Personal pdf file for
M.T. Guerra, J.E. Perobelli, M. Sanabria, J.A. Anselmo-Franci, W. De Grava Kempinas

With compliments of Georg Thieme Verlag

www.thieme.de

Long-term Effects of Perinatal Androgenization on Reproductive Parameters of Male Rat Offspring Androgenization and Male Rat Reproduction

DOI 10.1055/s-0033-1341434
Horm Metab Res 2013; 45: 586–592

For personal use only.
No commercial use, no depositing in repositories.

Publisher and Copyright
© 2013 by
Georg Thieme Verlag KG
Rüdigerstraße 14
70469 Stuttgart
ISSN 0018-5043

Reprint with the
permission by
the publisher only

Long-term Effects of Perinatal Androgenization on Reproductive Parameters of Male Rat Offspring Androgenization and Male Rat Reproduction

Authors M. T. Guerra[1], J. E. Perobelli[1], M. Sanabria[2], J. A. Anselmo-Franci[3], W. De Grava Kempinas[2]

Affiliations
[1] Graduate Program in Cell and Structural Biology, Institute of Biology, State University of Campinas – UNICAMP, Campinas, SP, Brazil
[2] Department of Morphology, Institute of Biosciences, UNESP – Univ Estadual Paulista, Botucatu, SP, Brazil,
[3] Department of Morphology, Stomatology and Physiology, School of Dentistry, University of São Paulo, USP – Ribeirão Preto, São Paulo, Brazil

Key words
- rat male offspring
- perinatal androgenization
- sperm counts
- sexual behavior
- fertility

Abstract

It is known that during sex differentiation, fetal androgens are critical determinants of the male phenotype. Although testosterone is necessary for normal development of male sexual behavior, perinatal androgen treatment can result in disruption of normal male sexual reproduction. Pregnant Wistar rats were administered either corn oil (vehicle) or testosterone propionate at 0.2 mg/kg from gestational day 12 until the end of lactation and the reproductive function of male offspring was evaluated at 90 (adulthood) and 270 (middle age) days of age. Perinatal androgenization in the rat provoked a reduction in sperm production and reserves in adulthood that did not affect fertility and did not persist at more advanced ages, as shown by the results at post-natal day 270. If perinatal androgenization promotes similar effects in humans of reproductive age, the results of the present work can impact male reproduction health, given the less efficient spermatogenesis and lower sperm reserves in the human epididymis, compared to rodents.

received 31.10.2012
accepted after second revision 28.02.2013

Bibliography
DOI http://dx.doi.org/10.1055/s-0033-1341434
Published online: April 2, 2013
Horm Metab Res 2013; 45: 586–592
© Georg Thieme Verlag KG Stuttgart · New York
ISSN 0018-5043

Correspondence
M. T. Guerra
Department of Morphology
Institute of Biosciences
UNESP
Distrito de Rubião Jr s/n
Botucatu – São Paulo
Brazil CEP 18618-000
Tel.: +55/14/3880 0487
Fax: +55/14/3880 6462
marinatguerra@hotmail.com

Introduction

It is known that the presence of androgens during sex differentiation is critical for the determination of the male phenotype [1]. In males, testosterone secreted by fetal and neonatal testis [2] reaches 2 important peaks: first between gestational day (GD) 18–19 [3] and again during the first hours after birth [4]. These surges have been shown to be important for sexual differentiation, resulting in male-typical sexual behavior in adulthood, establishment of gonadotropin secretion patterns, and also for various morphological indices [5].
Several researchers reported that although testosterone is necessary for normal development of male sexual behavior, perinatal androgen treatment of an intact male results in disruption of normal male sexual reproduction [6,7]. In humans and rodents, exposure to hormonally active chemicals during sex differentiation can produce a wide range of abnormal sexual phenotypes [8]. Among contaminant substances, preliminary studies have focused only on environmental estrogens, but there is a growing awareness that androgenic chemicals are widespread in the environment [9].
Structural and/or functional reproductive deficiencies subsequent to male neonatal androgen administration have been reported previously [10]. These detrimental effects include not only reduced testicular, prostate and seminal gland weights but also impairment of sexual activity. Although some studies demonstrated that androgenized male rats presented alterations in hormone levels [11], others showed no variations in this parameter [12].
Based on the previous evidences that early excess exposure to androgen may cause alterations in the sexual development of male rats, the present study aimed to evaluate the exposure of pregnant and lactating female rats to a low dose of testosterone propionate (TP) and assesses the possible long-term effects on sexual parameters of the androgenized male offspring.
Besides sex organ weights, fertility and sexual behavior, commonly evaluated on previous studies, our experiment includes a more complete assessment of male reproductive function by measuring sperm quality, testicular and epididymal histology and hormone assays, and a different window of exposure. The exposure period coincides with the critical window of reproductive system development, which continues after

Electronic reprint for personal use

birth [13]. During the developmental period, fetuses presented an incomplete machinery of DNA repair, lack of detoxification enzymes, primitive hepatic metabolism and high sensitivity to epigenetic alterations [14]. These facts generate in the fetuses a higher susceptibility to the action of environmental chemicals [15].

Materials and Methods

Animals and androgen exposure

Adult female (60 days of age) and male (90 days of age) Wistar rats were supplied by the Central Biotherium of the State University of São Paulo (UNESP). Two nongravid female rats were mated with one male and the day of sperm detection in the vaginal smear was considered gestational day – GD 0. The experimental protocol followed the Ethical Principles in Animal Research of the Brazil College of Animal Experimentation and was approved by the Institute of Biosciences/UNESP Ethics Committee for Animal Experimentation (Protocol no. 104/2009-CEEA).

Pregnant rats (n=16) were randomly allocated into 2 experimental groups: exposed group (TP-exposed, n=8) that received testosterone propionate (TP- DEG Ltd., CAS 57-85-2, 97% of purity) at the dose of 0.2 mg/kg, subcutaneously (s.c.), from GD 12 until post-natal day (PND) 21, and control group (n=8) that received corn oil (vehicle), following the same experimental protocol. Based on this experimental design, male pups were exposed to TP via placenta (during intrauterine period) and through milk (after birth). The period of the treatment coincided with the critical period of reproductive system development, which continues after birth [13]. TP was already used previously as an androgenic disruptor [6,16–18] and it provides a model of base testing studies for environmental androgens [17].

At PND 1 litters were weighed and reduced to 8 [19] and after the last day of maternal TP exposure, male pups were weaned and maintained until adulthood to perform the assessment of reproductive endpoints, described below. All the procedures were performed at 90 (adulthood) and 270 (middle age) days of age.

Organ collection

Male rats (n=8, one per litter) were weighed, euthanized by CO_2 inhalation followed by decapitation, between 8:00 and 10:00 AM. The right testis and epididymis, left vas deferens, ventral prostate, seminal gland (without the coagulating gland and full of secretion), and pituitary were collected, trimmed free of fat and weighed on a precision balance. Liver and kidneys – detoxifying organs – were also weighed.

Histological evaluation and sertoli cell numbers

The left testis and epididymis were fixed in Alfac solution (85% of which was composed of 80% alcohol, 10% of formaldehyde, and 5% of glacial acetic acid) and processed for histological analysis. Organs were examined by light microscopy following specific guidelines for toxicological studies [20]. The numbers of Sertoli cell nuclei were counted in 20 cross-sections of seminiferous tubules per rat ([19]; with modifications).

Hormonal analysis

After decapitation, trunk blood was collected and allowed to clot (4 °C). Serum was collected after centrifugation (4 °C, 20 min at 2400 rpm) and stored at −20 °C until analysis. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, progesterone, and testosterone concentrations were measured using a double-antibody radioimmunoassay (RIA) kit (National Institute of Arthritis, Diabetes and Kidney Diseases NIADDK, USA). All the samples were analyzed in the same assay to avoid inter-assay variability.

Sperm motility and morphology

Sperm motility was analyzed as described by Perobelli et al. [21] on spermatozoa collected from the right vas deferens. With the aid of a syringe and needle, sperm were recovered from the left vas deferens by flushing with 1.0 ml of saline formol. To morphological analysis, 200 spermatozoa per animal were analyzed in a phase-contrast microscope [22]. Morphological abnormalities were classified into head and tail morphology [23]. Sperm were also classified as to the presence or absence of the cytoplasmic droplet.

Daily sperm production per testis, sperm number, and transit time in the epididymis

Homogenization-resistant testicular spermatids (stage 19 of spermiogenesis) in the testis and spermatozoa in the caput/corpus and cauda epididymis were counted as described previously [24]. To calculate the daily sperm production (DSP), the number of spermatids at stage 19 was divided by 6.1 days, which is the duration of the seminiferous cycle when these spermatids are present in the seminiferous epithelium. The sperm transit time through the epididymis was determined by dividing the number of sperm in each portion by the DSP.

Sexual behavior and natural mating

Male rats (1 or 2 per litter) were placed individually with one sexually receptive adult female rat and the following parameters were observed for 40 min: latency to the first mount, intromission, and ejaculation; number of intromissions until the first ejaculation; latency of the first post-ejaculatory intromission; number of post-ejaculatory intromissions; and total number of ejaculations [25,26]. Males that did not mount in the initial 10 min were considered sexually inactive [27]. After completing the sexual behavior evaluation, paired animals were kept together for an additional 4 h. After this period, males and females were separated and vaginal smears collected to confirm that mating had occurred and to determine the GD 0.

Fertility test

Twenty days after mating (GD 20), naturally inseminated females were killed by CO_2 inhalation followed by decapitation. After collection of the uterus and ovaries, the numbers of corpora lutea, implants, reabsorptions, and live and dead fetuses were determined. From these results the following parameters were calculated: fertility potential (efficiency of implantation): implantation sites/corpora lutea × 100; rate of pre-implantation loss: [number of corpora lutea – number of implantations/number of corpora lutea] × 100; and rate of post-implantation loss: (number of implantations – number of live fetuses)/number of implantations × 100 [28].

Statistical analysis

According to the characteristics of each variable, parametric Student's *t*-test (mean ± S.E.M.) or nonparametric Mann-Whitney test [median (Q_1–Q_3)] was used. Differences were considered

Electronic reprint for personal use

Parameters	PND 90 (n=8 animals/group)		PND 270 (n=8 animals/group)	
	Control	TP-treated	Control	TP-Treated
Body weight (g)	386.38±13.72	286.63±11.85*	503.97±22.98	452.19±23.21
Testis (g)	1.56±0.03	1.23±0.05*	1.67±0.04	1.53±0.06
Testis (g)/100g	0.41±0.01	0.43±0.01	0.34±0.02	0.34±0.01
Epididymis (mg)	518.18±11.64	423.00±12.78*	672.27±14.04	639.37±27.63
Epididymis (mg)/100g	135.17±4.52	148.34±3.73*	135.70±5.74	142.53±4.98
Ventral prostate (mg)	386.40±13.68	291.25±23.24*	501.68±37.57	514.70±58.51
Ventral prostate (mg)/100g	101.58±6.34	102.15±7.65	99.99±6.91	116.78±14.79
Full seminal gland (g)	1.18±0.05	1.06±0.07	1.54±0.09	1.60±0.12
Vas deferens (mg)	81.06±4.23	70.79±4.09	111.67±3.50	99.62±4.25
Vas deferens (mg)/100g	21.03±0.95	24.88±1.46	22.69±1.38	22.52±1.75*
Pituitary (mg)	9.76±0.49	7.89±0.35*	9.29±0.91	15.16±4.54
Pituitary (mg)/100g	2.53±0.10	2.76±0.11	1.83±0.13	3.30±0.92
Kidneys (g)	2.63±0.10	1.95±0.08*	3.25±0.14	2.74±0.15
Kidneys (g)/100g	0.68±0.02	0.68±0.01	0.65±0.02	0.60±0.01
Liver (g)	11.90±0.41	10.11±0.38*	14.44±0.59	12.56±0.78
Liver (g)/100g	3.09±0.05	3.54±0.07*	2.88±0.08	2.77±0.07

Values are expressed as mean±S.E.M. Student's *t*-test. *p<0.05

Table 1 Body and organ weights of male offspring (PND 90 and 270) from control and TP-exposed groups.

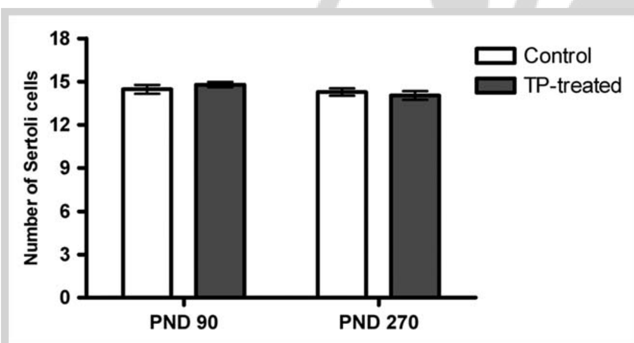

Fig. 1 Number of Sertoli cell nuclei of male offspring from control and TP-exposed groups (n=8 animals/group). Values are expressed as mean±S.E.M. Student's *t*-test.

significant when p<0.05. The statistical analyses were performed by GraphPad InStat (version 3.02).

Results

At PND 90, the exposure to TP provoked a diminution of body weight that was reflected in low weights of the testis, ventral prostate, pituitary and kidneys. In relation to the epididymis and liver, the male offspring from the TP-exposed group showed a significant decrease in absolute and relative weights. On the other hand, at PND 270, all the alterations found previously were no longer detectable (▶ **Table 1**).

Histopathology revealed that qualitative analysis of the epididymis and qualitative/quantitative evaluation of the testis were similar between experimental groups (data not shown). Furthermore, the numbers of Sertoli cell nuclei, at both tested ages, did not differ between experimental groups (▶ **Fig. 1**). However, epithelial vacuoles were found in the cauda epididymis of older animals (PND 270) from both experimental groups (69% of control group and 62% of TP-exposed group), but not in 90-day-old animals (▶ **Fig. 2**).

Analysis of hormonal status from male offspring at PND 90 revealed alterations in the serum levels of FSH and progesterone, which were found to be elevated and diminished, respectively. Again, these changes were not detected in the 270-day-old animals (▶ **Fig. 3**). At 90 and 270 days of age, the levels of estrogen, LH, and testosterone were similar between experimental groups. TP exposure caused a significant decrease in type C sperm at PND 270, but this alteration was not accompanied by any disturbance in other categories in the sperm motility evaluation. Sperm morphology analysis revealed similar percentages of normal and abnormal sperm between experimental groups whereas the cytoplasmic droplet was present in the majority of spermatozoa in both the control and TP-exposed groups (▶ **Table 2**).

The evaluation of sperm counts in the testis demonstrated that the number of mature testicular spermatids and DSP were diminished at PND 90. The sperm number in the cauda epididymis/g from TP-exposed group at PND 90 was significantly reduced when compared to controls. However, the sperm counts in the testis and epididymis at PND 270 were similar between experimental groups (▶ **Table 2**).

TP exposure was not able to impair the evaluated parameters in the sexual behavior test at either tested ages (▶ **Table 3**). Following the sexual performance assessment, the fertility test was performed in order to analyze the ability of androgenized male offspring to produce decedents, an endpoint also unaltered by perinatal androgenization (▶ **Table 3**).

Discussion

In rodents, the critical period of sexual differentiation begins in the last gestational phase and continues until the first week of post-natal life [29] and this event is determined by circulating levels of androgens during this specific period [30]. Androgen is aromatized in the brain into estradiol and is responsible for causing significant sexual dimorphism, which determines endocrine patterns and male behavior [31].

Typically, chemicals that adversely affect human sex differentiation also produce predictable alterations of this process in rodents [32]. Some compounds, known as endocrine disruptors, when administered to pregnant females and/or neonates may interfere with hypothalamic sexual differentiation of offspring [33].

There is a lack of agreement in the literature regarding the effect of androgenization on the reproductive function of male rats. Some researchers have reported reduced sexual behavior and

Electronic reprint for personal use

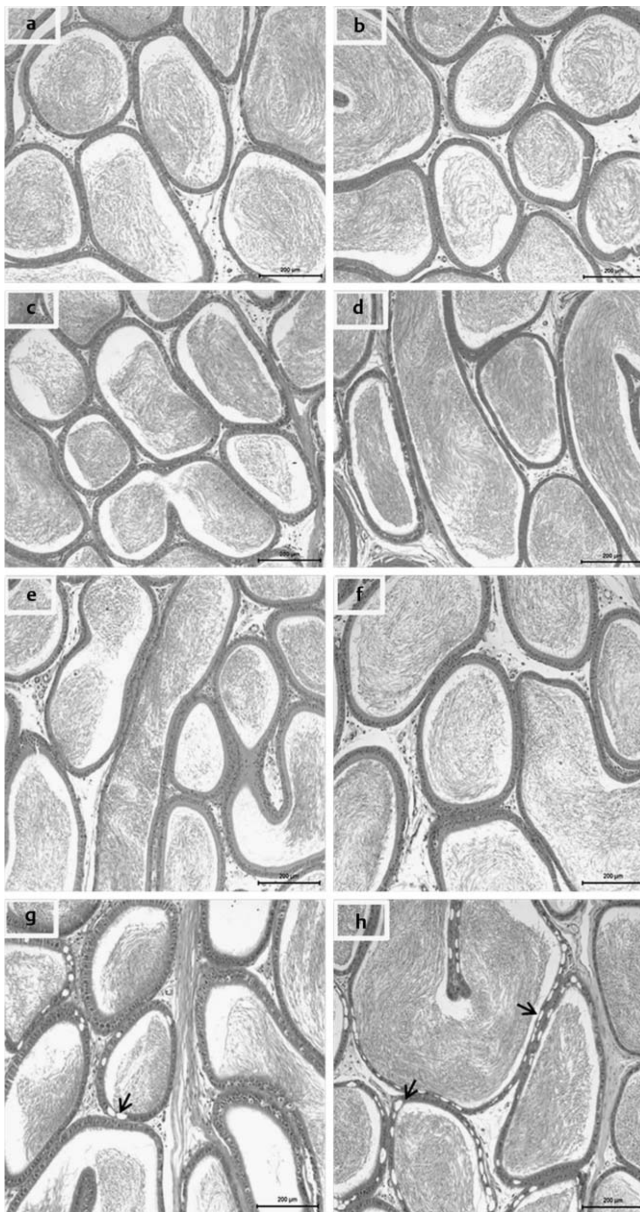

Fig. 2 Longitudinal sections of the epididymis from male rats from control and TP-exposed groups. **a**–**d** at PND 90 (**a** caput and **c** cauda of control group; **b** caput and **d** cauda of TP-exposed group); **e**–**h** at PND 270 (**e** caput and **g** cauda of control group; **f** caput and **h** cauda of TP-exposed group). Arrows: epithelial vacuoles; magnification: 200×; staining: **h** & **e**.

fertility [7, 18], whereas others found an absence of such alterations [17, 34]. The results presented in this experiment revealed impairment reflected in diminished weights of body and sex organs, hormone levels and sperm reserve at PND 90. These alterations, however, were not sufficient to alter the sexual behavior pattern or the capacity to produce decedents by natural mating.

Significant changes in absolute or relative male reproductive organ weights may constitute an adverse reproductive effect [26]. Wolf et al. [17] demonstrated that male body and reproductive organ weights were not affected by *in utero* TP administration. Contrary to these results, earlier experiments revealed that androgenized male rats presented smaller testes and sex accessory glands [7, 35]. In our experiment, the alteration in body weight at PND 90 may explain the low testicular, ventral prostate, kidney, and pituitary weights, but this fact was not responsible for the smaller epididymis and liver, since these organs were significantly decreased even when relative weights were compared to the control group. By PND 270 these parameters had all recovered.

Previous studies showed that such gonadotropic hormones as FSH, LH, prolactin, and androgens were not altered by neonatal testosterone exposure [7], but on the other hand, Rajfer and Coffey [35], Henley et al. [18] and, most recently, Cruz et al. [36] demonstrated lower levels of circulating androgens as a result of neonatal androgenization. In the present work, maternal TP administration did not alter testosterone levels, but provoked a reduction in progesterone levels and increase in FSH levels in the male offspring at 90 days, but not at 270.

FSH influences Sertoli secretion of inhibin, the major factor responsible for negative feedback of FSH release [37]. The significant increase in this hormone in 90-days-old TP-treated rats may suggest a dysfunction in these cells, despite normal testicular histology and Sertoli cell nuclei counts. Besides normal pituitary weight between experimental groups, we cannot also rule out the possibility of hypothalamus or pituitary dysfunction. More studies should be performed to characterize which cell type function is compromised by early androgenization.

Progesterone, which is secreted mainly by the adrenals in males, may play an important role in the normal expression of androgen-dependent sexual behavior in male mammals [38]. Thus, the decrease in progesterone levels in the current study may be due to a late functional alteration in the adrenal gland after early androgenization [39]. Androgen receptors have been characterized in the rat adrenal gland [40] and previous studies reported that neonatal sex hormones are responsible for organizational effects on the hypothalamic-pituitary-adrenal axis of male rats [41]. A more specific and profound evaluation should be performed in order to elucidate adrenal injury after perinatal TP exposure.

In aging experiments, Serre and Robaire [42] demonstrated the emergence of a localized region of the epididymal epithelium with large clear vacuoles in Brown Norway rats at 18 months of age, pointing to a signal of aging specific to the epididymis. In the present work, the vacuoles found in the cauda epididymis of the Wistar rats, at PND 270, in both experimental groups may reflect a degree of aging that accounts for our decision to characterize them as middle-aged. However, this alteration did not impair sperm function.

During most of their reproductive period, normal individuals present a uniform efficiency of sperm production. This efficiency is highly correlated with testicular weight and is influenced by age, environmental factors, hormonal status, and drugs [43]. Although our results showed, at PND 90, diminutions in testis weight, daily sperm production and in sperm reserves in the cauda epididymidis, the sperm production efficiency (number of sperm produced per day per gram of testicular parenchyma) was not altered by maternal TP treatment. These alterations did not persist on PND 270.

There is a heightened public awareness regarding the possibility that semen quality might be declining in the human population due to environmental factors [44]. Perturbation of the testicular and epididymal biochemical environments may occur after chemical exposure, altering sperm properties [26]. In the present study, androgenized male rats showed no alterations in sperm morphology or motility, suggesting that in utero and lactational TP exposure did not affect spermatogenesis or the post-testicu-

Electronic reprint for personal use

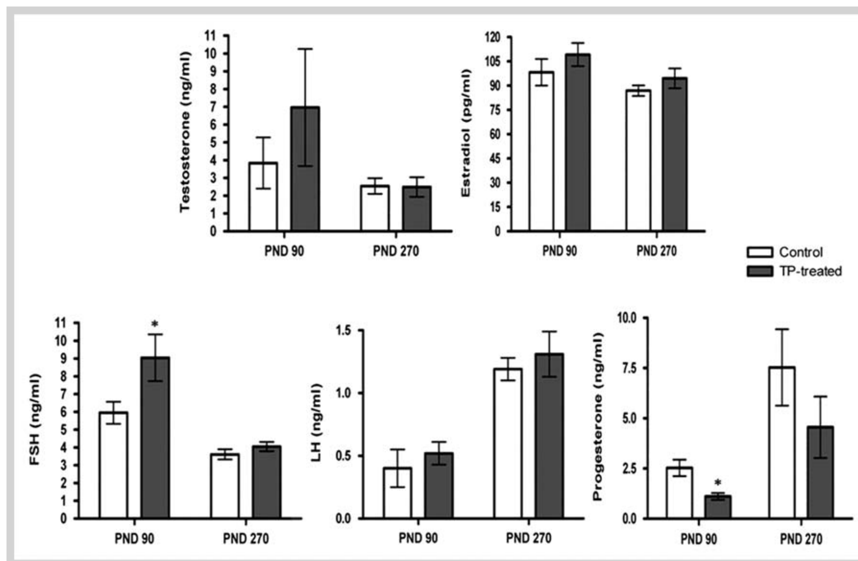

Fig. 3 Serum hormone levels of male rats from control and TP-exposed groups (n = 8 animals/group) at both studied ages. Values are expressed as mean ± S.E.M. Student's *t*-test. *p < 0.05.

Table 2 Sperm counts, motility, and morphology of male offspring (PND 90 and 270) from control and TP-exposed groups.

Parameters	PND 90 (n = 8 animals/group)		PND 270 (n = 8 animals/group)	
	Control	TP-treated	Control	TP-treated
Sperm counts				
Mature spermatid number (× 10^6/testis)	186.05 ± 4.93	147.25 ± 10.63*	185.74 ± 4.50	174.70 ± 11.46
Mature spermatid number (× 10^6/g testis)	153.40 ± 9.30	143.33 ± 7.40	134.72 ± 4.17	138.90 ± 9.48
Daily sperm production (× 10^6/testis/day)	30.50 ± 0.81	24.14 ± 1.74*	30.45 ± 0.73	28.64 ± 1.88
Relative daily sperm production (× 10^6/g testis/day)	25.15 ± 1.52	23.50 ± 1.21	22.09 ± 0.68	22.77 ± 1.55
Caput/corpus epididymis sperm number (× 10^6/organ)	105.82 ± 7.53	84.10 ± 11.08	124.16 ± 10.71	114.15 ± 13.24
Caput/corpus epididymis sperm number (× 10^6/g organ)	601.09 ± 47.84	586.09 ± 31.30	488.75 ± 19.44	481.41 ± 30.67
Sperm transit time in the caput/corpus epididymis (days)	3.51 ± 0.31	3.45 ± 0.31	4.08 ± 0.34	4.05 ± 0.46
Cauda epididymis sperm number (× 10^6/organ)	209.46 ± 13.95	176.84 ± 13.43	290.31 ± 16.93	238.96 ± 24.43
Cauda epididymis sperm number (× 10^6/g organ)	1776.90 ± 57.55	1520.60 ± 81.94*	1420.20 ± 75.38	1425.60 ± 42.38
Sperm transit time in the cauda epididymis (days)	6.91 ± 0.51	7.41 ± 0.54	9.48 ± 0.38	8.48 ± 0.86
Sperm motility (%)[a]				
Type A (motile, progressive)	83.00 (65.00–88.00)	77.00 (76.75–81.50)	86.00 (79.25–88.75)	83.00 (73.00–83.50)
Type B (motile, nonprogressive)	17.00 (12.00–25.00)	16.00 (12.00–19.25)	12.50 (8.50–18.25)	16.00 (15.00–20.00)
Type C (immotile)	3.00 (1.00–9.00)	5.00 (2.75–7.50)	3.50 (2.25–5.75)	2.00 (1.00–2.00)*
Sperm morphology (%)[a]				
Normal shaped sperm	75.00 (70.00–76.00)	69.25 (65.00–72.75)	66.50 (64.50–68.25)	64.00 (62.00–66.00)
Sperm with cytoplasmic droplet	65.00 (63.50–66.00)	64.00 (60.25–66.12)	67.77 (66.75–74.00)	68.00 (66.75–69.50)

Values are expressed as mean ± S.E.M. Student's *t*-test. *p < 0.05

[a] Values are expressed as median (Q_1–Q_3). Mann-Whitney test. *p < 0.05

lar maturation process, a finding supported by the normal sperm transit time through the epididymis.

In our present experiment, we observed an increased sperm transit time in the cauda epididymis at PND 270 when compared to 90 days-old animals; but despite this result, all experimental groups were able to fertilize female rats and generate decedents. Although previous research suggested that the delay in sperm transit through the cauda of epididymis diminishes their fertility potential [45], others showed that the spermatozoa that underwent a delay in passage through the epididymis and were therefore stored for a longer period, had a normal fertility potential [46].

Previous work have shown that supra-physiological levels of testosterone would pose a great disadvantage to male rats for successful mating [7, 18]. In the current experiment, maternal TP treatment did not impair sexual behavior of the male offspring. Moreover, the fertility test showed that early TP exposure did not affect the ability to produce offspring. Corroborating our results, Lumia et al. [16] and Cruz et al. [36] demonstrated that androgenized male offspring presented normal fertility and sexual behavior.

According to Zadina et al. [6], reproductive impairment caused by TP administration is due to estradiol, as a result of testosterone conversion. However, Frick et al. [47] demonstrated that estradiol benzoate, when administered neonatally to male rats provoked different results compared to TP exposure. Besides, Slob et al. [48] demonstrated that TP given to rat dam is metabolized by the dam and placenta into other androgens, of which androsterone (that cannot be converted in estrogens) is the most abundant in the fetus, followed by 3α-androstanediol and epiandrosterone. Based on this theory, we can assume that the reproductive effects followed by TP exposure are caused by androgenic activity rather than estradiol conversion.

We can conclude based on the present results, that although perinatal androgenization in the rat provokes a reduction in sperm production and reserves in adulthood, with no effects on

Electronic reprint for personal use

Table 3 Sexual behavior parameters of male offspring (PND 90 and 270, 1 or 2 per litter) from control and TP-exposed groups.

Parameters	PND 90		PND 270	
	Control n=11 animals	TP-treated n=8 animals	Control n=10 animals	TP-treated n=8 animals
Sexual behavior				
Number of mounts	4.00±1.41 (n=5)	4.80±1.24 (n=5)	2.57±0.68 (n=7)	2.14±1.32 (n=7)
Latency of the first intromission (s)	78.40±49.01 (n=5)	196.83±76.02 (n=6)	203.14±83.10 (n=7)	64.14±30.52 (n=7)
Number of intromissions until the first ejaculation	18.00±2.70 (n=5)	14.17±1.25 (n=6)	16.71±2.94 (n=7)	16.43±1.85 (n=7)
Latency of the first ejaculation (s)	807.40±171.22 (n=5)	699.00±203.01 (n=6)	628.75±152.53 (n=4)	1 008.20±884.00 (n=5)
Latency of the first post-ejaculatory intromission (s)	269.00±67.33 (n=5)	345.60±35.60 (n=5)	315.50±27.63 (n=4)	364.75±32.11 (n=4)
Number of post-ejaculatory intromissions	17.00±5.13 (n=5)	17.40±3.61 (n=5)	16.25±3.66 (n=4)	8.00±3.55 (n=6)
Number of ejaculations	2.20±0.37 (n=5)	2.33±0.42 (n=6)	2.00±0.68 (n=6)	1.57±0.43 (n=7)
Fertility test				
Gestation rate (%)	100	100	90	100
Fertility potential (%)[a]	100 (100–100)	100 (98.53–100)	100 (93.33–100)	100 (98.33–100)
Final body weight (g)	379.43±7.86	364.71±10.22	397.20±11.11	388.30±9.07
Uterus+fetuses weight (g)	66.77±3.59	68.11±3.50	85.16±4.33	83.09±2.22
Rat weight – (uterus+fetuses) (g)	312.66±5.60	296.61±8.24	312.04±7.94	305.2±8.77
Number of fetuses	12.82±0.70	13.63±0.75	12.89±0.51	12.50±0.33
Number of implantations	13.55±0.73	14.50±0.42	13.44±0.44	13.00±0.27
Number of corpora lutea	14.18±0.33	14.75±0.53	13.78±0.43	13.25±0.37
Fetuses weights (g)	3.20±0.05	3.12±0.09	4.91±0.15	5.00±0.05
Placenta weights (g)	0.53±0.01	0.52±0.02	0.52±0.02	0.53±0.01
Sex ratio (M:F)	1.13±0.14	1.81±0.68	1.36±0.31	1.26±0.28
Pre-implantational loss (%)[a]	0 (0–0)	0 (0–1.47)	0 (0–6.67)	0 (0–1.67)
Post-implantational loss (%)[a]	0 (0–10.83)	3.33 (0–8.69)	0 (0–7.14)	0 (0–7.28)

The numbers in parenthesis indicate the number of animals performing the behavior
Values are expressed as mean±S.E.M. Student's *t*-test
[a] Values are expressed as median (Q1–Q3). Manny-Whitney test

Electronic reprint for personal use

fertility, these effects do not persist at more advanced ages, as shown by the results at PND 270. Moreover, it is interesting to emphasize that although these animals present aging signs, their sperm and fertility parameters are comparable to those of PND 90 rats. If perinatal androgenization promotes similar effects in humans of reproductive age, the results of the present work can have an impact on male reproduction health, given the less efficient spermatogenesis and lower sperm reserves in the human epididymis, compared to rodents [49].

Acknowledgements

This work was supported by the State of Sao Paulo Research Foundation as part of a Doctoral scholarship (FAPESP, Grant 2009/00352-9). The authors are grateful to José Eduardo Bozano (CNPq, Grant 501339/2010-8) from the Department of Morphology, Bioscience Institute, UNESP, Botucatu/SP-Brazil, for excellent technical assistance.

Conflict of Interest

The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

References

1 *Jost A, Viger B, Prepin J, Perchellet JP.* Studies on sex differentiation in mammals. Recent Prog Horm Res 1973; 29: 1–4
2 *Quadros PS, Pfau JL, Goldstein AYN, De Vries GJ, Wagner CK.* Sex differences in progresterone receptor expression: a potential mechanism for estradiol-mediated sexual differentiation. Endocrinology 2002; 143: 3727–3739
3 *Weisz J, Ward IL.* Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 1980; 106: 306–316
4 *Corbier P, Kerdelhue B, Picon R, Roffi J.* Changes in testicular weight and serum gonadotropin and testosterone levels before, during, and after birth in the perinatal rat. Endocrinology 1978; 103: 1985–1991
5 *Bakker J.* Sexual differentiation of the neuroendocrine mechanisms regulating mate recognition in mammals. J Neuroendocrinol 2003; 15: 615–621
6 *Zadina JE, Dunlap JL, Gerall AA.* Modifications induced by neonatal steroids in reproductive organs and behavior of male rats. J Comp Physiol Psychol 1979; 93: 314–322
7 *Piacsek BE, Hostetter MW.* Neonatal androgenization in the male rat: evidence for central and peripheral defects. Biol Reprod 1984; 30: 344–351
8 *Gray LE Jr, Ostby JS, Kelce WR.* Developmental effects of an environmental antiandrogen: the fungicide vinclozolin alters sex differentiation of the male rat. Toxicol Appl Pharmacol 1994; 129: 46–52
9 *Radl V, Pritsch K, Munch JC, Schloter M.* Structural and functional diversity of microbial communities from a lake sediment contaminated with trenbolone, an endocrine-disrupting chemical. Environ Pollut 2005; 137: 345–353
10 *Swanson HE, Van der Werff ten Bosch JJ.* Sex differences in growth of rats and their modification by a single injection of testosterone propionate shortly after birth. J Endocrinol 1963; 26: 197–207
11 *Moguilevsky JA, Sacchi P, Rubinstein L.* Effect of neonatal androgenization on luteinizing hormone, follicle stimulating hormone, prolactin and testosterone levels in male rats. J Endocrinol 1977; 74: 143–144

12 *Morrison RL, Johnson DC.* The effect of androgenization in male rats castrated at birth. J Endocrinol 1966; 34: 117–123
13 *Wolf CJ, LeBlanc GA, Ostby JS, Gray LE Jr.* Characterization of the period of sensitivity of fetal male sexual development to vinclozolin. Toxicol Sci 2000; 55: 152–161
14 *Jones PA, Takai D.* The role of DNA methylation in mammalian epigenetics. Science 2001; 293: 1068–1070
15 *Wolf C Jr, Lambright C, Mann P, Price M, Cooper RL, Ostby J, Gray LE Jr.* Administration of potentially antiandrogenic pesticide (procymidone, linuron, iprodione, chlozolinate, p,p'-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethanesulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. Toxicol Ind Health 1999; 15: 94–118
16 *Lumia AR, Thorner KM, McGinnis MY.* Effects of Chronically high doses of the anabolic androgenic steroid, testosterone, on intermale aggression and sexual behavior in male rats. Physiol Behav 1994; 55: 331–335
17 *Wolf CJ, Hotchkiss A, Ostby JS, LeBlanc GA, Gray LE Jr.* Effects of prenatal testosterone propionate on the sexual development of male and female rats: a dose-response study. Toxicol Sci 2002; 65: 71–86
18 *Henley CL, Nunez AA, Clemens LG.* Exogenous androgen during development alters adult partner preference and mating behavior in gonadally intact rats. Horm Behav 2010; 57: 488–495
19 *Nassr AC, Arena AC, Toledo FC, Bissacot DZ, Fernandez CD, Spinardi-Barbisan AL, Pires PW, Kempinas WG.* Effects of gestational and lactational fenvalerate exposure on imune and reproductive systems of male rats. J Toxicol Environ Health A 2010; 73: 952–964
20 *Foley GL.* Overview of male reproductive pathology. Mechanisms of male reproductive organ toxicity. Toxicol Pathol 2001; 29: 49–63
21 *Perobelli JE, Martinez MF, Franchi CAS, Fernandez CDB, Camargo JLV, Kempinas WG.* Decreased sperm motility in rats orally exposed to single or mixed pesticides. J Toxicol Environ Health A 2010; 73: 991–1002
22 *Seed J, Chapi RE, Clegg ED, Dostal LA, Foote RE, Hurtt ME, Klinefelter GR, Makris SL, Perreault SD, Schrader S, Seyler D, Sprando R, Treinen KA, Veeramachaneni DN, Wise LD.* Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. Reprod Toxicol 1996; 10: 237–244
23 *Filler R.* Methods for evaluation of rats epididymal sperm morphology. In: Chapin RE, Heindel JH (eds.). Male reproductive toxicology. California: Academic Press, 1993; 334–343
24 *Fernandes GSA, Arena AC, Fernandez CDB, Mzercadante A, Barbisan LF, Kempinas WG.* Reproductive effects in male rats exposed to diuron. Reprod Toxicol 2007; 23: 106–112
25 *Ågmo A.* Male rat sexual behavior. Brain Res Protoc 1997; 1: 203–209
26 *Clegg ED, Perreault SD, Klinefelter GR.* Assessment of male reproductive toxicology. In: Hayes AW (ed.). Principles and methods of toxicology. Philadelphia: Taylor & Francis, 2001; 1263–1299
27 *Perobelli JE, Alves TR, Toledo FC, Fernandez CDB, Anselmo-Franci JA, Klinefelter GR, Kempinas WG.* Impairment on sperm quality and fertility of adult rats after antiandrogen exposure during prepuberty. Reprod Toxicol 2012; 33: 308–315
28 *Damasceno DC, Kempinas WG, Volpato GT, Consonni M, Rudge MVC, Paumgartten FJR.* Modelos experimentais – Laparotomia. In: Anomalias congênitas. Belo Horizonte: Coopmed, 2008; 13–21
29 *MacLusky NJ, Naftolin F.* Sexual differentiation of the central nervous system. Science 1981; 211: 1294–1303
30 *Hoepfner BA, Ward IL.* Prenatal and neonatal androgen exposure interact to affect sexual differentiation in female rats. Behav Neurosci 1988; 102: 61–65
31 *Sakuma Y.* Gonadal steroid action and brain sex differentiation in the rat. J Neuroendocrinol 2009; 21: 410–414
32 *Gray LE.* Chemical-induced alterations of sexual differentiation: a review of effects in humans and rodents. In: Colborn T, Clement C (eds.). Advances in modern environmental toxicology Vol XXI. Chemically-induced alterations in sexual and functional development: the wildlife/human connection. Princeton: Princeton Scientific, 1992; 203–230
33 *Gerardin DCC, Pereira OCM.* Reproductive changes in male rats treated with an aromatase inhibitor. Pharmacol Biochem Behav 2002; 71: 309–313
34 *Maqueo M, Kincl FS.* Testicular histomorphology of young rats treated with oestradiol 17β-benzoate. Acta Endocrinol 1964; 46: 25–30
35 *Rajfer J, Coffey DS.* Effect of neonatal steroids on male se tissues. Invest Urol 1979; 17: 3–8
36 *Cruz CD, Pereira OCM.* Prenatal testosterone supplementation alters puberty onset, aggressive behavior, and partner preference in adult male rats. J Physiol Sci 2012; 62: 123–131
37 *Norris DO.* The Endocrinology if mammalian reproduction. In: Norris DO (eds.). Vertebrate Endocrinology. San Diego: Academic Press, 1997; 357–406
38 *Witt DM, Young LJ, Crews D.* Progesterone and sexual behavior in males. Psychoneuroendocrinology 1994; 19: 553–562
39 *Kalra PS, Kalra SP.* Circadian periodicities of serum androgens, progesterone, gonadotropins and luteinizing hormone-releasing hormone in male rats: The effects of hypothalamic deafferentation, castration and adrenalectomy. Endocrinology 1977; 10: 1821–1827
40 *Rifka SM, Cutler GB Jr, Sauer MA, Louriaux DL.* Rat adrenal androgen receptor: a possible mediator of androgen-induced decrease in rat adrenal weight. Endocrinology 1978; 103: 1103–1110
41 *McCormick CM, Furey BF, Child M, Sawyer MJ, Donohue SM.* Neonatal sex hormones have 'organizational' effects on the hypothalamic-pituitary-adrenal axis of male rats. Dev Brain Res 1998; 105: 295–307
42 *Serre V, Robaire B.* Segment-specific morphological changes in aging brown Norway rat epididymis. Biol Reprod 1998; 58: 497–513
43 *Amann RP.* A Critical review of methods for evaluation of spermatogenesis from seminal characteristics. J Androl 1981; 2: 37–58
44 *Andersson AM, Jørgensen N, Main KM, Toppari J, Rajpert-DeMeyts E, Leffers H, Juul A, Jensen TK, Skakkebaek NE.* Adverse trends in male reproductive health: we may have reached a crucial 'tipping point'. Int J Androl 2008; 31: 74–80
45 *Orgebin-Crist MC.* Studies on the function of the epididymis. Biol Reprod 1969; 1: 155–175
46 *Kempinas WG, Suarez JD, Roberts NL, Strader L, Ferrell J, Goldman JM, Klinefelter GR.* Rat epididymal sperm quantity, quality, and transit after guanethidine-induced sympathectomy. Biol Reprod 1998; 59: 890–896
47 *Frick J, Chang CC, Kincl FA.* Testosterone plasma levels in adult male rats injected neonatally with estradiol benzoate or testosterone propionate. Steroids 1969; 13: 21–27
48 *Slob AK, den Hamer R, Woutersen PJA, van der Werff ten Bosch JJ.* Prenatal testosterone propionate and postnatal ovarian activity in the rat. Acta Endocrinol 1983; 103: 420–427
49 *Amann RP.* Detection of alterations in testicular and epididymal function in laboratory animals. Environ Health Perspect 1986; 70: 149–158

Electronic reprint for personal use