 1 minute at room temperature followed by a 1 min wash with Agilent Gene expression Wash Buffer II at 37°C. Slides were finally rinsed with Acetoniitrile for cleaning up and drying.

**Microarray Data Processing**

**Array analysis.** Microarray experiments with individual samples were performed according to standard MIAME guide lines http://www.mged.org/Workgroups/MIAME/miame.html. Hybridized arrays were scanned at 5 μm resolution on an Agilent DNA Microarray Scanner G2565BA. Data extractions and
Quantiﬁcation from images were done using Feature Extraction software Agilent Technologies. The extracted raw data were analyzed using GeneSpring Gs v 11.0.1 software from Agilent Technologies. Within each hybridization panel the 50th percentile of all measurements was used as a positive control of normalization for each gene. Per-membrane and per-gene intensity dependent normalization, two-way normalization, lowess normalization, averaging, exploratory analysis and signiﬁcant ratio analysis were performed on log transformed data using GeneSpring v.11.0.1 Agilent Technologies, Santa Clara, CA, USA. Pearson’s correlation coefﬁcients were computed to assess the reliability of data obtained from two separate hybridization runs for same RNA preparations and conﬁrmed the reproducibility assurance $P<0.01$ among hybridizations. Analysis of the data retrieved from separate chips with the same RNA samples yielded QC statistics highly concordant with that of the manufacturer, and it revealed more than 95% conﬁdence level.

Clustering analysis. It has been observed that there are thousands of genes for each observation in gene expression data and a few genes or group of gene may account much of the variation in whole data. Principal component analysis PCA is an unsupervised multivariate analysis tool that reduces the dimensionality of the data set by transforming to a new set of variables principal components, PCs and summarizes the data features [11]. The normalized and ﬁltered data were analyzed to characterize the global relationships of individual samples by unsupervised principal component analysis PCA and the exposed clustered groups were displayed in a three dimensional 3-D graph. The PCA algorithm in GeneSpring11.5.1 was applied to all annotated regressed, recrudescent and active Phase samples for all expressed genes to evaluate the similarity in gene expression patterns on the basis of underlying variability and cluster structures. Since PCA reduces data complexity in a rational way without any prior knowledge of the categories, it was used to determine if any intrinsic clustering or outliers existed within the data set [12].

To further evaluate the patterns of gene expression proﬁles in each sample belonging to different groups and/sub-groups, unsupervised hierarchical clustering of all samples using a standardized Pearson’s uncentered correlation vector with average linkage for distance measures was performed [13]. Sample to sample expression relationship and visualization in the form of dendrogram and heatmap respectively were yielded in hierarchical cluster analysis. While in $K$-means cluster analysis, gene expression levels were randomly assigned into distinct clusters and the average expression vector was computed for each cluster [13]. For every gene, the algorithm then computed the distance to all expression vectors, and moved the gene to the cluster whose expression vector was closest to it. The entire process was repeated iteratively until no gene products could be reassigned to a different cluster. The whole gene classiﬁed into different sets of clusters, $K$ clusters, according to their expression patterns and based on their average expression pattern in different samples, clusters of genes also identiﬁed as co-expressed genes.

Differential gene expression analysis. The fold-change is a measure of differential expression “signal”, whereas t-statistic is a signal standardized by the noise level, i.e., “signal-to-noise” ratio. The fold-change is an example of absolute effect size, whereas t-test a relative effect size. To maximum utilization of statistical information from the data, fold-change and t-statistic can be displayed simultaneously by volcano plots. Volcano plots allow easy comparison between the “double ﬁltering” gene selection criterion and “single ﬁltering” or “joint ﬁltering” criteria.

Normalized microarray data were subjected to one-way analysis of variance followed by pair-wise comparisons of expression values for each gene between active, recrudescent and regressed phase of reproductive cycle samples respectively. The resulting differential gene lists from each pair-wise comparison only included those genes that showed a fold change of $>2.0$ or higher and a $P<0.05$ by using a parametric Welch $t$ test with Benjamini-Hochberg multiple testing corrections for false discovery rate FDR [14]. All statistical analysis was performed using GeneSpring 11.0.1 software.

Gene ontology based functional analysis. The gene ontology GO based functional enrichment analysis was performed by using the co-expressed genes of three different clusters $K_1$, $K_2$, and $K_3$ identiﬁed in previous $K$-means cluster analysis [15]. Further networks and different functional enrichment analyses were done using gene lists obtained from the differential gene expression analyses between different sub-groups based on pre-deﬁned setting of a cut-off threshold of pFDR $p<0.05$ with the help of the GeneSpring11.0.1 software, GeneGo Metacore software Thomson Reuters, St. Joseph, MI, USA, DAVID tool http://david.abcc.ncifcrf.gov [16] and the Kyoto Encyclopedia of Genes and Genomes KEGG platform http://www.genome.jp/kegg/ for pathways analysis to link genomic information with higher order functional information.

Quantitative Real-Time Polymerase Chain Reaction Microarray data was validated by assessing the relative expression of some of the selected genes using SYBR green chemistry by Q-PCR as described earlier [17]. The expression of coronin, actin binding protein 1A Coro1a, Secreted acidic cysteine rich glycoprotein sparce, Inhibitor of growth family, member 1 Ing1, Vasohibin VASH1, Kringle containing transmembrane protein 1 Kremen 1, Casein kinase 2, alpha 1Csank2a1 and ﬁbronectin Fn was checked. The relative quantity was derived from the formula: Fold Change $=2^{\Delta \Delta C_{t}}$. For each sample, the calculated quantity was normalized with relation to the quantity found for GAPDH. The list of primers used in this study is given in Table 1.

Results

Evaluation of the Status of Spermatogenesis in the Wall Lizard Testis from Regressed, Recrudescent and Active Phase of Reproductive Cycle

Weight and size of wall lizard testis differed signiﬁcantly during various phases of reproductive cycle. The testis weighed $4–6$ mg, $20–25$ mg and $36–40$ mg during regressed phase, recrudescent phase and active phase respectively. Gonado-somatic Index GSI during regressed, recrudescent and active phase was $5.4 \times 10^{-4}$, $1.93 \times 10^{-2}$ and $3.4 \times 10^{-2}$ respectively. Light microscopic analysis of testicular sections from regressed, recrudescent and active phase of reproduction showed various phases of reproductive cycle. In the regressed phase seminiferous tubules shrank to the minimal size with no deﬁned tubule morphology. In this phase there are only retracted Sertoli cells and spermatogonia in the seminiferous tubule Figure 1A. No other stages of germ cells were seen in this phase. In the testis from recrudescent phase seminiferous tubule size enlarged with full size Sertoli cell and advanced meiotic germ cells. Elongated spermatids embedded in Sertoli cell cytoplasm were also seen in this phase Figure 1B. In the testis from active phase, diameter of the tubule became maximal. Sertoli cells were also seen in this phase Figure 1C. A detailed comparative description of all the phases of reproductive cycle is given in Table 2.
### Table 1. List of primers used in this study for the validation of microarray data by Q-PCR.

| Gene Name | Primer Sequence* | Primer length | Tm °C | Amplicon Size in base pairs | Accession No. |
|-----------|------------------|---------------|-------|----------------------------|---------------|
| Csnk1e    | **F** CAGTGTTGTATGGGGCTTT | 19            | 59.4  | 131                        | NM_013767.6  |
|           | **R** ACAGTCACACAAAGGCACTCAT | 20            | 58.7  |                            |               |
| Fn1       | **F** GACAATGCGCGTAGACCTG | 19            | 59.3  | 140                        | NM_010233.1  |
|           | **R** TCTAGCGGCGATGACAC   | 18            | 61.0  |                            |               |
| Sparc     | **F** TGGAGTTAGCCAGAGGGAAGT | 21            | 64.9  | 139                        | NM_009242.4  |
|           | **R** TCTAGCGGCGATGACAC   | 18            | 61.0  |                            |               |
| Coro1A    | **F** CTCAAGGATGGCTACGTGC | 19            | 62.5  | 129                        | NM_009898.2  |
|           | **R** CTCCAGCCCTTGACACGGTA | 19            | 62.6  |                            |               |
| VASH1     | **F** CTGGGATGAGTTGGGCTT  | 18            | 61.2  | 119                        | NM_177354.4  |
|           | **R** ATACCCCTTGCCCCTCACA | 19            | 61.7  |                            |               |
| Ing1      | **F** GTTACCGGTGCCTCTC    | 19            | 63.5  | 139                        | NM_011919.4  |
|           | **R** CTGACACTCGGAGCTAG    | 20            | 62.1  |                            |               |
| Kremen1A  | **F** GGTAAAGGCGAGGAGAGAG | 20            | 64.9  | 132                        | NM_032396.3  |
|           | **R** GTGAGGAGGAGGAGAGAG  | 20            | 62.9  |                            |               |

*F; Forward primer, R; reverse primer.

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Figure 1. Size and cross section of wall lizard testis. **A** Very small seminiferous tubules, retracted Sertoli cells and only spermatogonia are seen in regressed phase testis, black arrow. **B** Testis from recrudescence phase shows large seminiferous tubules, normal Sertoli cells and advanced germ cells, yellow arrow. **C** Testis from active phase is largest in size and sperm in seminiferous lumen can be noticed, red arrow. Bar = 10 μm. Note **A** = active phase; **B** = recrudescent phase and **C** = regressed phase.

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Microarray Analyses of Wall Lizard Whole Testis from Regressed, Recrudescent and Active Phase of Reproductive Cycle

The data from duplicate array, separate chips arrays with the same RNA samples, yielded high concordant with more than 95% confidence level with that of the manufacturer. The data from 6 samples, 2 samples from each active, recrudescent and regressed phase, which passed quality control parameters, has been deposited to NCBI via the Gene Expression Omnibus GEO data repository http://www.ncbi.nlm.nih.gov/geo/query/acc. The data deposited to NCBI-GEO can be accessed at GSE36505. The data from duplicate array, separate chips arrays with the same RNA samples, yielded high concordant with more than 95% confidence level with that of the manufacturer. The data from 6 samples, 2 samples from each active, recrudescent and regressed phase, which passed quality control parameters, has been deposited to NCBI via the Gene Expression Omnibus GEO data repository http://www.ncbi.nlm.nih.gov/geo/query/acc.

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Table 2. Description of the different reproductive phases of wall lizard testis.

| Reproductive Phase | Description | Duration from – to | Testis Weight | Animal Body weight | Gonado-Somatic Index |
|--------------------|-------------|--------------------|---------------|--------------------|----------------------|
| Regressed or Quiescent | Retracted Sertoli cell, presence of spermatogonia only, small testis size, shrunk seminiferous tubule | Late May – August | 5.0 mg | 9.3 gm | 5.4×10⁻⁴ |
| Recrudescent | Normal Sertoli cells and testis size, meiotic germ cells, normal seminiferous tubule | September – October22.5 mg | 11.7 gm | 1.9×10⁻³ |
| Active | Same as recrudescent except presence of mature sperm | November – April | 38.0 mg | 10.9 gm | 3.4×10⁻³ |

Cluster Analysis

Principal component analysis PCA. PCA is a mathematical tool that aggregates the correlated samples into new sets of variables/samples using the variability in the gene expression data. Unsupervised PCA based on the percentage variance in gene expression of the active, regresedence and regressed phase samples categorized into 2 main clusters. Figure 2 shows the graphical presentation of PCA. The distribution of genes differentially expressed between active and regressed phase in components 1, 2 and 3, while in between regresedence and regressed phase in components 1, 2 and 3, revealed considerable degree of expression homogeneity in their corresponding groups.

Hierarchic clustering analysis HCA. The results of the unsupervised HCA of the all 6 samples, 2 samples each from active, recrudescent and regressed phases, and semi-supervised hierarchal clustering analysis and heat map showed that gene expression pattern in active phase clustered closely with those in regressed phase and both of these clustered together with recrudescent phase samples. The heat map generated based on HCA showed that there was no significant variation among biological replicates Figure 3. This analysis confirmed that the expressions of biological replicates were well correlated with each other.

K-means cluster analysis. To identify the clusters of genes whose expression is regulated in a similar way throughout the samples, K-means entity based clustering tool was applied to all the expressed genes across all the 6 samples of regressed, recrudescent and active phase tests. As shown in Figure 4, 3 major groups named as clusters K-1, K-2 and K-3 were identified. The clusters distinguished themselves according to the percentage enrichment of gene ontology GO-based functionality with regard to biological processes, molecular functions, and cellular localization. Out of 60 K probe sets, the largest cluster cluster K-1 consisted of 38.5%, 35113 probes in which expression was highest in regressed phase. Of 35113 probe sets in cluster K-1, 4216, 12% were related to molecular functions, 23890, 68% to biological processes and 7027, 20% to cellular components. The probes of cluster K-1 were over represented in regressed phase. Cluster K-2 included 20.4%, 12215 probes whose expression was gradually increased from active to recrudescent phase. GO functionality showed that out of 12215 probes, 1710, 14% were related to molecular functions, 7329, 60% to biological processes and 3176, 26% to cellular components. These functions showed highest expression in recrudescent phase. Cluster K-3 contained 21.1%, 12672 probes out of which 3548, 28% were associated with molecular functions, 6083, 48% with biological processes and 3041, 24% with cellular components in GO functionality groupings.

Gene Filtering

Tests from regressed phase were considered as control group while recrudescent and active tests were considered as test groups. As shown in Volcano plot and Venn diagram Figure 5 total of 336 genes were differentially regulated in active Vs recrudescent group analysis, of which 74 and 262 genes showed the common and exclusive differential expression with other analyzed groups respectively. Total 547 differentially regulated genes were identified in active Vs regressed group analysis, of that 138 and 389 genes showed the common and exclusive differential expression with rest of the analyzed groups respectively. Highest number of genes, 1832, found differentially regulated in recrudescent Vs regressed phase analysis, of which 215 and 1617 genes showed the common and exclusive differential expression with rest of the analyzed groups respectively. Highest number of genes, 1832, found differentially regulated in recrudescent Vs regressed phase analysis, of which 215 and 1617 genes showed the common and exclusive differential expression with rest of the analyzed groups respectively. Highest number of genes, 1832, found differentially regulated in recrudescent Vs regressed phase analysis, of which 215 and 1617 genes showed the common and exclusive differential expression with rest of the analyzed groups respectively. Highest number of genes, 1832, found differentially regulated in recrudescent Vs regressed phase analysis, of which 215 and 1617 genes showed the common and exclusive differential expression with rest of the analyzed groups respectively. Highest number of genes, 1832, found differentially regulated in recrudescent Vs regressed phase analysis, of which 215 and 1617 genes showed the common and exclusive differential expression with rest of the analyzed groups respectively. Highest number of genes, 1832, found differentially regulated in recrudescent Vs regressed phase analysis, of which 215 and 1617 genes showed the common and exclusive differential expression with rest of the analyzed groups respectively. Highest number of genes, 1832, found differentially regulated in recrudescent Vs recrudescent and recrudescent phase compared with active phase testis samples. 148 genes were commonly regulated in between active Vs regressed and recrudescent Vs regressed phase samples, while 64 genes showed the common expression in between active Vs recrudescent and recrudescent Vs regressed phase samples. Of all, only 3 genes, Gm2800, Fam76b and Gm11546, were commonly regulated in all the analyzed groups during three phases. These genes are hypothetical in nature and have not yet been annotated for coding specific protein. The complete list of differentially expressed genes in each group is given in supplementary Tables S2, S3, S4, S5, S6. The differentially regulated genes having least p values and higher fold changes in between active, recrudescent and regressed phases were further funnel out based on the functional involvement in lizard spermatogenesis and reproduction, important genes are listed in Table 3.
Gene Ontology GO Enrichment Analysis

The GeneGo Metacore, GeneSpring 11.0.1 and DAVID software's have been employed for Gene Ontology analysis. Further conditional test was used to enrich more specific GO terms. The cohort of the genes identified in pair-wise analysis in between active, recrudescence and regressed phase testis samples were considered for further GO analysis. The GO terms cytoskeleton, cell-cell junctions, transcription, osmo-regulation, differentiation, cell cycle, and niche maintenance showed the involvement during various phases of lizard reproductive cycle Table 4. Genes involved in cytoskeleton, cellular growth, apoptosis, initiation of transcription, cell division and regulation of cellular metabolism were over represented in active and recrudescence phase. Genes playing important roles including negative regulation of transcription, stem cell niche maintenance, inhibition of cell growth and negative regulation of metabolism were upregulated in regressed phase Figure 6. Three transcription factors, Sp1, HNF4-α and c-Myc have shown to be prominently expressed in both recrudescence and active phase samples. These transcription factors regulate a number of other important genes involved in downstream signaling cascade. Figure 7 shows interaction of these transcription factors with other genes.

Validation of Microarray Data by Quantitative Real Time Polymerase Chain Reaction

The transcripts of some selected differentially expressed genes were further quantified using q RT-PCR for microarray data validation. The relative expressions of 7 genes, Ing, Coro1A, Vasohibin, Spare, Kremen 1, Casein Kinase1 and Fibronectin were validated by quantitative real time PCR in active phase compared with regressed phase testis samples. Coro1A and Spare were down-regulated in active phase as compared to regressed phase, while rests of 5 genes were found to be up-regulated in active phase. The histogram in Figure 8 represents that all the genes considered for validation showed similar expression profile in both real time PCR and microarray analysis. Although the extent of expression varies between microarray and Real Time PCR data yet the trend remain similar.
Discussion

Many features of the reptilian testis resemble with mammalian testis. Reptiles are the first group of animals adapted successfully to terrestrial life outside water owing to the ability of internal fertilization. Insightful studies of reptilian reproduction elucidate our understanding of differences between oviparous and viviparous animals [18]. In the evolution, only squamate reptiles and mammals show viviparity [19]. Exhibition of amniotic membrane places reptiles in close proximity to birds and mammals. Unlike amphibians and fishes, lizard testis consists of tubular, seminiferous tubules, and interstitial spaces similar to mammalian testis [20]. Seminiferous tubules are covered with a non-cellular basement membrane and these cells are called as fibroblast/myoid-like peritubular cells Ptc. Ptc are absent in anamniotes and they make their first phyletic appearance in the reptilian testis [2]. Microarray studies on reptilian models are negligible and very limited. This is the first report of high throughput microarray analysis of whole testis of any reptilian organism. PubMed search for “reptilian microarray” on 15.09.2012 returned with only one result in which cDNA microarray was employed to analyze gene expression in Australian snake venom glands [21].

The knowledge of gene sequences is a prerequisite for employing microarray technique to study gene expression [22]. However, use of microarray technique is restricted to few model organism species such as human, rat, mouse etc. because largely their genome has been sequenced making it possible to fabricate microarrays for such organisms [23]. Unfortunately, sequenced genomes are not available for most of the reptilian species; hampering the use of microarray to assess large scale gene expression in these species [18]. The very high cost and huge amount of work involved in developing and producing a DNA array or microarray for a new species is prohibitive for most researchers working in comparative biology. The alternative is to explore the use of heterologous array hybridization, screening for gene expression in one species, using an array developed for another species [24]. Such heterologous or crossspecies microarray hybridization analyses have been executed to study differential gene expression in several non-model species [25–32]. It is challenging to study gene expression in developing organs due to its dynamic nature [33]. During a reproductive cycle, the cellular components of wall lizard testis undergo dramatic changes in number, differentiation and transcription in cells. Although whole organ microarray cannot represent cell specific gene expression

Figure 3. Unsupervised hierarchical clustering. The two-way representation of unsupervised HCA of the expression levels, in logarithmic scale, of all the target probes/genes, Y-axis, in each sample, each column, and their clustering based on expressional distance Pearson correlation coefficient between samples in dendrogram formation, X-axis. Heat map shows the gene expression pattern in defined colour range. I Represents the unsupervised HCA of the all 6 samples, 2 samples each from active, recrudescence and regressed phases and ii showed the semi-unsupervised hierarchical clustering analysis and heat map of average expression of the replicates in all three groups. Note A = active phase; B = recrudescent phase and C = regressed phase.
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[34–36], it can provide overall gene expression profile in various stages of reproductive cycle, active, recrudescent & regressed phase. The limitations in isolating various cell types from lizard testis, especially during regressed phase, due to its compactness, and small size restricts comparison of cell specific gene expression. However, transcriptome analyses of regressed, recrudescent and active phase testis of wall lizard can describe key changes in gene expression within testis during these phases.

Testes from regressed phase were collected during late June, recrudescent from early October and active phase from late March. Histological analysis and the total RNA obtained from equal amount of tissue from all groups represented physiological activity of testes from different phases of reproductive cycle. 8 fold lesser amount of RNA in regressed testes as compared to that by recrudescent and active phases indicates that in the regressed phase of breeding, testes displays severe quiescence and becomes physiologically inactive Table S1. The results of hierarchical clustering analysis, HCA, and K-mean clustering of all samples revealed a high order of sample homogeneity. The resulting gene lists from each pair-wise comparison only included the genes that showed a fold change of 2.0 or higher and a \( P\leq0.05 \) by using a parametric Welch t test with Benjamini-Hochberg multiple testing corrections for false discovery rate [14]. The real time RT-PCR based quantitative analysis for expression of 7 target genes from all 6 samples selected on the basis of microarray information validated the array data with a high degree of concordance. K-mean entity based cluster analysis divided the entire 60 K genes expression profile in three clusters. The cluster K-1 included highest number of genes 58\% whose expression pattern was similar in active and recrudescent phase and higher in regressed phase. In the cluster K-3 there were 23\% genes whose expression pattern was opposite to what that was in cluster K-1. In cluster K-3, expression was similar in active and recrudescent phase and was least in regressed phase. Around 20\% genes made the cluster K-2 in which there was no significant change in expression of genes between active and regressed phase and expression was highest in recrudescent phase.

During a reproductive cycle, lizard testis shuttles between severe quiescent to active states of spermatogenesis. In regressed phase there is no physiological activity whereas testis resumes activity in recrudescent phase leading to its peak in active phase. This study provides a changing landscape of regressed, recrudescent and active phase testis at gene level. Functional analysis reveals drastic increase in differentially regulated genes in recrudescent phase as compared to regressed phase, which is further maintained in active phase. This change in gene expression is due to reactivation of cellular functions in recrudescent phase. In the recrudescent phase, Sertoli cell regain its shape and proliferate to provide full size to testis. Sertoli-Sertoli cell junctions make blood-Testis-Barrier BTB leading to compartmentalization. Concomitantly, spermatogonia begin proliferation by mitosis to increase in number and meiotically to reach up to elongated spermatid stage [37]. Cell division is an important event in recrudescent and active state testis. Sertoli cells divide mitotically to make optimum number of...
Table 3. Lists of the differentially regulated genes identified from microarray data in recrudescent and active phase samples compared with regressed phase samples.

| Gene Name | Probe Set ID | Gene ID | Gene Description | Gene expression as compared to Regressed Phase |
|-----------|--------------|---------|------------------|-----------------------------------------------|
|           |              |         |                  | Recrudescent Phase | Active Phase |
| Aass      | GT_Mm_44k_51_P483544 | 30956   | Aminoadipate-semialdehyde synthase nuclear gene encoding mitochondrial protein | –1.01 | –4.34 |
| Aifm2     | GT_Mm_44k_52_P2800     | 71361   | Apoptosis-inducing factor mitochondrion associated 2 (Aifm2), transcript variant 1 | 2.51 | 3.68 |
| Anapc7    | GT_Mm_44k_51_P442481   | 56317   | Anaphase promoting complex subunit 7 | 3.40 | 1.96 |
| Cdx1      | GT_Mm_44k_51_P318999   | 12590   | Caudal type homeo box 1 | 0.52 | 3.68 |
| Cep63     | GT_Mm_44k_51_P149313   | 28135   | Centrosomal protein 63 | 3.57 | –0.27 |
| Crnkl1    | GT_Mm_44k_52_P440102   | 66877   | Crooked neck-like 1 (Drosophila) | 2 | 5.94 |
| Csnk2a1   | GT_Mm_44k_51_P397768   | 12995   | Casein kinase 2, alpha 1 | 2.30 | 4.37 |
| Ddx1      | GT_Mm_44k_51_P387220   | 104721  | DEAD(Asp-Glu-Ala-Asp)box polypeptide 1 | 0.30 | 3.26 |
| Dhx32     | GT_Mm_44k_51_P285997   | 101437  | DEAH (Asp-Glu-Ala-His) box polypeptide 32 | 4.02 | 0.47 |
| Elavl4    | GT_Mm_44k_51_P503722   | 15572   | ELAV (embryonic lethal, abnormal vision, Drosophila)-like | 2 | 4.14 |
| Elmo1     | GT_Mm_44k_51_P306129   | 140580  | Engulfment and cell motility homolog (C. elegans) | 2 | 3.79 |
| Epc1      | GT_Mm_44k_52_P117197   | 13831   | Enhancer of polycomb homolog 1 (Drosophila) (Epc1), transcript variant 2 | 1.89 | 4.27 |
| Fat1      | GT_Mm_44k_51_P350252   | 14107   | FAT tumor suppressor homolog 1 | 2 | 4.74 |
| Gadd45g   | GT_Mm_44k_51_P315904   | 23882   | Growth arrest and DNA-damage-inducible 45 gamma | 3.85 | –1.48 |
| H3F3b     | GT_Mm_44k_52_P61786    | 15081   | H3 histone, family 3B | 1.08 | 0.27 |
| Hira      | GT_Mm_44k_51_P125395   | 15260   | Histone cell cycle regulation defective homolog A | 3.74 | 3.24 |
| Ing1      | GT_Mm_44k_51_P403631   | 26356   | Inhibitor of growth family, member 1 | 5.06 | 2.39 |
| Kremen1   | GT_Mm_44k_51_P390239   | 84035   | Kringle containing transmembrane protein 1 | 2 | 3.52 |
| Leprotl1  | GT_Mm_44k_52_P395220   | 68192   | Leptin receptor overlapping transcript-like 1 | 3.12 | 5.79 |
| Moap1     | GT_Mm_44k_51_P475291   | 64113   | Modulator of apoptosis 1 | 0.75 | 2.36 |
| Msi2      | GT_Mm_44k_52_P628060   | 76626   | Musashi homolog 2 (Drosophila) | –2.37 | –1.36 |
| Myst1     | GT_Mm_44k_51_P103757   | 67773   | MYST histone acetyltransferase 1 | 0.46 | 3.24 |
| Nfat5     | GT_Mm_44k_51_P133509   | 54446   | Nuclear factor of activated T-cells 5 | 1.57 | 2.39 |
| Pax5      | GT_Mm_44k_51_P122855   | 18507   | Paired box gene 5 | 3.28 | 4.79 |
| Pcdh10    | GT_Mm_44k_52_P78439    | 18526   | Protocadherin 10 (Pcdh10), transcript variant 3 | –2.91 | –6.58 |
| Ptc1      | GT_Mm_44k_51_P127435   | 19206   | Patched homolog 1 | 1.13 | 4.51 |
| Ptgir     | GT_Mm_44k_52_P621368   | 19222   | Prostaglandin I receptor | –3.20 | –1.18 |
| Raver1    | GT_Mm_44k_52_P333352   | 71766   | Ribonucleoprotein, PTB-binding 1 | 1.56 | 3.15 |
| Runx2     | GT_Mm_44k_51_P230942   | 12393   | Runt related transcription factor 2 | –8.49 | –4.86 |
| Serpine2  | GT_Mm_44k_51_P268094   | 20720   | Serine (or cysteine) peptidase inhibitor, clade E, member 2 | 5.01 | 1.36 |
| Sirt6     | GT_Mm_44k_51_P01331    | 50721   | Regulation 2, homolog 6 (S. cerevisiae) sirtuin 6 (silent mating type information | –3.53 | –0.82 |
| Sic2a8    | GT_Mm_44k_51_P358171   | 56017   | Solute carrier family 2, (facilitated glucose transporter), member 8 | 3.61 | 4.91 |
| Sp3       | GT_Mm_44k_52_P522264   | 20687   | Trans-acting transcription factor 3 (Sp3), transcript variant 1 | –0.15 | 0.82 |
| Sycp3     | GT_Mm_44k_51_P01569    | 20962   | Synaptopemal complex protein 3 | 3.17 | 4.65 |
| Trps1     | GT_Mm_44k_51_P240384   | 83925   | Trichorhinophalangeal syndrome 1 | 2.54 | 3.14 |
| Tubb1     | GT_Mm_44k_50653        | 545846  | Tubulin, beta 1 | 4.27 | 6.51 |
| Ube2d3    | GT_Mm_44k_52_P229709   | 66105   | Ubiquitin-conjugating enzyme E2 D 3 (UBC4/S homolog, yeast) | 3.97 | 5.01 |

*Negative sign represent that genes are down regulated while others are up-regulated in between analyzed conditions.

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cells to give final size to the testis and hold appropriate number of germ cells. Germ cells divide both mitotically and meiotically to produce sperm. Active phase is similar to recrudescent phase except the presence of mature sperm in seminiferous tubule lumen. Down regulation of negative regulators of transcription in active and recrudescent phase, meant up regulation of these genes
### Table 4. Functional category enrichment analysis based on Gene Ontology terms.

| Biological Process                  | GO Term ID | Counts | % of Differentially Regulated genes | p-Value |
|-------------------------------------|------------|--------|------------------------------------|---------|
| Cell junction                       | GO:0030054 | 8      | 7.4                                | 0.009   |
| Cellular homeostasis                | GO:0019725 | 6      | 2.04                               | 0.56    |
| Spermatogenesis                     | GO:0007283 | 3      | 1.02                               | 0.89    |
| Positive regulation of growth      | GO:0045927 | 4      | 3.3                                | 0.004   |
| Cytoskeleton                        | GO:0005856 | 13     | 10.83                              | 0.02    |
| Steroid binding                    | GO:0005496 | 7      | 0.46                               | 0.06    |
| Positive regulation of cytokine biosynthetic process | GO:0042108 | 7      | 0.46                               | 0.07    |
| Homeostasis of number of cells     | GO:0048872 | 14     | 0.92                               | 0.05    |
| Germ cell development              | GO:0007281 | 13     | 0.86                               | 0.05    |
| Gene silencing                      | GO:0016458 | 6      | 0.39                               | 0.03    |
| Cell fate commitment                | GO:0045165 | 8      | 0.53                               | 0.9     |
| Tissue remodeling                   | GO:0048771 | 3      | 0.19                               | 0.85    |
| Negative regulation of gene expression | GO:0010629 | 37     | 2.4                                | 0.01    |
| Cell division                       | GO:0051301 | 3      | 4.5                                | 0.2     |
| Apoptosis                           | GO:0006915 | 30     | 1.9                                | 0.78    |
| Transcription initiation            | GO:0006352 | 3      | 2.4                                | 0.02    |
| GTPase regulator activity           | GO:0030695 | 37     | 2.4                                | 0.02    |
| Negative regulation of macromolecule metabolic process | GO:0010605 | 8 | 3.9 | 0.21 |
| Blood vessel morphogenesis          | GO:0048514 | 7      | 2.3                                | 0.07    |

% of DR refers to the percent of differentially regulated transcripts falling under the term; p-value is the raw p-value from Fishers exact test. GO term analysis was done using DAVID bioinformatic tool for functional analysis (http://david.abcc.ncifcrf.gov/).

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**Figure 6. Functional clustering of biologically important genes.** Heat map showing changes in expression of genes related to some biologically important functional categories during active, recrudescence and regressed phase of wall lizard reproductive cycle. Heat maps were constructed using average of raw signals for each gene in microarray data.

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Functional analysis of differentially regulated genes attributes gene functions during various phases of reproductive cycle. The prominent groups of genes showing change in expression during progression through reproductive cycle are in regressed phase. 

Figure 7. Interactome and networks. Interaction of three transcription factors, HNF-4, c-Myc and Sp1, with other important pathway candidate genes. These three transcription factors are prominently expressed commonly in both recrudescent and active phase.

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related to cyto-architecture maintenance, apoptosis, transcription, cell division, differentiation, gamete development, DNA repair, cell growth, metabolism, stem cell niche and maintenance of cellular homeostasis. These are discussed below under separate subheadings for better understanding.

Genes Related to Cyto-architecture
The expression of genes involved in cytoskeleton maintenance Actg, Act7b, Csn, Clasn, Dynlt3, Krt17, Limd3, Nas1, Palld, Tll3, and Tuba8 and cell-cell junctions Cadm3, Clda10a, Ldn19, and Sppn was profound in recrudescent and active phases. Tubulin [38,39], actin [40], Palld [41], and Raver1 [42] are implicated in cytoskeleton organization, cellular events such as mitosis, cellular organization, transport, focal adhesions and motility. The claudins are established junctional proteins essential for maintenance of spermatogenic epithelium [43]. Expression of these genes in recrudescent and active phase tests indicated progressive development of cytoskeletal structure bearing large number of differentiating germ cells.

Genes Related to Apoptosis
Programmed cell death is the prominent feature of spermatogenic testes. Sertoli cell mediated apoptosis is an essential event to maintain a static ratio of germ cells per Sertoli cell [44]. Upregulation of apoptosis related genes Aifm2, Bel10, Card10, Elmo1, Fastd5, Puc12, Sqt31, and Sik4 in recrudescent and active phases tests showed importance of programmed cell death in these phases. Elmo1 is a pivotal gene involved in Sertoli-cell mediated apoptosis of spermatogenic germ cells [45]. It is also important to note that during regressed phase, no apoptotic activity is inhibited because the germ cell differentiation is at hold.

Genes Related to Muscle Band
The expression of large number of muscle band proteins Ank2, Slmnap, Sphk2, Smtnl1 and Tbn in regressed phase was associated with the occurrence of multilayered peritubular cell over seminiferous tubule. In the recrudescent and active phase, peritubular cells’ layer become single layered. Multiple layers during regressed phase are probably necessary for protecting seminiferous tubule from damage.

Genes Related to Homeostasis of Number of Cells
Development of cell population in testes is tightly controlled. Genes involved in maintenance of optimum number of cells were Ireh2, Bygfn, Sox5, H2r7, Coro1a [46], Ankl1, Libr8, Ikbg, Houb6 [47], Mtap7 [48], Kif1 [49] upregulated in recrudescent and active phase. These genes determine number of cells required in a tissue or organ by regulating cytoskeleton, cell division, cell-cell interaction and microtubule organization.

Genes Related to Cell Division and Cell Growth
Controlled cell division and regulated cell growth is essential phenomenon in testis to maintain proper testis size and adequate sperm output. Genes regulating cellular growth, cell cycle and protein biosynthesis were Rambpl [50,51], Nedd9 [52], Ingl [53,54], Sit2 [55], CEP63 [56], Lgl1 [57] and Fli [58,59]. These genes are important in regulating proliferation, differentiation, adhesion, migration, and signal transduction through interactions with other cellular proteins. Upregulation of these genes in recrudescent and active phase suggested critical role of these genes in the cell division and growth in spermatogenic tests.

Contrary to upregulation of growth and cell division promoting genes in recrudescent and active phase, genes keeping check on cell growth Nnk3-1, Apo, Sli, Scc5, Lkb1 were upregulated in regressed phase. These genes, Nnk3 [60,61], Lkb1 Liver Kinase B1 [62] and Sli [63] play an important role in negative regulation of cell growth. Since cell growth and division is completely arrested in regressed phase, these genes are crucial for maintaining quiescent state of testis.

Genes Related to Transcription
Transcriptional machinery of testis restarts in recrudescent phase of testis after a hiatus of nearly 3 months and maintained in active phase. Active and recrudescent testes are actively engaged in transcription and genes crucial in initiation and regulation of transcription were Pax5 [64–66], Dmbt32 [67], Mesi1 [68] and Nfil3 [69,70] are up-regulated. These genes play critical role in initiation and maintenance of active transcriptional machinery in the cell. Expression of these genes may allow recrudescence of regressed tests.

Contrary to recrudescent and active phase, regressed phase is the most inactive phase of breeding cycle. In this phase, tests entered in severe quiescence leading to complete cessation of cellular activity. Higher expression of genes involved in transcriptional silencing or negative regulation of gene expression turns off the transcriptional activity in regressed tests. The genes involved in transcriptional silencing were Cbhip [71], Trrc48 [72], Jarid2 [73–75] and N2f14 and Spt5. These genes are global transcriptional repressors that balance cell lineage choices during embryonic development and silencing and degradation of the miRNA-targeted mRNAs. H3f3a is a transcriptional regulatory gene expressed in all the phases of reproductive cycle with highest expression in regressed phase, indicating its role in controlling regulation of transcription.

Genes Related to the Maintenance of Niche for Spermatogonial Stem Cell
In the regressed phase tests, only spermatogonia and retracted Sertoli cells are present. Genes involved in cell fate commitment Smo, Aiol1, Eshb4, Neurol, Sma2, Sox6, Ptx1, and Tlx1 and chemokine signaling pathway Sox1, Rasgpl2, Ikbg, Cdk27a, Gnb5, Cxcr4, H1rap, H2r7, Csf1r, Gm1bl and cytokine biosynthesis Ieod, Eng, Bel, Tbk1, Glom, Cdk29b, Cdk27 were up-regulated in regressed phase. All these genes are crucial for maintenance of stem cells in

Figure 8. Validation of microarray genes by quantitative Real Time PCR, qPCR. Similar trends with high degree of concordance are represented in between Q-PCR and array data. Filled black bar represent the microarray data while hollow bar is for qPCR data.

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their respective niches and later their differentiation into specialized cell; sperm in case of testis [76]. CXCL12 is expressed by Sertoli cells and its ligand CXCR4 is expressed by germ cells [77]. Interaction between the chemokine CXCL12, Sdf1 and the G-protein coupled receptor CXCR4 is responsible for the maintenance of adult stem cell niches and migration of primordial germ cells. Cx33 encoded Hpl is required for proper germ-cell renewal and survival in the testes [78]. Lin28 is a human homolog of lin-28 of nematode Caenorhabditis elegans and is crucial in deriving stem cells from somatic cells [79]. Expression of a constitutively activated form of β-catenin in postnatal Sertoli cells causes male infertility via progressive deterioration of seminiferous tubules, germ cell loss, and testicular atrophy [80]. The transcription factors Axl1, Ehhh4, Neurod1 plays crucial role in cell fate determination [81,82]. Upregulation of these genes in regressed phase indicated that spermatogenesis are carefully maintained during this dormant phase in their respective niche by Sertoli cells.

Genes Related to Osmoregulation

Osmo-regulation is an essential function of active testis. Tonicity-responsive enhancer binding-protein Tonebp or Nrfl5, which belongs to the Rel/Nfl family of transcription factors, plays a critical role in osmoregulation by controlling the expression of osmoprotective genes [83,84]. Higher expression of these genes in recrudescent and active phases showed importance of osmoregulation during normal spermatogenesis.

Genes Related to Cellular Differentiation

Genes involved in cell differentiation Fzs, Fzd3, Hspa1a, Sphk2, Sqstm1, Pyp3r1, Pml1, Pdh1, Ragap1, Sp1, and Sp3 were up-regulated in active phase tests. Many of these genes were part of serine/arginine-rich protein family, Sr proteins, and Sp family of proteins. The Sr proteins Srsf3 and Srsf1 bind histone H3 tail to control cell cycle progression [85,86]. Sp1 belongs to a larger family of factors which bind G/C box elements to either activate or repress transcription. The Sp family of proteins is ubiquitous and tissue-restricted transcription factors found to regulate the promoters of several genes, including cell-cycle regulated genes [87,88]. These genes play important role in cellular differentiation including those of Sertoli cells and germ cells through regulation of cell cycle.

Conclusion

This study documents the global change in gene expression pattern across different phase of spermatogenesis in the wall lizard, Hemidactylus flaviviridis, tests. Data analysis revealed that active and recrudescent phase testis expresses genes involved primarily in “cytoskeleton maintenance”, “apoptosis”, “metabolic pathways”, “transcription initiation” and “cellular growth and differentiation”. Contrary to that, in the regressed phase, the up-regulated genes were mainly involved in stem cell niche maintenance, transcriptional repression, and negative regulation of growth and maintenance of terminally differentiated state.

Regressed phase is a unique condition where cellular activities reach to standstill. Regressed phase may serve as model for infertility where only spermatogonial stem cells are present and require maintaining their potential to proliferate. Detailed analysis of genes regulated in regressed phase may give us clue about causes of such quiescent situation. The genes expressed in recrudescent phase may prove to be crucial to treat such quiescent conditions as in recrudescent phase genes important for re-initiation of cellular activity are expressed. Our data indicates that the genes involved in spermatogonial stem cell niche maintenance were expressed in regressed phase testis of lizard. This shows conservation of genes involved in basic cellular functions in tests from reptiles to mammals. Present study provided knowledge about several group of genes associated with onset of spermatogenesis. In depth studies of functional genomics studies using transgenic mice over expressing or shutting down of functions of these genes selected out of our microarray data will strengthen our knowledge in understanding genes required for governing germ cell differentiation and sperm production.

Supporting Information

Table S1 Quantification of total RNA extracted from equal amount of tissue (in duplicate) from Active, Recrudescent and Regressed phase testis of wall lizard. (DOC)

Table S2 Complete list of genes commonly expressed in both active phase and recrudescent phase as compared to regressed phase. (XLS)

Table S3 Complete list of genes expressed only in active phase as compared to regressed phase. (XLS)

Table S4 Complete list of genes expressed only in recrudescent phase as compared to regressed phase. (XLS)

Table S5 Complete list of genes commonly expressed between active phase compared to recrudescent phase and recrudescent phase compared to regressed phase. (XLS)

Table S6 Complete list of genes expressed only in active phase as compared to recrudescent phase. (XLS)

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Author Contributions

Conceived and designed the experiments: SSM UR MG. Performed the experiments: MG AM. Analyzed the data: MG MAK SSM. Contributed reagents/materials/analysis tools: MG AM. Wrote the paper: MG SSM UR.

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