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Effects of long-term release GnRH agonist “deslorelin” on testicular HSP expression, accessory sex glands and testicular functions in adult male rats

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ABSTRACT

The objective of the present was to determine the effect of long-term release GnRH agonists “deslorelin” on suppression and restoration of testicular and accessory sex glands functions, and expression of HSP in testes of adult male rats. A group of twenty-eight male rats and fifty-six female rats were kept for eleven months. The male rats were subdivided into treatment (n = 18; deslorelin, an analogue of GnRH, 4.7 mg, S.C; six months) and control (n = 10; untreated), and the adult female rats were introduced with either treatment or control male rats at the 2nd, 6th and 11th months post implant insertion. At 6th month of deslorelin implants insertion, six male rats from treatment and five rats from control group were sacrificed. The remaining (twelve treatment and five control) male rats were sacrificed at 11 months. The testicular dimension were measured monthly in both treatment and control rats. The blood samples were collected for testosterone and HSP70 antibody, whereas, the testes and accessory glands were isolated for histological examination at each sacrificial time. The results showed that testicular dimension were significantly lesser in treatment group until 9 months post treatment. HSP70 protein expression was negligible at 6 months in treatment group but its intensity increased in spermatids 11 months of treatment similar to control group. Significantly lower testosterone concentrations with poor semen quality, and smaller litter size were observed in treatment group. The histological picture of accessory sex glands and seminiferous tubules shown a variable integrity in treatment group than control at 6 months implant insertion. In conclusion, the subcutaneous application of 4.7 mg of the GnRH-analogue deslorelin represents a practicable, like in the female rats, method to suppress testicular, accessory sex glands functions, testicular HSP expression and fertility in male rats. Moreover, the suppressive effects of deslorelin, continued until 11th months after removal of the implant.

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1. Introduction

Suppression of reproductive functions in male animals is a practical regimen for population control [1,2]. In this regard, blocking of gonadotropin releasing hormone (GnRH) through downregulation of receptors in pituitary by using long-term release GnRH agonist treatment, is one of the applied protocols for male contraception. In this protocol initially LH and FSH release is higher and then reduced due to the downregulation of GnRH receptors in pituitary by long-term release of GnRH agonists which in turn inhibit the reproductive functions [3,4]. The suppression of reproductive functions including suppression of sexual behavior [5,6], complete cessation of spermatogenesis [7,8] and loss of fertility [9] has been reported in swine, canine and feline species.

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Alike to other companion animals, the rats are kept as pet. Mostly the non-surgical contraception of pet rats is desired by the owners. As the surgical neutering is costly and involves post-operative complications, therefore, not preferred by the pet owners In this scenario the application of less invasive method like long-term release of GnRH implants, is the protocol of choice for contraception in rats [10,11].

The duration of reproductive function inhibition is variable in different species which depends upon implant insertion time, dose rate and individual animal response [5,8,9]. The insertion of implants affect the testosterone production which in turn suppress the male behavior for inconsistent durations [12,13]. It is also been observed that the semen characteristics and fertility are not initially influenced after implant insertion [12,14]; however, the long-term application has prominent effects on the sperm variables [6,8,9]. Similarly, the histological view of testes initially do not show any morphological alteration in response to long-term release GnRH treatment as it is observed in later stages of treatment [12,13,15].

Generally the stress enforce the cells to prepare specific proteins i.e. heat shock proteins, for the regulation of cellular functions [16,17]. Some of the proteins are contributing in vital functions of testes particularly HSP60 and HSP70 [18]. HSP60 is detected at the developmental stages of spermatogonia and primary spermatocytes; moreover, it is observed in Sertoli cells of testes. Similarly, HSP70 protein expression is observed in spermatocytes and spermatids phases during spermatogenesis process [16]. The low expression of HSPs is a clear indication of spermatogenesis arrest and subsequent infertility [19]. Previously numerous reports have been documented regarding the application of long-term release GnRH agonists in suppression of testicular functions in male [2,3,5,7,9]; however, there is no single available report about HSPs expression in testes after the use of long-term release GnRH agonists in males. In this background, there is dire need to explore answers whether the long-term release GnRH agonist treatment completely suppress spermatogenesis in conjunction with accessory glands activity or affect the HSP protein expression in testes. In the current study, the hypothesis was tested that long-term application of deslorelin implants downregulates the anterior pituitary and gonadal function and post withdrawal of implants resumes spermatogenesis and fertility in adult rats. The objectives of the present study were to determine the effects of long-term deslorelin implants on spermatogenesis, testicular histology and accessory glands activity. Furthermore, concentration of HSP70 protein was examined to determine their expression during and after implant removal in testes of adult male rats.

2. Materials and methods

2.1. Animals

The experimental animals were kept in Yuzuncu Yil University lab animal house during the study period. The twenty-eight male rats of age 3–4 months with body weight 250–300 g and fifty-six female fertile non-pregnant females were selected. Prior to execution of experiments, the ethical approval was obtained from the Animal Ethics Committee, Yuzuncu Yil University, Van, Turkey (approval number 2013/05 and 16.05.2013).

2.2. Study design

The male rats were randomly divided into two group; treatment ($n = 18$, Suprelorin, deslorelin acetate, 4.7 mg, S.C.) and control group ($n = 10$). The procedure of implant insertion was performed under local anesthesia (Lidocaine hydrochloride 2%; Bela Pharm GmbH & Co. Vechta, Germany). The implants were placed subcutaneously in paraumbilical area for period of six months. The control groups did not receive any treatment and observed for same duration. At the time of implants removal (6 months), the rats (six from treatment and five from control group) were killed, whereas, remaining male rats, control ($n = 05$) and treatment ($n = 12$) groups, were monitored until the end of study (11 months) and sacrificed (Fig. 1).

2.3. Introduction of female rats and pregnancy diagnosis

The female rats of known fertility, were introduced into cages of treatment and control male rats at 2, 6 and 11 months of implants insertion. One male and two female rats were kept together for about 15 days. After isolation, the female rats were monitored for enlargement of abdomen and mammary gland development. After 15 days, fetuses' presence were confirmed through palpation in pregnant rats and their nest preparation activity for neonates, was noted.

2.4. Blood sampling and storage

At each sacrificial time, at 6 months or 11 months of implants insertion, the rats were treated with Xylazine (Xylazin HCL 2 mg/kg, Rompun, BAYER, intraperitoneal route), and (Ketamine HCL 13 mg/kg Ketalar, PFİZER intraperitoneal route). After induction of anesthesia, the rats were bleed for serum samples and sera were harvested by centrifuging the blood samples at 3000 rpm for 10 min. The sera samples were stored at −20 °C until analysis. The stored samples were later processed for testosterone and anti-HSP 70 antibodies analysis.

2.5. Measurement of the testicular dimensions

Measurement of the left and right testicles dimensions (length and width including the scrotal skin) were made using Vernier caliper at every month in submitted experimental groups by a single trained person.

2.6. Histologic evaluation of the testes and accessory glands

Following six and eleventh months of GnRH implant administration, the rats were sacrificed humanly and, the testicles and accessory glands were isolated. After the removal of extra fat or tissue from the testes, testicular weight and epididymal sperm

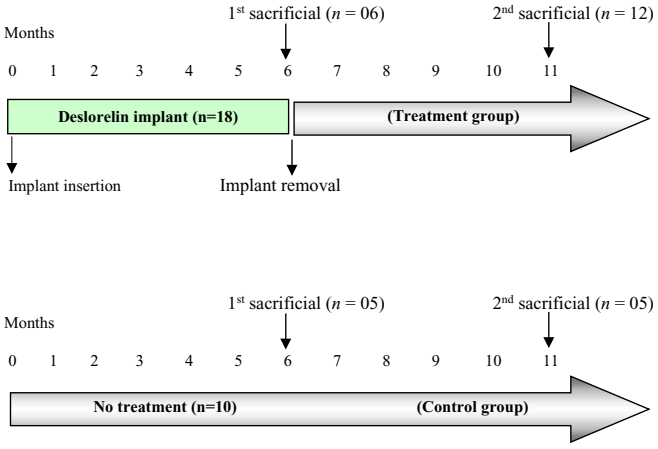


Fig. 1. Timeline for the application GnRH agonist implants (4.7 mg deslorelin, Suprolerin®) in male rats (treatment and control groups).

Table 1
HSP 70 concentration and serum testosterone (ng/ml) in control ($n = 10$) and treatment ($n = 18$) male rats at 6th and 11th months following GnRH analogue implant insertion (Mean ± S.D).

Variables	Time after implant insertion (months)	Control	Treatment
HSP 70	**6**	1.52 ± 0.24	1.76 ± 0.45
	11	1.21 ± 0.31	1.44 ± 0.37
Testosterone (ng/ml)	**6**	0.97 ± 0.53[a]	0.08 ± 0.12 [b,Y]
	11	4.51 ± 2.02[a]	0.80 ± 0.54[a,X]

The letters (a, b) indicate the difference across the groups.
The letters (X, Y) indicate the difference within groups.

variables were observed. Afterwards, the testes and accessory sex glands tissues were observed for histological examinations and immunohistochemistry. For histological observations, the tissues were fixed in Bouin's solution whereas 10% formal tampon was used to fix the tissue for immunohistochemistry. After routine tissue histological procedures, the paraffin blocks were prepared, sliced into 5–6 μm thickness sections and later sections were stained through Triple staining method. After triple staining procedure, the slides sections were observed under bright microscope for histological changes.

After fixing the tissues in Bouin's solution for 24 h, the fixed tissues were processed for serial alcohol and xylene washings and paraffin blocks were prepared. The fixed tissue block were sectioned into 6 μm thickness slices and were stained through Mallary Triple stain. The stained section slides were observed under bright microscope. In the testes slides, the seminiferous tubules dimensions (diameter, lumen and epithelium thickness) were determined. These dimensions were determined using 20× objective in each group prepared slides and randomly 20 tubules per section were recorded. The Leica ICC50 microscope and LAS V4.3 was used to convert the micrometer readings.

Above mentioned procedures were followed for the accessory glands histological slides preparation and immunohistochemistry observations.

2.7. Immunohistochemistry for HSP 70

For immunohistochemistry examination, the tissue specimens were fixed in Buoin's solution for 24 h. A sections of 4 μm thickness sections were processed for HSP70 activity through ABC method staining. The sections were deparafinized and later incubated in 3% H_2O_2 for endogenous peroxidase blockage for 30 min. 0.01 M citric acid (pH 6.0) was used for the retrieval of antigen. For nonspecific stain blockage, the ¼ goat serum was used. The sections were stained with 1/80 dilution (abcam HSP70) at 4 °C for primary antibody. The slides were stained in biotin for 45 min followed by streptavidin staining for 45 min and in AEC (Zymed) for 45 and 10 min wheras Hematoxylin was as counter stain. Negative control primary antibodies were used for other phase removal.

2.8. Statistical analysis

The data were analyzed for mean, standard deviation, median, minimum and maximum values. Mann-Whitney *U* test was used to compare the parameters between the treatment and control. Wilcoxon test was applied to determine the variance between the groups at different time intervals. All tests had an alpha level of 0.05 as the measure of statistical significance. All the tests were performed using SPSS (ver.20).

3. Results

Following deslorelin implantation, no tissue reaction or infection were observed at site of implant placement in male rats.

3.1. Testosterone measurement

The results of testosterone concentration indicate that it was significantly reduced ($p < 0.05$) in treatment (0.08 ± 0.12 ng/ml) group than control (0.97 ± 0.53 ng/ml) group after 6 months of implant insertion. Similar ($p > 0.05$) fashion of testosterone concentration was observed at 11 months post implant insertion between treatment (0.80 ± 0.54 ng/ml) group and control (4.51 ± 2.02 ng/ml) groups (Table 1).

3.2. Pregnancy rate and litter size (pups number delivered by pregnant females)

The results presented in Table 2 shown that there was a significant difference ($p < 0.05$) in pregnancy rate and litter size between treatment and control groups at 2 and 6 months of trial. However, pregnancy rate and number of pups delivered by female rats, mated by treatment and control, were similar at 11 months.

3.3. Testicular dimensions

The results of testicular measurements (width and lengths) after GnRH implant insertion were given in Table 3. There was significant ($p < 0.05$) reduction in testicular length and width in treatment group compared to control group during each observation point except at 1, 2, 9 and 11 months post implant insertion.

3.4. Retrieved testes and accessory sex glands size

The retrieved testes after killing at 6 and 11 months of trial, shown significantly smaller testicular dimensions in treatment than control group, whereas, testicular dimensions were larger comparatively at 11th month than 6th months in treatment group. Similar observation was made for testes weight in treatment and control groups (Table 4).

The size of accessory sex glands isolated from experimental rats at 6 or 11th month of trial, was significantly lower in treatment

Table 2
Comparison of pregnancy rate (%) and litter size (pups number delivered by pregnant rats, mean ± SD) from female rats exposed to control and treatment adult male rats at 2nd, 6th and 11th months following GnRH analogue implant insertion.

	Month after implant insertion					
	2		6		11	
	Treatment	Control	Treatment	Control	Treatment	Control
Pregnancy rate (%)	3/36 (8.3)	8/20 (40)	1/36 (2.7)	3/20 (15)	1/24 (4.1)	1/10 (10)
Litter size (Mean pups number delivered by pregnant rats)	1.56 ± 3.71	7.50 ± 4.81	0.01 ± 0.001	2.60 ± 4.55	0.01 ± 0.001	1.40 ± 3.13
P-value	$P < 0.05$		$P < 0.05$		$P > 0.05$	

$P < 0.05$ indicate the significance at each observation point between the experimental groups.

Table 3
Comparison of testicular dimensions including scrotal skin (width and lengths; Mean ± SD) after GnRH analogue implant insertion in control (n = 10) and treatment (n = 18) groups of adult male rats.

Groups	Months after GnRH implant insertion								
	1	2	3	4	5	6	8	9	11
Testes width (cm)									
Control	0.45 ± 0.05^{a}	0.50 ± 0.12^{a}	0.92 ± 0.13^{a}	1.01 ± 0.12^{a}	1.01 ± 0.15^{a}	$0.44 \pm 0,02^{a}$	$1.07 \pm 0,12^{a}$	1.03 ± 0.06^{a}	0.83 ± 0.29^{a}
Treatment	0.54 ± 0.05^{b}	0.52 ± 0.09^{a}	0.48 ± 0.07^{b}	0.49 ± 0.07^{b}	0.59 ± 0.18^{b}	0.69 ± 0.22^{b}	0.48 ± 0.05^{b}	0.55 ± 0.21^{b}	0.35 ± 0.09^{b}
Testis length (cm)									
Control	1.85 ± 0.13^{a}	1.75 ± 0.14^{a}	2.29 ± 0.10^{a}	2.29 ± 0.19^{a}	2.74 ± 0.24^{a}	0.97 ± 0.04^{a}	2.32 ± 0.35^{a}	2.25 ± 0.30^{a}	1.85 ± 0.30^{a}
Treatment	1.94 ± 0.05^{a}	1.84 ± 0.22^{a}	1.06 ± 0.15^{b}	1.09 ± 0.15^{b}	1.86 ± 2.88^{b}	1.41 ± 0.61^{b}	1.04 ± 0.12^{b}	1.36 ± 0.67^{a}	1.24 ± 0.48^{a}

Different letters (a, b, c) superscripts at each observation point (month) along the same column across the groups indicate the significance ($P < 0.05$)

than control group.

3.5. Sperm parameters

Sperm attributes presented in Table 4, indicate that GnRH implant insertion influence the sperm motility, sperm concentration and abnormal sperm rate both at 6 and 11 months of treatment; however, control group showed the normal values for studies sperm parameters.

3.6. Histological findings in testes

Seminiferous tubules (diameter, lumen and epithelium size) of treatment and control groups were presented in Table 4. The seminiferous tubules diameter, lumen and epithelium thickness decreased significantly in treatment group at 6 month observation point. However, no difference was noted in seminiferous tubules diameter, lumen or epithelium thickness at 11 month of observation between treatment and control rats.

Seminiferous tubules had the normal different developmental phases (spermatogonia, primary spermatocyte, secondary spermatocyte, spermatids and sperm) of spermtogenic cells in control group. Area between these germ cells was occupied by achromatic nuclei containing Sertoli cells. Between seminiferous tubules, Leydig cells were clearly observed (Fig. 2A).

In treatment group at 6 month, histological slides of testes shown the sparse or distorted arrangement in seminiferous tubules with significantly smaller tubular diameter. The tubules epithelium decreased, whereas, the tubules lumen measurement was not possible at different points. The seminiferous tubules had variable and undistinguishable spermtogenic cells stages. In germinal epithelium, only spermatogonia and primary spermatocytes were detected. Sertoli cells were similar to the control group. Moreover, no sperm were seen in tubules lumen (Fig. 2B).

In treatment group at 11 months, the seminiferous tubules had dilatation with morphological changes. The seminiferous tubules had irregular cellular arrangement with morphological deformities. Sertoli and Leydig cells were similar as observed at 6 months in treatment group (Fig. 2C).

3.7. Accessory sex glands

The histological picture of prostate gland, seminal vesicles and bulbourethral glands (Cowper's glands) were presented in Figs. 3–5.

In control group, there were larger alveoli structures in prostate glands. The alveoli epithelium was composed of single prismatic layer and the space between the alveoli was occupied by fibromuscular stroma (Fig. 3A). In treatment group at 6 months, the alveolar epithelium of alveoli was smaller with a marked curve towards one side and maximum area was infiltrated by fibromuscular stroma (Fig. 3B). In treatment group at 11 month, the alveoli diameter increased with single layer of prismatic epithelium corresponding to the control group (Fig. 3C).

In control group, the seminal vesicles were highly convoluted with irregular lumen. There were primary and secondary foldings and epithelium components. Tunica muscularis and tunica adventia layers were observed (Fig. 4A). In treatment group at 6 months, the seminal vesicles had only primary folds, whereas, the secondary folds were short with narrow lumen. Moreover, the epithelium area was also narrower due to tunica muscularis and tunica advetita layers presence (Fig. 4B). In treatment group at 11 month, the epithelial area was alike to control group with primary and secondary foldings. Tunica muscularis and tunica adventitia

Table 4
Effect of GnRH analogue implant insertion on testicular and sperm parameters in treated ($n = 18$) and untreated ($n = 10$) adult male rats.

Variables	Control	Treatment (months after implant insertion)	
		6	11
Testes weight (gms)	2.7 ± 0.63^{a}	1.20 ± 0.12^{b}	1.96 ± 0.21^{a}
Seminiferous tubules			
Diameter (μm)	349.56 ± 5.14^{a}	246.96 ± 86.07^{b}	349.83 ± 41.52^{a}
Lumen (μm)	130.66 ± 30.24^{b}	48.29 ± 41.04^{c}	188.0 ± 49.87^{a}
Epithelium thickness (μm)	98.31 ± 9.77^{a}	82.4 ± 26.04^{b}	95.0 ± 19.43^{a}
Sperm variables			
Motility (%)	78.03 ± 17.65^{a}	26.10 ± 22.15^{b}	18.33 ± 9.25^{b}
Concentration (10^{8}/mL)	3.22 ± 0.78^{a}	1.04 ± 0.42^{b}	1.22 ± 0.65^{b}
Dead sperm (%)	8.21 ± 1.42^{b}	54.27 ± 4.25^{a}	66.18 ± 4.99^{a}
Total abnormalities (%)	12.9 ± 7.6^{b}	26.37 ± 14.5^{a}	48.36 ± 20.06^{a}
Head abnormalities (%)	1.22 ± 2.02^{b}	13.85 ± 2.6^{a}	16.65 ± 8.25^{a}
Acrosome abnormalities (%)	0.6 ± 0.2^{b}	4.27 ± 1.66^{a}	17.21 ± 6.36^{a}
Mid-piece abnormalities (%)	6.86 ± 2.2^{a}	2.19 ± 1.1^{b}	4.4 ± 2.1^{b}
Tail abnormalities (%)	4.22 ± 3.9^{b}	6.06 ± 2.02^{b}	10.1 ± 4.66^{a}

Different letters (a, b, c) superscripts at each observation point (month) along the same row across the groups indicate the significance ($P < 0.05$).

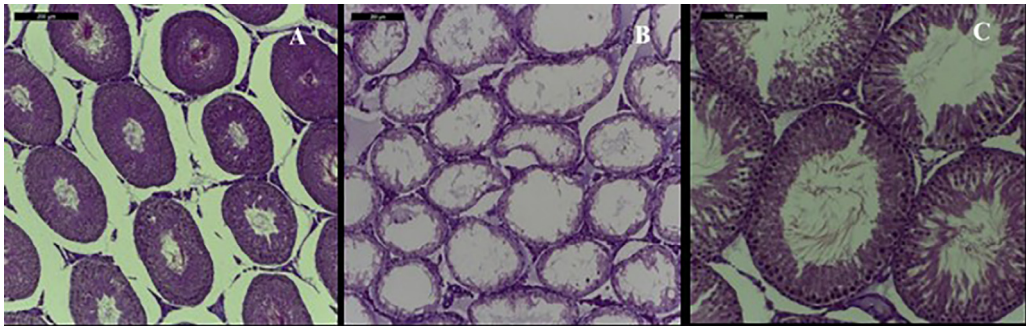


Fig. 2. Microscopic view of [A: Control group, 200 μm], [B: Deslorelin group at 6 months, 200 μm] and [C: Deslorelin group at 11 months, 100 μm] testes of rat. Panel (A) indicate relatively normal texture of seminiferous tubules, panel (B) shows the sperm free seminiferous tubules with sparse arrangement with vacuoles and panel (C) shows the normal seminiferous tubules with different germ cells generations.

layers arrangement was similar to control group (Fig. 4C).

The Cowper's gland had tubuloalveolar structures with multiple secretory units surrounded by smooth and skeletal muscles and connective tissues in control group (Fig. 5A). In treatment group, the Cowper's gland at 6 month, the secretory area reduced as because of connective tissues infiltration. The secretory glands area was decreased and clearly differentiated by the alveoli. The amount and area of secretory units was decreased dramatically (Fig. 5B). In treatment group at 11 month, the Cowper's gland secretory units became more tubuloalveoler with lesser connective tissue area. Smooth and skeletal muscles arrangement was same as observed in control group (Fig. 5C).

3.8. HSP 70 expression in testes

The expression of HSP70 in the seminiferous tubules was observed during the different phases of spermatogenesis. The HSP70 expression was more prominent at the post-meiotic phase of spermatids development. There was no expression of HSP70 in seminiferous tubules at 6 months observation in treatment group; however, fewer primary spermatocytes shown positive reaction for HSP70 (Fig. 6A). In contrast, higher expression of HSP70 positive spermatids during post-meiotic phase were seen in treatment group at 11 months alike to control group (Fig. 6B). However, the HSP 70 concentration did not change ($P > 0.05$; Table 1) across the treatment and control groups either at 6 or 11 month of post implant administration (Fig. 6C).

4. Discussion

The current study is the first report which focused on the testicular morphology, accessory glands integrity, hormonal pattern, HSPs expression and sperm features prior and after the long-term release GnRH agonist, deslorelin, treatment in male rats. In this study, the deslorelin treatment duration extended for six months and this duration was expected a long spell for suppression of HPG axis and reproductive activity. It was observed that long-term deslorelin application has significantly reduced the testicular and accessory glands size, lowered down the testosterone level, altered the HSPs expression in testes, and deteriorated the sperm quality; moreover, these overt reproductive features were persisted for next few months in treated male rats.

In the present study the long-term deslorelin treatment suppressed the circulating testosterone which correspond directly to reduction in testicular mass. This relation could be good indicator to assess the spermatogenesis inhibition. Current suppressed testosterone profile following long-term deslorelin was similar as documented earlier in different species [8,20,21]. This marked reduction in testosterone level probably related to suppressed release of FSH and more particularly LH secretion [4,21]. Moreover, the serial blood sampling for testosterone, after long-term deslorelin application with its association to testes morphometry, could be futuristic study to observe the behavioral level of testosterone in deslorelin exposed males.

The rats implanted with GnRH agonist did not show any increase in testicular size and had a significantly smaller testes compared with control rats between third to ninth months of study. These variation might be directly linked to the testicular parenchyma particularly the Sertoli cells number [22]. The Sertoli cells shown a smaller variation than Leydig cells during the neonatal stage [23] and this variation is more notably associated with down-regulation of GnRH receptors by deslorelin long-term treatment. A substantial increase in testes size was happened when the GnRH analogue long-term treatment diminishes. Previous reports also

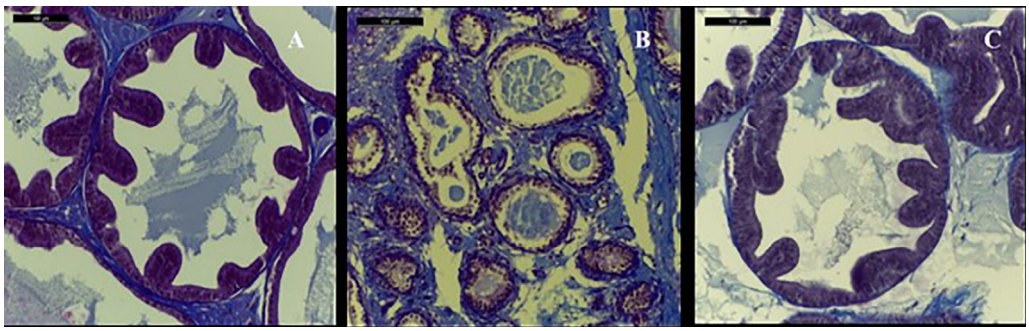


Fig. 3. Microscopic view of [A: Control group], [B: Deslorelin group at 6 months] and [C: Deslorelin group at 11 months] prostate glands of rat. Panel (A) indicate relatively normal alveoli with prismatic epithelium, panel (B) shows the smaller epithelium with fibromuscular stroma and panel (C) shows the alveoli epithelium with a single prismatic layer.

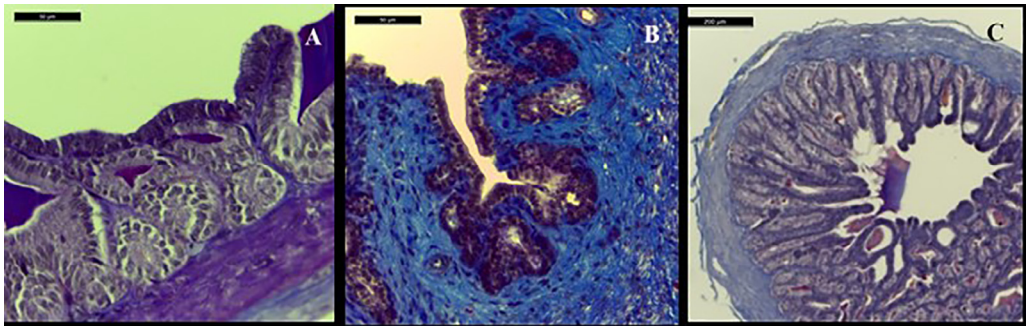


Fig. 4. Microscopic view of [A: Control group], [B: Deslorelin group at 6 months] and [C: Deslorelin group at 11 months] seminal vesicle of rat. Panel (A) indicate highly convoluted with multiple primary and secondary folding along prismatic epithelium. The panel (B) shows only primary and secondary folds with short and narrow lumen and panel (C) shows the similar primary and secondary folding with an increasing lumen.

shown the similar pattern of change in testes volume following the treatments by long-term release GnRH analogues [8,20,21,24,25]. The whole treatment groups had suppressed testicular volumes, represented as neonates, until unless they were under influence of submitted doses. FSH is involved Sertoli cells proliferation and following 2–3 months of implants removal testes size increased due to proliferation of Sertoli cells. It could be experimented by providing the exogenous FSH to treatment group after implant removal to observe the effects. The attribute lacks in the present study is relationship of testes size and testosterone, FSH and LH levels measurements, prior and after the implants removal or different time points during the trial. Testes size were reduced by long-term treatment with a GnRH agonist and testes regained their normal size when its effects eliminated were the salient features of the present study. Moreover, this tool might be effective to delay the testicular growth and development by suppressing the FSH or LH release from pituitary gland. The long-term release GnRH treatment downregulate the GnRH receptors in pituitary and suppress the sexual activity in female and male animals for variables durations [26,27]. Similarly their resumption of sexual activity and return to normal fertility post GnRH analogue therapy is also variable [28,29]. Its efficacy depends upon the course of implants insertion [29,30], type of analogue used [26] its bioavailability [31], individual variations [26,30] and growth phase [22]. It has also been noted that there is great variability in resumption of sexual activity and it is more species specific [28–30] and, its efficacy remains continue from months to years [32].

The histological testicular findings of treated rats were true reflection of testosterone hormonal depletion. The current findings are in line to the observation made earlier in different species treated with GnRH analogues [4,29–35]. The presence or absence of abnormal germ cells stages in seminiferous tubules is an indication of infertility following long-term release GnRH analogues application, whereas, proper spermatogenesis was seen during the later phase of trail. Smaller diameter of seminiferous tubules has association with Sertoli cells proliferation and disintegrated population of germ cells in seminiferous tubules. In addition, the long-term release GnRH treatment notably affect the sperm variables in rats. The earlier reports also documented the marked suppression over a time and restoration in semen quality following implants withdrawal [25]. It has observed that the sperm quality suppression was dose dependent and normal sperm appeared in the ejaculate after recovery of testosterone levels following GnRH implant removal [35]. Although the main aim is to suppress the sexual activity but restoration after short or long-term is also required if male needed for the future breedings or fertility trials.

In male animals, the seminal vesicles has a role to provide the seminal fluid for maximum volume of semen. The steroids are essentials for the normal physiology of accessory sex glands and any sort of immunization against the steroids decrease the production of seminal fluid which results in low viscosity seminal fluid that is resorbed readily [36]. Similarly, reduction in bulbo-urethral gland is also related with immunocastration; however, thick walled and high viscous contents are indicative of slow reduction in bulbo-urethral gland [37]. The immunization lead to the flattening of the glandular epithelia of seminal vesicles with an increase at stromal mass. In prostate gland, the reduction of tall columnar secretary epithelia and an increase in stromal tissue compared to secretary epithelia are the sequel of immunization [38]. In the present study, the continuous low levels of testosterone due to long-term deslorelin application resulted in diminution of weight and secretory epithelial area of accessory sex glands in rats. However, normal histological architecture of accessory sex glands was observed when the implants were removed and the steroids and testes attained the capacity to secrete the steroids.

HSP proteins are normally detected in cells and have the role of

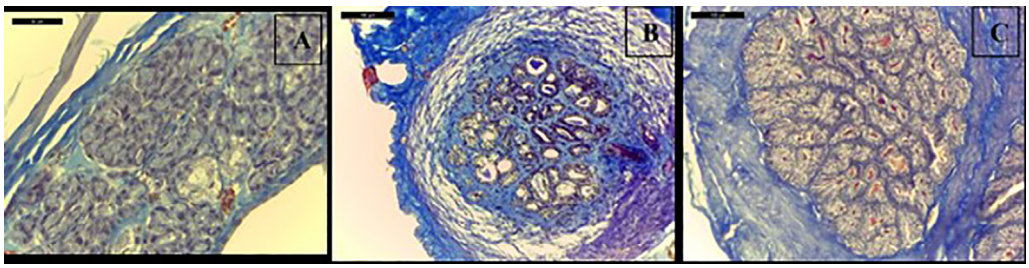


Fig. 5. Microscopic view of [A: Control group], [B: Deslorelin group at 6 months] and [C: Deslorelin group at 11 months] Cowper's gland of rat. Panel (A) represent the tubuloalveolar structures with multiple secretory units. The panel (B) indicate the smaller secretory area occupied by connective tissues and panel (C) shows the increasing tubuloalveolar structures along multiple secretory glands.

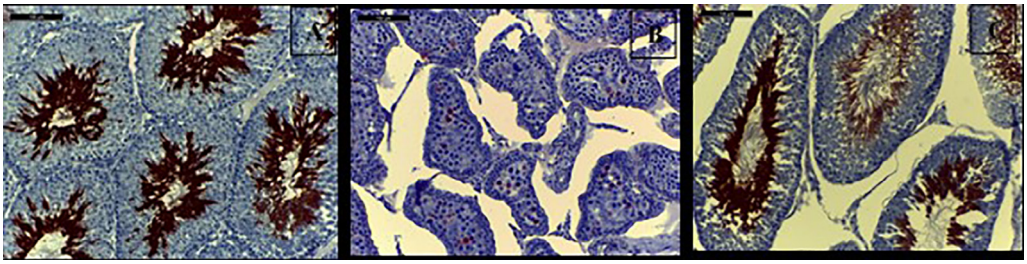


Fig. 6. Microscopic view of [A: Control group], [B: Deslorelin group at 6 months] and [C: Deslorelin group at 11 months] HSP70 in rat seminiferous tubules. Panel (A) shows the higher expression of HSP70 positive spermatids during post-meiotic phase. The panel (B) indicates HSP70 positive reaction only in a fewer primary spermatocytes and panel (C) shows the similar expression of HSP70 as in control (Panel A).

cytoprotection under stressful conditions. HSPs are involved in transport system of cytoplasm, nucleus, endoplasmic reticulum and mitochondrial protein synthesis. Sudden heat change, pH change, oxygen deficiency, stress factors improved its expression for normal cell functioning. HSP70, is synthesized in most of the tissues during pathological conditions, however, it formation is common phenomenon in testes during stage of mitotic proliferation of spermatogonia, meiotic development of spermatocytes and, post-meiotic development of spermatids and maturation of spermatozoa [39]. In the present study, it was first time observed that long-term release GnRH analogue implant suppressed the HSP70 protein expression in treated compared to untreated rats. HSP70 protein expression clearly indicated the inhibition of spermatogenesis or inappropriate development in rat testes. The higher expression of HSP70 during post-meiotic phase of spermatids development after 11 months of deslorelin treatment is an indication of the resumption of spermatogenesis process. This variation in expression of HSP70 during and after long-term treatment by GnRH analogue depicts that application of long-term release GnRH implants might be involved in up- or down-regulation of HSP70 protein in testes.

In the current study, fertility of GnRH treated rats was also tested. The data of fertility also corroborate the other studied parameters like testes morphology, testicular histological view, sperm attributes, hormonal and protein expression. Pregnancy rate and litter size in submitted female against treated males were nadir when after the insertion of deslorelin implant. Similar observations were made earlier in a study [28]. In future, inclusion of larger number of female against treated and control males could provide better results with less variability.

5. Conclusions

In conclusion, application of GnRH implant (4.7 mg deslorelin, Suprolerin®) has long-term castrating effects in rats. It affects the morphology of testes and accessory gland, suppress the HSP70 expression, and decrease the testosterone and sperm quality. Its effects continues for extended time even after removal of implants and resumption in sexual activity and fertility occurs within 6 months of implant removal.

Conflicts of interest

The authors declare that there is no conflicts of interest in the content of the article.

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