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Modulatory effects of Selenium on Spermatogenesis: Involvement of transcription factors CREB and CREM

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Abstract

In the testicular compartment, cAMP response element binding protein (CREB) and cAMP response element modulator (CREM) transcription factors are important transducers of hormonal signals into the induction of gene expression. Selenium (Se) is essential for male fertility. It has been shown earlier in our lab that selenite modulates Nuclear Factor κB, Activator Protein-1 and Stress Activated Protein Kinase pathways in the testicular mileau. Keeping all these in view, the present study was designed to explore further the intracellular pathways involved in spermatogenesis, by analyzing the transcription factors, CREB and CREM under Se status conditions. Also, the levels of Leutinising hormone, Follicle stimulating hormone and Testosterone were monitored to gain insight into the hormonal circuitry involved in the process. Mice were fed yeast based Se deficient, adequate and excess diet for 8 weeks after which they were sacrificed for further analysis. It was seen that Se deficient diet fed animals showed a reduction in testicular Se content alongwith decrease in glutathione peroxidase (GSH-Px) activity. On the other hand Se levels and GSH-Px activity increased in Se excess diet fed group. There was a significant decrease in the mRNA and protein expression of CREB and CREM after Se deficient and excess diet feeding. Also, there was a decrease in the levels of serum Testosterone, LH and FSH alongwith altered testicular histoarchitecture. Thus, spermatogenesis is altered by alteration in Se status. The altered Se status lead to changes in the levels of steroid hormones and the testicular circuitry was hampered. This lead to changes in the basal transcription machinery as demonstrated previously and in addition, the transcription factor CREB and CREM also showed changes in their expression. Thus, the study lead to a conclusion that spermatogenesis is a developmentally regulated program, and is under the control of complex hormonal circuitary which leads to modulation in the various signaling pathways governing cell survival and apoptosis depending upon the physiological conditions.

Keywords: Selenium; CREB; CREM; LH; FSH; Testosterone; Spermatogenesis.

1. Introduction

Spermatogenesis is controlled by a complex network of endocrine, paracrine, and autocrine signals. In response to the hypothalamic gonadotropin releasing hormone, the pituitary gland secretes two hormones, luteinizing hormone, and follicle stimulating hormone (FSH) that are involved in the regulation of spermatogenesis. Luteinizing hormone regulates the testosterone secretion by somatic Leydig cells located in the interstitium between seminiferous tubules. FSH acts on Sertoli cells, the only somatic cells inside the seminiferous tubule, by stimulating signaling, gene expression and secretion of peptides and other signaling molecules [1].

In Sertoli cells, the cAMP response element binding protein (CREB) transcription factor is an important transducer of FSH signals into the induction of gene expression [2]. Recent studies suggest that CREB activity in Sertoli cells is required for spermatogenesis; overexpression of a CREB mutant in Sertoli cells *in vivo* results in apoptosis and elimination of germ cells [3]. Transcription factors of the CREB family are involved in the regulation of gene expression in response to a number of signaling pathways [4]. Activation of gene transcription by CREB is dependent on the phosphorylation of a single serine within the phosphorylation domain (P box or kinase-inducible domain, KID) by cAMP-dependent protein kinase A, PKA [5]. CREB is expressed in nearly all tissues tested thus far. However, in the testis, investigators have identified a number of novel alternative splice variants that result in the synthesis of mRNAs encoding multiple isoforms of CREB [6;8]. In the rat testis, these alternatively spliced CREB mRNAs are spermatogenic, cycle dependent and expressed during development of the germ and Sertoli cells [9], indicating that the CREB isoforms may be major players in spermatogenesis.

The transcription factor cAMP response element modulator (CREM) is highly expressed in male germ cells [10] and is known to regulate the expression of several post-meiotic genes, such as the transition proteins and protamines, and is likely the key regulator of gene expression during spermatogenesis [11; 12]. Targeted disruption of the CREM gene blocks the differentiation program in the first step of spermiogenesis [13;14]. These findings indicate a crucial role of CREM in post-meiotic germ cell differentiation, linking the action of hormonal stimuli to direct regulation of spermatogenesis genes [15].

CREB and CREM belong to the basic domain-leucine zipper (bZip) class of proteins. These factors bind, as homo- or heterodimers, to a DNA sequence known as the cyclic AMP responsive element, which is present in the regulatory region of various target genes [16;17].
Spermatogenesis can be disrupted at various stages in conditions of infertility. Such conditions can have a genetic basis [18;19], can also be due to defects in reproductive hormone production or action [20], or alternatively, be induced by exposure to environmental or chemical toxicants [21; 22].

One such essential environmental trace element is selenium (Se) which is present in both prokaryotic and eukaryotic cells. It has been linked to regulatory functions in cell growth, cell survival, cytotoxicity and transformation [23]. Selenium appears to modulate such cellular activities presumably by acting on proteins important for signal transduction [24]. We have previously demonstrated that selenite activates the NF-κB and SAPKs signaling pathways, whereas it represses the AP-1 signaling pathway [25;29].

Also, since the reproductive organ appears to be a priority tissue for Se accumulation [30], previous studies have demonstrated that Se deficiency and excess conditions give rise to testicular structural [31] and functional disturbances and deficiency of the element causes oxidative stress in the organ due to diminished antioxidant property of this element as a co-factor of GSH-Px [32]. At higher dose levels, Se has various deleterious effects on various organs including testes.

Thus, keeping all these in view, the present study was designed to explore further the intracellular pathways involved in spermatogenesis, by analyzing the transcription factors, CREB and CREM under Se status conditions. Also, the levels of LH, FSH and

Testosterone were monitored to gain insight into the hormonal circuitry involved in the process.

2. Materials and Methods

2.1 Chemicals

Sodium selenite (Na_2SeO_3), 2,3-diaminonapthalene (DAN), Agarose, Ethidium bromide and Proteinase K were purchased from Sigma-Aldrich (St. Louis, MO, USA). TRI-reagent and one-step RT-PCR kit were obtained from Molecular Research Centre (Inc. Cincinnati; Ohio) and QIAGEN, respectively. Oligonucleotides were synthesized by Sigma-Aldrich. Molecular biology grade chemicals for RNA isolation e.g. chloroform, isopropanol, ethanol and formaldehyde were purchased from Amresco, Ohio (USA)

Antibody against CREB and CREM were purchased from Santa Cruz Biotechnology, Santa Cruz, CA (USA), peroxidase conjugated anti-rabbit secondary antibody and anti-mouse β-actin were purchased from Sigma-Aldrich, St Louis (USA). All other chemicals and reagents used in the present study were of analytical grade and were procured from the Indian manufacturers.

2.2 Animal procurement and treatment schedule

Male Balb/c mice in the weight range of 20-25g were used in the present study. Animals were procured from the Central Animal House, Panjab University, Chandigarh (India). They were housed in a standard animal facility under controlled temperature and 12/12 hr light/dark cycle with food and water provided *ad libitum*. The proposed study was cleared from the Instituitional Animal Ethics Committee.

To make different Se status animals *viz.* 0.02ppm, 0.2 ppm and 1 ppm, in different groups (Group I, II and III respectively), the mice were kept on yeast-based diet. The yeast-based diet usually contains 0.02 ppm Se and hence animals fed on this diet were considered Se deficient animals (Group I). For Se supplemented groups, Se was added at 0.2ppm (Group II; adequate level) and 1ppm (Group III; excess level) as sodium selenite to Se deficient diet.

2.3 Selenium Deficient Diet

Selenium deficient diet with inactivated Baker's yeast as a protein source was prepared in the laboratory itself according to the composition given by Burk [33]. The diet contained inactivated torula yeast (inactivated by autoclaving) 30%, sucrose 56.99%, corn oil 6.67%, mineral mix 5%, vitamin mix 1%, dl-methionine 0.3% and vitamin E 0.04%. Se adequate and excess diets were prepared from Se deficient diet by supplementing it with 0.2ppm and 1ppm of Se respectively as sodium selenite. After completion of diet feeding schedule of 4 and 8 weeks, animals were sacrificed by cervical dislocation under anesthesia (50 mg/Kg body weight of Sodium Phenobarbital), testes were removed and used for various analyses.

2.4 Selenium estimation

Selenium levels in testis were estimated as previously described [34]. The method is based on the principle that Se in tissues on acid digestion is converted into selenous acid; which on reaction with aromatic-o-diamines such as 2,3-diamine naphthalene (DAN) leads to the formation of 4,5-benzopiazselenol, which displays brilliant lime green fluorescence. Briefly, 100mg of testis tissue was acid digested in concentrated HNO_3 on a sand bath at approx. 100^oC in digestion flasks fitted with long air condenser to prevent any Se vapour loss. A known amount of digest was reacted with aqueous solution of DAN (precleaned of impurities with cyclohexane). The product formed (4,5-benzopiaselenol) was extracted completely with cyclohexane and quantitated on fluorescence spectrophotometer (Perkin Elmer, USA) using 366nm as excitation and 520 nm as emission wavelength. Sodium selenite was used as a standard for this assay.

2.5 Biochemical estimations

10% (w/v) tissue homogenates were prepared in 50mM Tris-HCl (pH 7.4) under ice-cold conditions. The homogenates were then centrifuged at 10,000rpm for 30 minutes. The supernatant (post mitochondrial fraction, PMF) thus obtained was collected for the biochemical estimations described below.

2.6 Se-dependent glutathione peroxidase activity

Activity of glutathione peroxidase (GSH-Px) was assayed by the coupled enzyme procedure with glutathione reductase using H_2O_2 as substrate [35]. The assay was carried out in testis PMF. The activity was expressed as μmoles of NADPH oxidized /min/mg protein. Total protein was estimated by the method of Lowry et al [36].

2.7 Hormone Analysis

Concentration of testosterone in serum was measured by DRG Testosterone ELISA. Serum concentrations of leutenizing hormone (LH) and follicle stimulating hormone (FSH) were determined by immunoenzymometric assay (ELISCAN Diagnour).

2.8 RNA isolation

Total RNA was isolated from mice testis using TRI-REAGENT (Mol Res. Centre, Inc, Ohio, USA). 50mg testis tissue from different treatment groups was homogenized in 0.5ml of TRI-REAGENT using hand homogenizer in 1.5ml polystyrene microfuge tubes. The samples were kept at room temperature for 5min after which 80μl chloroform was added. This was mixed vigorously for about 15sec and the homogenates were then kept at room temperature for 10min followed by centrifugation at 12,000 rpm for 15min at 4°C. Following centrifugation, the upper colorless aqueous phase containing RNA was collected. In order to precipitate RNA, isopropanol was added and after mixing, the samples were kept at room temperature for 10min. Thereafter, samples were spun at 12,000 rpm for 10min at 4°C. RNA precipitate so obtained was washed by adding 75% ice-cold ethanol and spinning at 7500g at 5min at 4°C. After removing the ethanol, the RNA pellet was briefly air-dried (not completely) and then dissolved in DEPC treated water. Purity, integrity and concentration of the isolated RNA were checked by taking absorbance at 260 and 280nm and finding their ratio. Concentration of RNA was estimated by using A_{260}=1=40 μg/ml.

2.9 Primer designing and synthesis for RT-PCR analysis

For the RT-PCR analysis, primers for the following genes i.e. CREB and CREM were searched from the literature and were got synthesized from Sigma Aldrich (USA). Primer sequences for different genes are given in Table 1.

Table 1: Sequence of Primer pairs used

Gene	Primer Pair	Reference
CREB	Sense 5' ACT GGC TTG GCA CAA CCA GA 3' Antisense 5' GGC AGA AGT CTC TTC ATG ATT 3'	[47]
CREM	Sense 5' TGG TAA GTT GCC ATG TCA CC 3' Antisense 5' ATG ACC ATG GAA ACA GTT GAA TC 3'	[48]
β-actin	Sense 5' ATC CGT AAA GAC CTC TAT GC 3' Antisense 5' AAC GCA GCT CAG TAA CAG TC 3'	[49]

2.10 RT-PCR procedure

RT-PCR was done using specific primers for the respective genes. RT-PCR for β-actin was also done alongwith to rule out the experimental errors. QIAZEN one step RT-PCR kit was used for the purpose. Two μg of total RNA was used in RT-PCR reaction from different groups. To this, the following reagents were added as follows: 10 μl 5x Qiagen one step RT-PCR buffer, 2 μl dNTP mixture, 5 μl each of forward and reverse primers

(10 µM stock), 2 µl enzyme mix, and 1 µl RNase inhibitor (1 U/µl). Finally PCR grade RNase free water was added to make the total volume 50 µl. The components were mixed with gentle vortex and centrifuged to collect all the components at the bottom of the tube. The PCR reaction was performed in the thermal cycler (Techne Ltd, England) using following conditions: RT reaction was performed at 50^{o}C for 50 min and activation at 94^{o}C for 15 min. PCR was followed by 35 cycles of 94^{o}C (denaturation) for 45 sec, 56^{o}C (annealing) for 45 sec, 68^{o}C (extension) for 1 min. Finally, the products were incubated at 68^{o}C for 5 min to extend any incomplete single strands. To authenticate the results from RT-PCR (a semi-quantitative method) analysis, initially RT-PCR for various genes was carried at 20, 25, 30, and 35 cycles. Progressive increase in products was obtained in all the cases and hence finally the RT-PCR was done with the samples at 35 cycles only.

Final PCR products formed were analyzed on 1.5% agarose gel electrophoresis and densitometric analysis of the bands was done by Image J software (NIH, USA). Mean of four independent densitometric analyses of PCR product bands were determined for comparison of each analysis.

Table 2. Selenium levels and glutathione peroxidase activity in mice testis after 8 weeks of diet feeding schedule

	8 weeks		
	Se deficient (Group I)	**Se adequate (GroupII)**	**Se excess (Group III)**
Selenium (µg Se/g tissue) *Testis*	0.47 ± 0.02***	0.75 ± 0.02	0.96 ± 0.03***
Glutathione peroxidase (µmoles NADPH oxid/min/mg protein) *Testis*	49.75 ± 1.10***	116.24 ± 3.52	134.15 ± 1.43

*The values are mean ±SD of four independent observations. *, **, *** represent p<0.05, p<0.01, p<0.001, respectively.*

2.11 Quantitation of CREB and CREM in testis by ELISA

10% (w/v) tissue homogenates were prepared in 50mM Tris-HCl (pH 7.4) under ice-cold conditions. The homogenates were then centrifuged at 10,000rpm for 30 minutes. The supernatant (post mitochondrial fraction, PMF) thus obtained was quantitated for protein by Lowry method and collected for ELISA. 2.5µg protein was loaded onto ELISA strip wells in carbonate buffer and kept overnight at 4˚C. The wells were then blocked with 1% BSA for 1hr at 37˚C. After washings with PBS- Tween20, the wells were incubated with respective primary antibodies (1:500) for 2hrs at 37˚C. After washings, the wells were incubated with secondary antibody (1:500) for 2hrs at 37˚C. Then the substrate, 2,2'-azino-di-3-ethylbenzthiazoline

sulfonic acid (ABTS) in citrate buffer was added along with H_2O_2 and kept in dark for 20 mins. The color obtained was quantitated at 405nm.

2.12 Light Microscopy

Testes were fixed immediately in Zenker's fixative. Next, the tissue was dehydrated in ascending series of alcohol (30%, 50%, 70%, 90% and 100%) for 1hr each. Traces of mercury were removed by placing the tissue pieces in iodine solution (prepared in 70% alcohol) during dehydration at 70% alcohol level. For embedding, the dehydrated samples were placed in benzene (30min), then sequentially in 1:1 benzene: wax (1hr) and then given two changes of 1hr each in pure wax before finally embedding in wax. 5μm thick sections were obtained using a manual hand driven microtome and transferred to the glass slides. These were dewaxed in xylene, rehydrated in descending series of alcohol and stained with haematoxylin and eosin (H and E). Stained sections were mounted in DPX after dehydration and viewed under a light microscope.

2.13 Statistical analysis

The difference between Means ± Standard Deviations (SD) for control and treated groups were examined by using the Student's t-test for unpaired values. Statistical difference of p-value at the level of 0.05 or less was considered significant.

3. Results

3.1 Selenium levels

Selenium was estimated in the testis of mice from all the treatment groups after 8 weeks of diet feeding (Table 2). In testis, a significant decrease in Se levels was observed in Se deficient group I ($p<0.001$) as compared to the respective Se adequate group II after 8 weeks of diet feeding schedule. However, significant increase in Se levels was observed in the Se excess supplemented group III ($p<0.001$) as compared to the respective Se adequate group II (Table 2).

Table 3. Protein expression of CREB and CREM by ELISA after 8 weeks of diet feeding schedule

	Se Deficient	Se Adequate	Se Excess
CREB A(405nm)	0.281 ± 0.01***	0.642 ± 0.01	0.314 ± 0.01***
CREM A(405nm)	0.235 ± 0.01***	0..762 ± 0.01	0.278 ± 0.01***

*The values are mean ±SD of six independent observations. *, **, *** represent $p<0.05$, $p<0.01$, $p<0.001$, respectively.*

3.2 Glutathione Peroxidase (GSH-Px)

GSH-Px activity was measured in testis (Table 2). A significant decrease ($p<0.001$) was observed in the selenium deficient group I when compared to the selenium adequate group II. On the contrary, significant increase was observed in selenium excess group III ($p<0.001$) as compared to the selenium adequate group II.

3.3 Hormone Analysis

Significant decrease in the levels of serum testosterone and FSH was observed in selenium deficient and selenium excess group after 8 weeks of diet feeding schedule (Table 3).

Similarly, after 8 weeks of selenium deficient and selenium excess diet feeding in separate groups, significant reduction the levels of serum LH was observed.

Table 4. Changes in the levels of serum Testosterone, LH and FSH after 8 weeks of diet feeding schedule

	Se Deficient	Se Adequate	Se Excess
T (ng/ml)	0.44 ± 0.01***	0.68 ± 0.01	0.46 ± 0.02***
LH (ng/ml)	1.2 ± 0.01***	2.4 ± 0.03	1.5 ± 0.01***
FSH (ng/ml)	0.4 ± 0.02***	0.58 ± 0.02	0.46 ± .001***

*The values are mean ± SD of six independent observations. *, **, *** represent p<0.05, p<0.01, p<0.001, respectively*

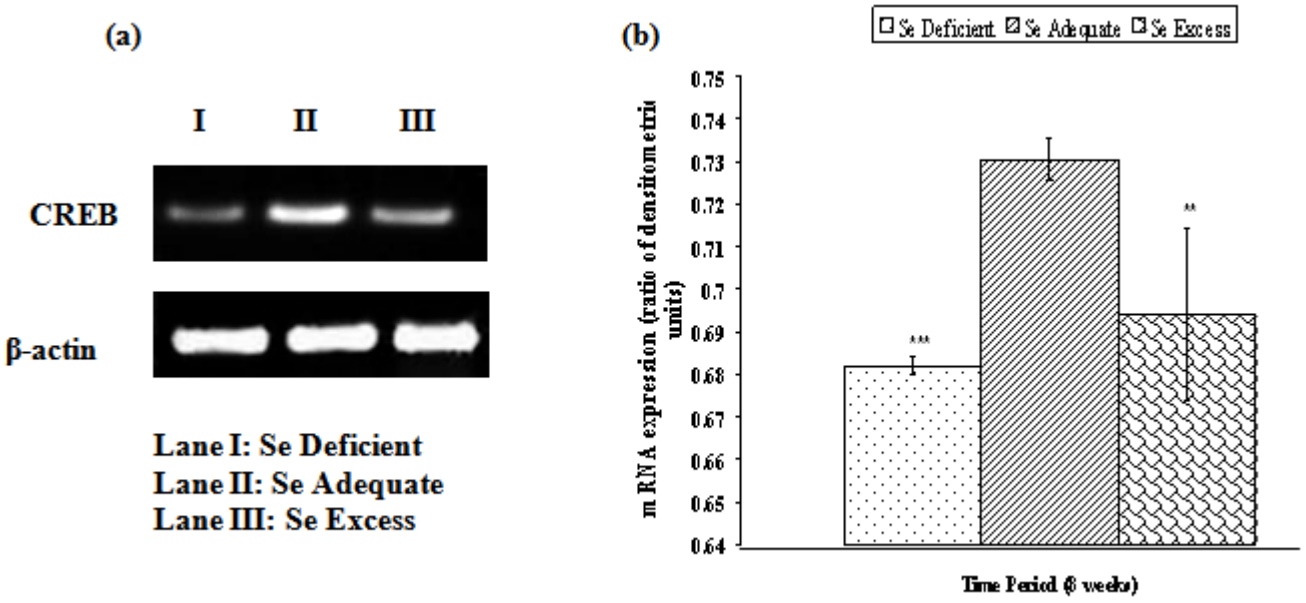

Figure 1. Effect of modulation in selenium status on the mRNA expression of CREB (a) and its densitometric analysis. (b) Lane I- Se Deficient (8 weeks), Lane II- Se Adequate (8 Weeks), Lane III- Se Excess (8 Weeks). The values are mean ± SD of four independent observations. *, **, *** represent p<0.05, p<0.01, p<0.001, respectively.

3.4 mRNA expression studies

Total RNA preparation was quantitated by finding the A_{260} and quality of RNA was also checked by finding the A_{260}/A_{280} which was found to be near 1.9. RT-PCR was initiated with the expression of the products of β-actin in total RNA in all the treatment groups at both the intervals. No change in the expression of the mRNA was observed in all the treatment groups.

RT-PCR products and their densitometric analysis for CREB are represented in Fig 1a, b. There was a significant decrease in the mRNA expression of CREB in the Se deficient group as compared to the Se adequate group at both the treatment intervals. Similarly decrease in

expression was also observed in Se excess group as compared to the Se adequate diet fed group.

CREM gene expression and densitometric analysis of RT-PCR products are shown in Fig 2a,b. There was a significant decrease in the mRNA expression of CREM in the Se deficient as well as Se excess group as compared to Se adequate group at 8 weeks of diet feeding.

3.5 Protein expression studies

CREB and CREM were quantitated in the PMF of testis in various groups of treated animals using ELISA. Progressive significant decrease in the levels of CREB and CREM was observed in Se deficient and excess groups as compared to Se adequate group (Table 4).

3.6 Histological studies

Histological changes in the testis after haematoxylin and eosin (H and E) staining of the paraffin sections was studied in all the treatment groups (Fig 3).

Mice fed Se adequate diet (group II) showed normal testicular histology (Fig 3b). All the stages of transformation of seminiferous epithelia from spermatogonia to mature spermatozoa were seen in the tubules of mice fed diet containing adequate levels of Se for 8 weeks.

Shrinkage of the seminiferous tubules was evident in Se deficient group I (Fig 3a) compared to its respective Se adequate group II (Fig 3b). Basement membrane was intact but the germ cell height was reduced. The lumen was almost completely devoid of spermatozoa. In Se excess group III (Fig 3c) shrinkage of the seminiferous tubules was observed with disorganization of germ cells. The lumen size was reduced and appeared almost completely devoid of spermatozoa.

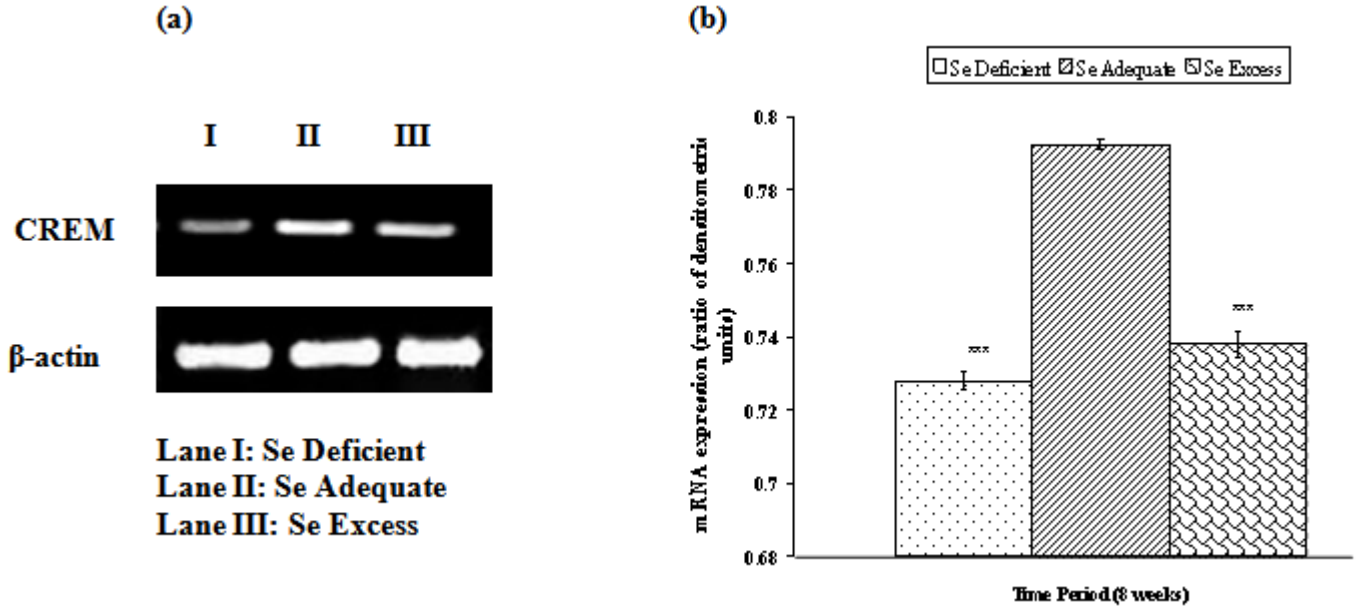

Figure 2. Effect of modulation in selenium status on the mRNA expression of CREM (a) and its densitometric analysis. (b) Lane I- Se Deficient (8 weeks), Lane II- Se Adequate (8 Weeks), Lane III- Se Excess (8 Weeks). The values are mean ± SD of four independent observations. *, **, *** represent $p<0.05$, $p<0.01$, $p<0.001$, respectively.

4. Discussion

The present study aimed at exploring further the molecular mechanism involved behind regulation of spermatogenesis by Se. The establishment of Se status in the animals under study was confirmed by measuring the testicular Se levels and the enzyme activity if glutathione peroxidase, GSH-Px.

A decrease in Se levels in Se deficient group and an increase in the Se excess group confirmed the establishment of Se status in the animals, as also reported elsewhere by previous work from our laboratory [29].

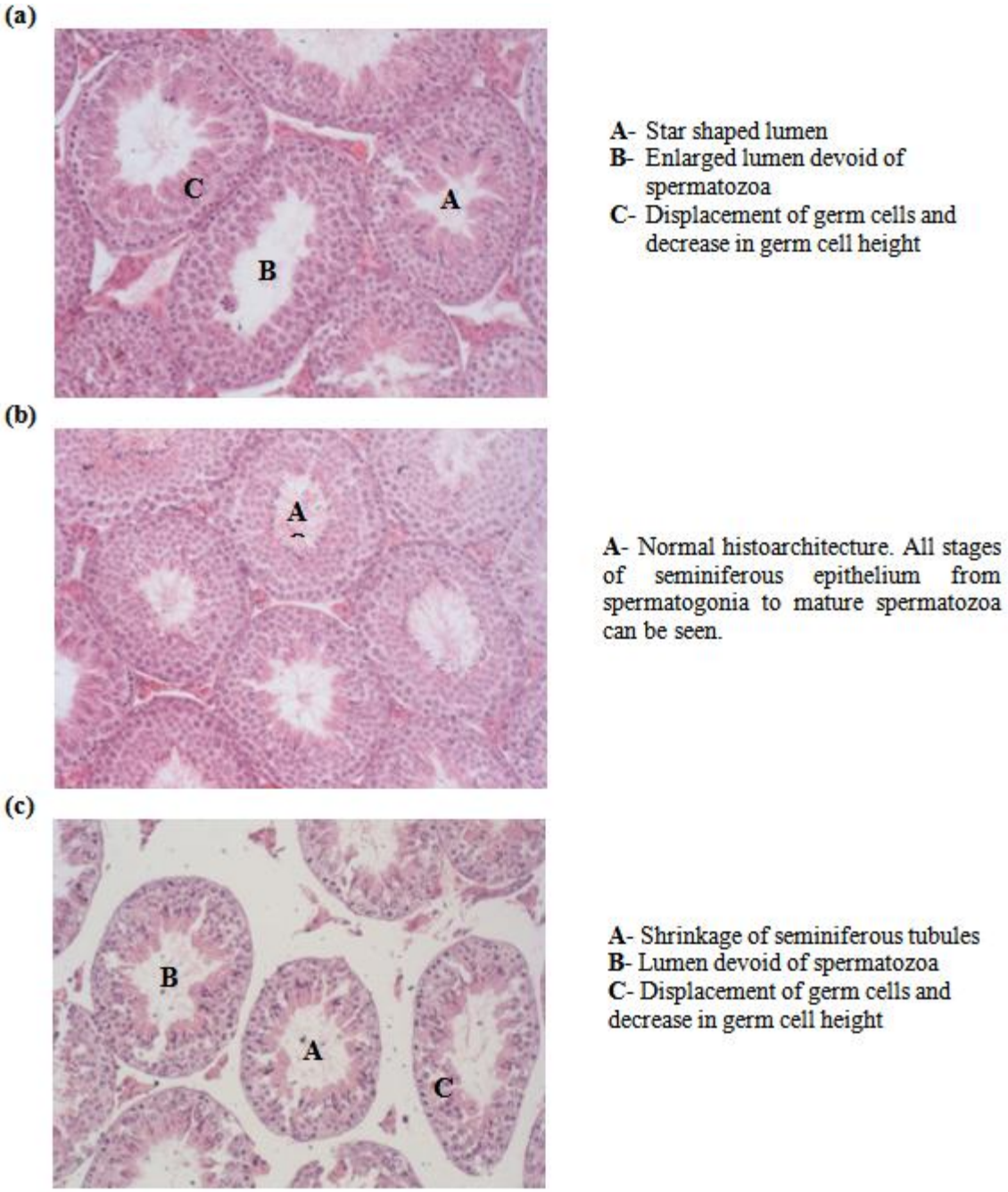

Figure 3. Photomicrograph showing H/E staining in testis paraffin sections of mice fed respective diet for 8 weeks. a)- Se Deficient (8 weeks), b)- Se Adequate (8 weeks), c)- Se Excess (8 weeks).

GSH-Px activity decreased in Se deficient group. Decreased Se levels [29] concomitant with the decrease in GSH-Px activity might contribute to building up of oxidative stress in this group. In Se excess group, increase in GSH-Px activity was observed after 8 weeks interval.

After 8 weeks of Se deficient and excess diet feeding schedule, the hormonal analysis revealed

decrease in the levels of LH, FSH and Testosterone. It has been reported that the entire process of spermatogenesis is regulated by gonadotropins released from the pituitary gland and sex steroids produced within the testis—the pituitary-testicular axis [1]. Alteration of this regulation results in profound changes in testicular morphology and sperm production [37]. In the present study, altered Se status could be responsible for change in the levels of these hormones, which finally culminates in altered testicular morphology as demonstrated in the present study.

The binding of the glycoprotein FSH to receptors on Sertoli cells stimulates adenylyl cyclase, causing an increase in intracellular levels of cAMP [38]. An increase of intracellular cAMP results in the activation of cAMP dependent protein kinase A, which phosphorylates the cAMP regulatory element-binding protein (CREB). mRNA expression analysis revealed decreased expression of CREB in Se deficient and excess groups. This decrease could be explained on the basis of decrease in the FSH levels observed under Se status conditions; following decrease in FSH levels, cAMP dependent protein kinase A was not activated, thus phosphorylation of CREB was not achieved.

Also, significant decrease in the mRNA expression of CREM was observed in Se deficient and excess groups in the present study. It has been reported that male mice lacking a functional CREM gene are infertile due to round spermatid maturation arrest [13, 14], suggesting that CREM is important for male fertility. CREM expression in male germ cells is phosphorylation independent and is achieved by ACT-Activator of CREM in testis [39]. It was found that ACT mRNA expression decreased in Se deficient and excess groups (data not shown). ACT is absent or at least drastically reduced in mice lacking a functional CREM gene and in men with round spermatid maturation arrest, in which CREM has been demonstrated to be only barely detectable or completely absent [40]. This observation also suggests that CREM plays an important role in ACT transcriptional regulation.

Transcription factor CREM is also involved in increase in steroid synthesis which is essential for steroid hormone synthesis [41]. Thus, the decrease in the levels of testosterone, LH and FSH in Se deficient and excess groups could also be explained on the basis of decrease in CREM expression in these groups.

It has been shown previously that [42] male mice lacking CREM expression are sterile because of stage-specific arrest of sperm maturation as the spermatids undergo apoptosis. A role for CREB in nerve growth factor-dependent survival has been clearly demonstrated for the sensory ganglia [43]. *In vitro* experiments performed on postnatal sympathetic neuronal cultures indicated that nerve growth factor-dependent survival requires CREB-mediated gene expression. Also, it has been demonstrated that germ and sertoli cell apoptosis is induced by withdrawl of FSH and Testosterone respectively [44].

Previous studies in our laboratory [26,27] have demonstrated that Se deficient and excess conditions modulated the intracellular transcription factors and stress activated protein kinases that finally culminated in the induction of testicular apoptosis resulting in diminished reproductive potential of male mice.

The results obtained in the present study are in agreement with the previous work suggesting that spermatogenesis is altered by alteration in Se status. The altered Se status lead to changes in the levels of steroid hormones and the testicular circuitry was hampered. This lead to changes in the basal transcription machinery as demonstrated previously and in addition, the transcription factor CREB and CREM also showed changes in their expression.

Thus, the study lead to a conclusion that spermatogenesis is a developmentally regulated program, and is under the control of complex hormonal circuitary which leads to modulation in the various signaling pathways governing cell survival and apoptosis depending upon the physiological conditions.

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