Acta Scientiarum. Biological Sciences
ISSN: 1679-9283
eduem@uem.br
Universidade Estadual de Maringá
Brasil

Mafra Cajú, Fernanda; D'Angelo Queiroz, Gian Carlo; Torres, Sandra Maria; Mendes Tenório, Bruno; Silva Júnior, Valdemiro Amaro

Opioid system manipulation during testicular development: results on sperm production and sertoli cells population

Acta Scientiarum. Biological Sciences, vol. 33, núm. 2, 2011, pp. 219-225
Universidade Estadual de Maringá
.png, Brasil

DOI: 10.4025/actascibiolsci.v33i2.5940

Opioid system manipulation during testicular development: results on sperm production and sertoli cells population

Fernanda Mafra Cajú*, Gian Carlo D'Angelo Queiroz, Sandra Maria Torres, Bruno Mendes Tenório and Valdemiro Amaro Silva Júnior

*Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, 52171-900, Dois Irmãos, Recife, Pernambuco, Brazil. *Author for correspondence. E-mail: fmc@hotmail.com*

ABSTRACT. The Sertoli cell has fundamental importance to the development and maintenance of spermatogenesis, as well as it has a directly proportional numerical relationship to sperm production. The proliferative period of this cell in rats occurs between 13 days pre-natal and 21 days pos-natal, when is established the final population in adult animals. The Leydig cell can modulate the Sertoli cell proliferation during fetal and neonatal period through β-endorphin. The manipulation of opioidergic system can promote changes in parameters related to development of nervous, endocrine and reproductive systems. By the way, the main purpose of this present work was to compare the effects of the blockade of opioid receptor blocking in Sertoli cells using naltrexone (50 mg kg^{-1}) during fetal and neonatal period in Wistar rats. According to the results, the manipulation of opioidergic system during pre-natal period reduced the total length of seminiferous tubule and Sertoli cell population in adult rats, but sperm production was normal because this cell has had a compensatory response for spermatozoids support capacity.

Keywords: Naltrexone, testis development, spermatogenesis, Wistar rats.

RESUMO. Manipulação do sistema opioidérgico durante o desenvolvimento testicular: consequência sobre a produção espermática e a população de células de sertoli. As células de Sertoli têm fundamental importância para o desenvolvimento e manutenção da espermatogênese, bem como possuem uma relação numérica diretamente proporcional com a produção espermática. O período proliferativo destas células em ratos ocorre entre 13 dias pré-natal e 21 dias pós-natal, resultando na definição da população de células de Sertoli nos animais adultos. As células de Leydig podem modular a proliferação das células de Sertoli durante o período fetal e neonatal por meio da β-endorfina. A manipulação do sistema opioidérgico durante esta fase pode promover alterações em parâmetros relacionados com o desenvolvimento dos sistemas nervoso, endócrino e reprodutivo. Em virtude disto, o objetivo do presente trabalho foi comparar os efeitos do bloqueio de receptores opioides nas células de Sertoli, utilizando o naltrexone (50 mg kg^{-1}), durante o período proliferativo destas células em ratos Wistar. De acordo com nossos resultados, a manipulação do sistema opioidérgico durante o período pré-natal reduziu o comprimento total de túbulos seminíferos e a população de células de Sertoli em ratos adultos, porém, a produção espermática foi normal pela resposta compensatória desta célula na capacidade de suporte para espermatozoides.

Palavras-chave: Naltrexone, desenvolvimento testicular, espermatogênese, ratos Wistar.

Introduction

In adult animals testis the Sertoli cells form a stable population. The growth of Sertoli and germ cells populations occurs at different times, inversely correlated and regulated by specific factors to each population (ORTH, 1993). In fetal period occurs the largest expansion of Sertoli cells population, however, from birth there is a decline in proliferation of these cells (ORTH, 1982). The number of germ cells in adults depends on the size of the Sertoli cells population. Therefore, the perinatal period is fundamental for the development of a quantitatively normal spermatogenesis in adult (ORTH et al., 1988).

FSH (follicle-stimulating hormone) is probably the most important replication regulator of Sertoli cells population during fetal and neonatal testicular development (FRANÇA et al., 2005). The determination of this gonadotropin levels may be related to an endocrine and paracrine control exercised jointly by Sertoli and Leydig immature cells, which can indirectly influence the final size of Sertoli cells population (ORTH, 1993; SHARPE,

1994). Leydig cells can influence Sertoli cells function in immature and adult animals by peptides derived from POMC (proopiomelanocortin), such as β-endorphin. This opioid, whose production is directly controlled by luteinizing hormone (LH), acts as paracrine inhibitor of Sertoli cell function (ROSER, 2008).

Naloxone is an opioid antagonist often used in fertility studies because of its ability to modulate the release of several hormones involved in testicular function, such as somatotropin, somatostatin, prolactin and GnRH (gonadotropin-releasing hormone) (AURICH et al., 2003). Several studies demonstrated that manipulation of the opioid system, using naloxone or naltrexone, during the development of nervous system change parameters related to opioid function in rats (CABO; VIVEROS, 1997; DE CASTRO et al., 1993).

Considering that β-endorphin may influence the Sertoli cells proliferation in prenatal period (DESJARDINS; EWING, 1993). The present work aimed to compare the effects of opioid receptors blockade using naltrexone hydrochloride, a potent opioid receptors antagonist with systemic action, during the proliferation period of fetal and neonatal Sertoli cells.

Material and methods

Pregnant Wistar rats (*Rattus norvegicus*, *var. Albinus*) were obtained from biotery of the Laboratory of Physiology in Federal Rural University of Pernambuco. These animals were divided into control (n = 4) and treated (n = 8). From 13 until 21 days of gestation was applied daily saline or 50 mg kg^{-1} of naltrexone hydrochloride (Revia®). Neonatal rats were randomly selected to compose the following groups: I) treated prenatal with 50 mg kg^{-1} of naltrexone (13-21 days of gestation, PN group, n = 8), II) treated pre and postnatal with 50 mg kg^{-1} of naltrexone (13 days of gestation to 21 postnatal days, PN-PoN group, n = 8); III) control group, exposed to the same experimental conditions except for the presence of naltrexone (n = 8). Group I received daily postpartum physiological solution of NaCl 0.9% until 21 postnatal days and group III (control) received NaCl 0.9% from 13 days of gestation until 21 postnatal days. All solutions were injected intraperitoneally.

The experimental protocol used in this study was approved by the ethics committee of the Department of Animal Morphology and Physiology - Federal Rural University of Pernambuco (CEEUA nº 0004/2004 - DMFA / UFRPE).

The animals were weighed daily from 1 to 21 postnatal days and subsequently weighed weekly until 90 postnatal days (balance BEL Engineering MARK 500 / BRA ± 0.001 g). At the end of experimental period, were measured the mean weight of left and right testes, left and right epididymis, seminal vesicle and gonadosomatic index (GSI). The GSI can be described as a relative factor of the mean weight of both testes in terms of total body weight.

At 90 postnatal days, rats were anesthetized (10 mg kg^{-1} xylazine 2% and 90 mg kg^{-1} of ketamine 10%) (HILLYER; QUESENBERRY, 1997) and perfused intracardiac with glutaraldehyde 4% in sodium phosphate buffer 0.01 M, pH 7.4. The testes, epididymis and seminal vesicles were removed and weighed. The testes were cut into fragments of 2 mm thick and placed back in the same solution of perfusion. Testicular fragments were processed for inclusion in plastic resin of glycol methacrylate, cut into 4 μm thick and stained with toluidine blue / sodium borate.

Histometric analyses were carried out initially through the measurements of tubular diameter and height of seminiferous epithelium. These measurements were taken at 100x, using linear micrometer reticle (10 mm / 100 - Olympus), calibrated with a micrometer standard. The mean tubular diameter and mean height of seminiferous epithelium were obtained from the measurement of fifteen tubules shaped as circular as possible.

Volumetric data of the testicular parenchyma composition were obtained using point counting for systematic allocation through micrometer graticule (Olympus) with 441 intersection points on histological preparation of the testis in 400x magnification. Fifteen fields were counted randomly, totaling 6615 points for each animal. The volume of each component of the testis, expressed in μL, was established from the product of the volume density of testicular components (%) and net weight of testis (mg). Due the density of testis is approximately 1.03 to 1.04 (ROCHA et al., 1999), the testis weight was considered equal to its volume. The value of testicular net weight was obtained by subtraction of 6.5% (correspondent to albuginea and mediastinum) of gross testicular weight (RUSSELL; FRANÇA, 1995). The total length of seminiferous tubules (TL) was expressed in meters and estimated

from the following formula (ATTAL; COUROT, 1963; DORST; SAJONSKI, 1974):

$$TL = \frac{TVS}{\pi R^2}$$

TVS = Total volume of seminiferous tubules; πR^2 = Cross sectional area of seminiferous tubules (R = tubular diameter / 2).

The estimate of the various cell types that make up the seminiferous epithelium at stage 7 of the cycle, classified according to acrosomal method (RUSSELL et al., 1990), was made from counts of germ cells nuclei at the stage of spermatocyte I in pre-leptotene (PL), spermatocyte I in pachytene (PQ), round spermatids (RS) and Sertoli cells nucleoli. To this purpose, we used 5 cross sections of seminiferous tubules for each animal.

With exception of Sertoli cells nuclei, the obtained counts were corrected for nuclear diameter and thickness of histological section, using the formula of Abercrombie (1946) modified by Amann and Almquist (1962):

$$Corrected\ Number = Obtained\ Count \times \frac{Histological\ Section\ Thickness}{Histological\ Section\ Thickness + \sqrt{\left(\frac{ND^2}{2}\right) - \left(\frac{ND^2}{4}\right)}}$$

Mean nuclear diameter (ND) represents the mean of 10 nuclei diameters, this parameter was measured through a linear micrometer lattice (10 mm / 100 - Olympus) using increase of 1000x. The numbers of Sertoli cells were corrected for the nucleolar diameter and histological section thickness. The Sertoli cells index was obtained from the ratio between the corrected nucleus number of round spermatids and Sertoli cells nucleolus corrected number. Total population of Sertoli cells per testis was obtained from the corrected number of Sertoli cells nucleoli per cross section of seminiferous tubule in stage VII and the total length of seminiferous tubules per testis, according to the formula described by Hochereau-de-Reviers and Lincoln (1978):

$$SC\ Testis\ Number = \frac{Tot.\ \text{Leng.}\ Sem.\ Tub. \times Cor.\ Num.\ SC.\ Nucl.\ Cross\ \text{Section}}{Histological\ Section\ Thickness}$$

SC Testis Number = Number of Sertoli cells per testis; Tot. Leng. Sem. Tub. = Total length of seminiferous tubules (μm); Cor. Num. SC Nucl. Cross Section = Corrected number of Sertoli cells nucleoli per cross section; Histological Section Thickness (μm).

The Sertoli cell index (SCI) was obtained from the ratio between the number of round spermatids per cross section and the number of Sertoli cells nucleoli per cross-section of seminiferous tubules.

Daily sperm production per testis (DSP) was obtained according to Rocha et al. (1999) and Silva Júnior et al. (2006):

$$DSP = \frac{Total\ SC\ Number \times \text{RS}\ Number \times \text{Relative Frequency}}{Stage\ VII\ Duration}$$

DSP = Daily sperm production; Total SC Number = Total number of Sertoli cells per testis; RS Number = Number of round spermatids per cross-section of tubule at stage VII; Relative frequency = Relative frequency of stage VII; Stage VII duration in days (SILVA JÚNIOR et al., 2006).

Daily sperm production per gram of testis (DEP/g) was obtained from the ratio between DSP and the net weight of testis.

The obtained values were statistically analyzed in computer program STATISTICA 6.0 (Statsoft©, Inc. 2001) to assess the trend of normality. Shapiro-Wilk test was performed to check the normality tendency of the data. Due to normal trend of the results, we proceeded using the program InStat 3.05 (GraphPad Software, Inc. 2000) through the use of analysis of variance parametric test (ANOVA one way) and Tukey post-hoc test to normal data. If the data were not normal, we used the nonparametric test of Kruskal-Wallis and Dunn post-hoc test. The data were expressed as mean and (±) standard deviation. All statistical analyses were outlined for $p < 0.05$.

Results and discussion

According to the data presented in Figure 1, we observed an increase in body weight between day 3 and 15, as well as between 17 and 21 days after birth, in the group that continued to receive 50 mg kg^{-1} of naltrexone hydrochloride after birth (Figure 1). The present study showed an increased body weight due to the blockade of opiate receptors during the treatment period before and after birth, confirming the results observed by McLaughlin et al. (1997). According to these authors, daily dose of naltrexone could interfere with the delicate balance of opioid growth factors and receptor ζ (zeta), both probably related to cell proliferation and consequently to body and organs development. The findings of body weight observed in the present study confirm the findings of Silva Júnior et al. (2006) that using naloxone hydrochloride, an opioid antagonist less potent than naltrexone, observed an increased weight gain in neonatal rats treated during the same period.

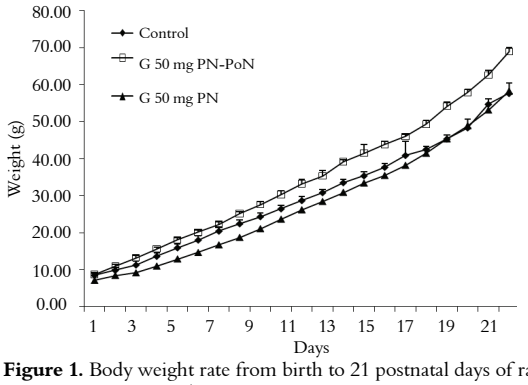

Figure 1. Body weight rate from birth to 21 postnatal days of rats treated with 50 mg kg^{-1} of naltrexone and controls.

During weekly weight measurements (21 to 90 postnatal days), we observed that from postnatal day 28 until the end of the experiment at 90 days, the weight of animals treated with naltrexone during pre and postnatal period did not differ compared to control group. Furthermore, in same time interval, the weight of animals receiving naltrexone in pre and post-natal period was superior to the animals that received the same drug only in prenatal period (Figure 2).

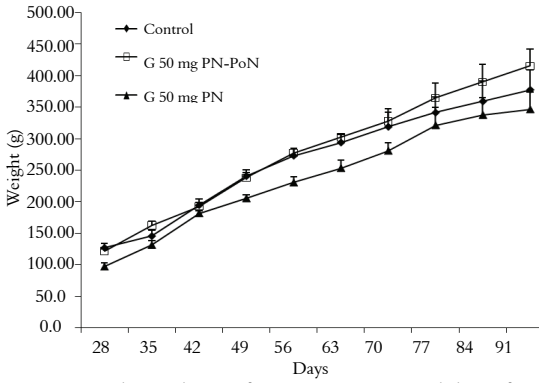

Figure 2. Body weight rate from 28 to 90 postnatal days of rats treated with 50 mg kg^{-1} of naltrexone and controls.

Organs weighed at the time of perfusion such as testis, epididymis and seminal vesicles showed no difference between the experimental groups (Table 3).

Table 3. Biometric parameters of control group and groups treated with naltrexone pre-natal and pre and postnatal up to 90 days of age (mean ± standard deviation).

	Control (n = 8)	G50 mg PN-PoN (n = 8)	G50 mg PN (n = 8)
Body Weight (g)	377.6 ± 27.30ab	415.1 ± 25.54a	346.4 ± 25.91b
Testicular Weight (g)	1.54 ± 0.39	1.72 ± 0.09	1.57 ± 0.07
Epididymis Weight (g)	0.56 ± 0.05	0.59 ± 0.03	0.54 ± 0.04
Seminal vesicle Weight (g)	1.38 ± 0.10	1.38 ± 0.30	1.41 ± 0.18
GSI (%)	0.40 ± 0.11	0.41 ± 0.04	0.46 ± 0.04

Different letters in same line indicate significant difference ($p < 0.05$).

Table 4 shows the data for testes net weight and histomorphometric parameters at 90 postnatal days. According to the results, there was no significant difference between experimental groups related to net weight of testis.

Tubular diameter and seminiferous epithelium height are used as important parameters to evaluate the spermatogenic activity in experimental and toxicological trials (BLANK et al., 1986; ORTH et al., 1988). Tubular diameter and seminiferous epithelium height significantly increased in animals treated with 50 mg kg^{-1} of naltrexone during the pre and postnatal period compared to control animals. This increase was not observed in animals that received the same drug only in the prenatal period.

The Sertoli cells number, as well as the number of round spermatids per cross-section of seminiferous tubules did not differ between experimental groups. However, it is important to note that there was a tendency of increase of approximately 20% in the number of round spermatids in groups that received the opioid antagonist. This fact was reflected in the support capacity of supporting cells, known as Sertoli cells index (SCI). According to França et al. (2005), Sertoli cells index reflects the functional efficiency of this cell, being expressed through the ratio between the number of elongated spermatids supported by the Sertoli cells. This index increased in animals treated with different protocols using naltrexone hydrochloride, however, only animals that received the antagonist during prenatal period showed a statistically significant increase, approximately 30%, compared to control animals (Table 4).

The total length of seminiferous tubules has a direct correlation with structural parameters such as testicular weight, total Sertoli cells population and daily sperm production (FABBRI, 1990). In the present work, the total length of seminiferous tubules and the Sertoli cells population per testis have been reduced due the administration of opioid antagonist in prenatal period. According to the results in Table 4, administration of naltrexone hydrochloride in pregnant rats via intraperitoneal in prenatal period, promoted a 25% reduction in total length of seminiferous tubules and 20% of the Sertoli cells population compared to control animals. A possible reason for the reduction of total seminiferous tubules length may be associated with the increased tubular diameter, and consequently, the increase of seminiferous tubule area (μ^2) in animals treated with naltrexone in prenatal period. Changes to these morphometric parameters directly influence the total length of seminiferous tubules. These results differ from those found by Silva Júnior et al. (2006), who observed an increase in

total length of seminiferous tubules and Sertoli cell population in rats treated with 8 μg of naloxone hydrochloride per gram of body weight associated with a reduced tubular diameter.

Daily sperm production (DSP) per testis and per gram of testis has not changed with the use of opioid antagonist. This fact can be related to adaptation in the support capacity of Sertoli cells.

Table 4. Biometric parameters of testicular parenchyma in controls rats and treated prenatal and pre and postnatal with naltrexone up to 90 days of age. (mean ± standard deviation).

	Control (n = 8)	G50 mg PN-PoN (n = 8)	G50 mg PN (n = 8)
Testicular Net Weight (g)	1.42 ± 0.36a	1.61 ± 0.09a	1.47 ± 0.06a
Tubular Diameter (μ)	332.5 ± 26.4a	360.7 ± 13.5b	349.4 ± 15.2ab
Epithelium Height (μ)	103.7 ± 7.7a	113.3 ± 6.3b	109.0 ± 6.9ab
Sertoli Cells / Tubule Cross Section	10.4 ± 1.53a	9.95 ± 1.58a	9.22 ± 1.40a
Round Spermatid / Tubule Cross Section	60.0 ± 21.1a	77.7 ± 14.7a	72.9 ± 5.5a
Sertoli cells index (SCI)	5.7 ± 1.7a	7.9 ± 1.4ab	8.0 ± 1.13b
Total seminiferous tubule length (m)	17.7 ± 2.6a	14.6 ± 1.04ab	13.2 ± 1.4b
Sertoli cells population (x10^7)	3.93 ± 1.6a	3.6 ± 0.7ab	3.1 ± 0.7b
DSP (x10^6)	19.1 ± 5.2a	21.4 ± 4.95a	16.0 ± 7.1a
DSP/g (x10^6)	12.0 ± 5.02a	13.2 ± 2.64a	12.3 ± 1.80a

Different letters in same line indicate significant difference ($p < 0.05$).

Table 5 shows the data concerning the population of germ and Sertoli cells per cross section of tubule at stage VII of seminiferous epithelium cycle in control and treated groups. According to the results, we have not found any influence of the opioid antagonist on cell population at this stage of the cycle.

Table 5. Cell population per cross-section of seminiferous tubule in stage VII of the cycle in control rats and treated prenatal and pre and postnatal period with 50 mg kg^{-1} up to 90 days of age (mean ± standard deviation).

	Control (n = 8)	G50 mg PN-PoN (n = 8)	G50 mg PN (n = 8)
Sertoli Cells	10.37 ± 1.53a	9.96 ± 1.58a	9.22 ± 1.40a
Pre-leptotene Spermatocyte I	25.77 ± 1.98a	25.55 ± 3.08a	26.66 ± 0.65a
Pachytene Spermatocyte I	25.32 ± 7.74a	31.33 ± 5.98a	29.98 ± 2.8a
Round spermatids	60.0 ± 21.1a	77.71 ± 14.7a	72.91 ± 5.5a

Different letters in same line indicate significant difference ($p < 0.05$).

Endogenous opioid peptides have a well-defined role in neuroendocrine system, given that regulate the activity of gonadotropin releasing neurons (GnRH) and consequently secretion of follicle stimulating hormone (FSH), luteinizing hormone (LH), gonadal steroid production and sexual behavior (RUSSELL et al., 1998). FSH is the main mitogenic factor responsible for proliferation of Sertoli cells (FRANÇA et al., 2005). Therefore, the reduction of adequate levels of this hormone can negatively influence the testis final size and sperm production. These parameters are determined by Sertoli cells population during the critical period of testicular development (ORTH et al., 1988; ORTH, 1993).

Naloxone, a powerful opioid antagonist, acts in central levels on receptors μ, δ and κ (ZHOU et al., 1990; RUSSELL et al., 1998) producing a large increase in outflow of GnRH in normal male and females rats, giving support to the role of opioids in tonic inhibition of gonadotropins. Gerendai et al. (1983) observed the local effect of naloxone injected intratesticular in neonatal rats through the increased testicular weight which occurred due to the higher number of sex cord at 10 days old.

In the present research, the blockade of opioid receptors systematically maintained through a naltrexone daily dose during the critical period of neonatal testicular development did not promote a increased number of Sertoli cells in treated animals. According to Laxmi and Vijayan (1998), chronic systemic administration of m-enkephalin and β-endorphin reduced plasma levels of gonadotropins and exerted an inhibitory effect on thymidine incorporation ^{3}[H] in testicular tissue, showing that this opioid reduced the proliferative activity of gonadal cells. The author also observed an opposite effect in animals treated with naloxone or antiserum to N-acetyl-β-endorphin, suggesting a direct effect of opioid peptides / naloxone in gonads or central nervous system.

In testis, the functional modulation exerted by β-endorphin has consequences that are closely related to ontogenetic differences in the action of this peptide in central release of hipofisitrofic hormones in begin of puberty and sexual maturation (CICERO et al., 1986). During perinatal and prepubertal period, this opioid peptide has paracrine modulating action on Sertoli cells proliferation and maintenance of testicular quiescence (GERENDAI et al., 1983; MORRIS et al., 1987; FABBRI et al., 1988). In sexually mature individuals, β-endorphin modulates the spermatogenic process, which is controlled synergistically by FSH and testosterone (CHANDRASEKHAR; BARTKE, 1992).

In an earlier study, Silva Junior et al. (2006) observed that the increase in daily sperm production per testis and spermatogenic process efficiency was attributed to the increase in Sertoli cells number, justifying the testicular effects of naloxone on opioid receptors located in Sertoli cells and the probable increase in FSH levels during the neonatal period. To this purpose, the authors used 2 daily doses with 8 mg kg^{-1} of naloxone hydrochloride in intervals of 12 hours. This observation confirmed the influence of opioid peptides during this phase of testicular

development. However, in this experiment, the use of an opioid antagonist with prolonged effect in dose 6 times higher than that used by Silva Júnior et al. (2006) not had the expected effect in Sertoli cells population.

The FSH receptor is a transmembrane protein belonging to the large family of GTP-binding proteins (protein-G). The binding of FSH to its receptor results in stimulation of protein-G, which activates adenylate cyclase associated with membrane, causing elevation of intracellular cyclic-AMP (SIMONI et al., 1997). In our results, the use of naltrexone hydrochloride did not produce the expected effect, probably due to unbalance in FSH production, number of receptors in Sertoli cells or both. There are several cellular mechanisms used to neutralize the overstimulation of this chemical messenger in Sertoli cell. In intact animals or in cultured Sertoli cells, chronic stimulation by FSH induces desensitization and down-regulation of intracellular signaling for this hormone. This phenomenon is evidenced by depression in cyclic-AMP production (MAGUIRE et al., 1997) and involves multiple steps in the pathway of signal transduction for FSH, including: (i) uncoupling of FSH receptor effector system induced by phosphorylation of serine or threonine residues in C-terminal intracellular domain of G protein-linked receptor; (ii) decrease in FSH receptor mediated, at least in part, by extensive clustering and internalization of hormone-receptor complex and reduced synthesis of protein receptor through decreased transcription and half-life of mRNA for FSH receptor (HECKERT; GRISWOLD, 1992; FORESTA et al., 2004).

Pharmacologically, naltrexone hydrochloride has more prolonged effect and greater ability to block opioid receptors compared to naloxone hydrochloride (BRUNTON, 2006). Therefore, the higher dose per body weight, more prolonged effect of the drug and the action of cellular mechanisms used to neutralize the overstimulation may have resulted in the difference observed between the results obtained in these experiments.

In Table 4 we can observe the increase of 29% in Sertoli cells index in PN group. The same trend was observed for PN-PoN group compared to control, but no statistical difference was found. The Sertoli cells index is the best reflection of the functional efficiency of Sertoli cells and is characterized by the number of germ cells supported by this cell. França et al. (2005) suggests that within a certain limit for each species, the support capacity of Sertoli cells to germ cells is relatively flexible. Therefore, our results indicate that the adaptability of Sertoli cells to support more spermatids can maintain the daily sperm production, despite the reduction of Sertoli cells population in animals treated with naltrexone during prenatal period.

Conclusion

In the present study, Sertoli cells population at 90 postnatal days was reduced in rats treated during prenatal period in response to manipulation of the opioid system, however, sperm production was normal due to an adaptive response in Sertoli cell ability to support the sperm.

References

ABERCROMBIE, M. Estimation of nuclear populations from microtome sections. **Anatomical Records**, v. 94, n. 94, p. 238-248, 1946.

AMANN, R. P.; ALMQUIST, J. O. Reproductive capacity of dairy bulls. VIII. Direct and indirect measurement of testicular sperm production. **Journal of Dairy Science**, v. 45, n. 1, p. 774-781, 1962.

ATTAL, J.; COUROT, M. Développement testiculaire et établissement de la spermatogénèse chez le taureau. **Annals Biology Biochemistry and Biophysical**, v. 8, n. 3, p. 219-241, 1963.

AURICH, J. E.; KRANSKI, S.; PARVIZI, N.; AURICH, C. Somatostatin treatment affects testicular function in stallions. **Theriogenology**, v. 60, n. 1, p. 163-174, 2003.

BLANK, M. S.; MANN, D. R.; DAUGHERTY, D. T.; SRIDIRAM, R.; MURPHY, J. R. Central, stereoselective receptors mediate the acute effects of opiate antagonists on luteinizing hormone secretion. **Life Science**, v. 39, n. 17, p. 1493-1499, 1986.

BRUNTON, L. L. **Goodman and Gilman's the pharmacological basis of therapeutics**. 11th ed. New York: McGraw-Hill Incorporated, 2006.

CABO, C.; VIVEROS, M. P. Effects of neonatal naltrexone on neurological and somatic development in rats of both genders. **Neurotoxicology and Teratology**, v. 6, n. 19, p. 499-509, 1997.

CHANDRASEKHAR, V.; BARTKE, A. The influence of β-endorphin on testicular endocrine function in adult rats. **Biology of Reproduction**, v. 47, n. 1, p. 1-5, 1992.

CICERO, T. J.; SCHMOEKER, P. F.; MEYER, E. R.; MILLER, B. T.; BELL, R. D.; CYTRON, S. M.; BROWN, C. C. Ontogeny of the opioid-mediated control of reproductive endocrinology in the male and female rat. **The Journal of Pharmacology and Experimental Therapeutics**, v. 236, n. 3, p. 627-633, 1986.

DE CASTRO, R. M.; CABRAL-FILHO, J. E.; COSTA, J. A.; COSTA, F. B.; GALLINDO, M. A.; HECKSHER, C. A. Neonatal treatment with naloxone causes permanent hyperalgesia in rats. **Brazilian Journal of Medical Biology Research**, v. 7, n. 26, p. 747-751, 1993.

DESJARDINS, C.; EWING, L. L. **Cell and molecular biology of the testis**. New York: Oxford University Press, 1993. p. 25-26.

DORST, V. J.; SAJONSKI, H. Morphometrische untersuchunhen am tubulussystem des schweinehodens während der postnatalen entwicklug. **Monatsh Verterinary Medicine**, v. 29, p. 650-652, 1974.

FABBRI, A. The role and regulation of testicular opioids. **Trends in Endocrinology Metabolism**, v. 1, n. 3, p. 117-120, 1990.

FABBRI, A.; KNOX, G.; BUCZKO, E.; DUFAU, M. L. β-endorphin production by the fetal Leydig cell: regulation and implications for paracrine control of Sertoli cell function. **Endocrinololgy**, v. 122, n. 2, p. 749-755, 1988.

FORESTA, C.; BETTELLA, A.; SPOLAORE, D.; MERICO, M.; ROSSATO, M.; FERLIN, A. Suppression of the high endogenous levels of plasma FSH in infertile men are associated with improved Sertoli cell function as reflected by elevated levels of plasma inhibin B. **Human Reproduction**, v. 6, n. 19, p. 1431-1437, 2004.

FRANÇA, L. R.; AVELAR, G. F.; ALMEIDA, F. F. L. Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. **Theriogenology**, v. 63, n. 2, p. 300-318, 2005.

GERENDAI, I.; NEMESKÉRI, A.; CSERNUS, V. Naloxone has a local effect on the testis of immature rats. **Andrologia**, v. 15, n. 4, p. 398-403, 1983.

HECKERT, L.; GRISWOLD, M. D. Expression of the FSH receptor in the testis. **Recent Progress in Hormone Research**, v. 48, p. 61-78, 1992.

HILLYER, E. V.; QUESENBERRY, K. E. **Ferrets, rabbits and rodents**: clinical medicine and surgery. New York: W. B. Sounders Company, 1997.

HOCHEREAU-DE-REVIERS, M.-T.; LINCOLN, G. A. Seasonal variation in the histology of the testis of the red deer, *Cervus elaphus*. **Journal of Reproduction and Fertility**, v. 54, p. 209-213, 1978.

LAXMI, N. A.; VIJAYAN, E. Effects of chronic systemic administration of peptides, naloxone and N-acetyl β-endorphin antiserum on gonadotropins and testicular function in the rat. **Indian Journal of Experimental Biology**, v. 36, n. 4, p. 361-366, 1998.

MAGUIRE, S. M.; TRIBLEY, W. A.; GRISWOLD, M. D. Follicle-Stimulating Hormone (FSH) regulates the expression of FSH receptor messenger ribonucleic acid in cultured Sertoli cells and in hypophysectomized rat testis. **Biology of Reproduction**, v. 56, n. 5, p. 1106-1111, 1997.

McLAUGHLIN, P. J.; TOBIAS, S. W.; LANG, C. M.; ZAGON, I. S. Chronic exposure to the opioid antagonist naltrexone during pregnancy: maternal and offspring effects. **Physiology and Behavior**, v. 3, n. 62, p. 501-508, 1997.

MORRIS, P. L.; VALE, W. W.; BARDIN, C. W. β-endorphin regulation of FSH-stimulated inhibin production is a component of a short loop system in testis. **Biochemical and Biophysical Research Communications**, v. 148, n. 3, p. 1513-1519, 1987.

ORTH, J. M. Proliferation of Sertoli cells in fetal and postnatal rats: a quantitative autoradiographic study. **Anatomical Record**, v. 203, n. 4, p. 485-492, 1982.

ORTH, J. M. Cell biology of testicular development in fetus and neonate. In: DESJARDINS, C.; EWING, L. L. (Ed.). **Cell and molecular biology of the testis**. 1st ed. New York: Oxford University Press, 1993. p. 3-42.

ORTH, J. M.; GUNSALUS, G. L.; LAMPERTI, A. A. Evidence from Sertoli-cell depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. **Endocrinology**, v. 3, n. 122, p. 787-794, 1988.

ROCHA, D. C. M.; DEBELJUK, L.; FRANÇA, L. R. Exposure to constant light during testis development increase daily sperm production in adult Wistar rats. **Tissue and Cell**, v. 3, n. 31, p. 372-379, 1999.

ROSER, J. F. Regulation of testicular function in the stallion: An intricate network of endocrine systems. **Animal Reproduction Science**, v. 107, n. 3-4, p. 179-196, 2008.

RUSSELL, J. A.; BROWN, C. H.; CÁRON, R. W. Endogenous opioids. In: KNOBIL, E.; NEIL, J. D. (Ed.). **Encyclopedia of Reproduction**. San Diego: Academic Press, 1998. p. 1043-1061.

RUSSELL, D. L.; ETTLIN, R. A.; SINHA HIKIM, A. P.; CLEGG, E. D. Mammalian spermatogenesis. In: RUSSELL, D. L.; ETTLIN, R. A.; SINHA HIKIM, A. P.; CLEGG, E. D. (Ed.). **Histological and histopathological evaluation of the testis**. Bolesta: Cache River Press, 1990. p. 1-40.

RUSSELL, L. D.; FRANÇA, L. R. Building a testis. **Tissue Cell**, v. 27, n. 2, p. 129-147, 1995.

SHARPE, R. M. Regulation of spermatogenesis. In: KNOBIL, E.; NEIL, J. D. (Ed.). **The physiology of reproduction**. 2nd ed. New York: Raven Press, 1994. p. 1363-1434.

SILVA JÚNIOR, V. A.; VIEIRA, A. C. S.; PINTO, C. F.; DE PAULA, T. A. R.; PALMA, M. B.; AMORIN, M. J. A. A. L.; AMORIN JÚNIOR, A. A.; MANHÃES-DE-CASTRO, R. Neonatal treatment with naloxone increases the population of sertoli cells and sperm production in adult rats. **Reproduction Nutrition Development**, v. 46, n. 2, p. 157-166, 2006.

SIMONI, M.; GROMOLL, J.; NIESCHLAG, E. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. **Endocrine Reviews**, v. 18, n. 6, p. 739-773, 1997.

ZHOU, Z. F.; XIAO, B. L.; ZHANG, G. Y.; ZHUANG, L. Z. A study of the effect of B-EP and naloxone on the function of the hypothalamo-pituitary-testicular axis of the rat. **Journal of Andrology**, v. 11, n. 3, p. 233-239, 1990.

Received on December 5, 2008.
Accepted on November 17, 2009.