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Leptin effects on testis and epididymis in the lizard *Podarcis sicula*, during summer regression

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ABSTRACT

In this study we assessed the effect of leptin treatment on testicular morphology, spermatogenesis, Peroxisome Proliferator Activated Receptor (PPAR) α, 17β-hydroxysteroide dehydrogenase, 17β-estradiol and testosterone levels in the testis and blood of the lizard *Podarcis sicula* at the beginning of summer regression before entering the refractory period, when lizards no longer respond to hormonal and environmental stimuli. Lizards treated with five injections of leptin showed seminiferous tubules with germinal cells at all stages and wider lumina with respect to the controls. After 10 injections, the diameter of the lumina increased compared to the controls and 5 injection-group. After 10 injections plus 20 days before the sacrifice, the seminiferous tubules with open lumina and germinal cells were less abundant than in the 5 and 10 injection-groups. In all groups, the epididymis epithelium was higher than in the controls, with mitosis and binucleated cells. In both the control and treated animals secondary spermatocytes and spermatids were immunoreactive to leptin receptor and PPARα. In treated animals the interstitial cells and peritubular fibrocytes were also leptin receptor immunoreactive, while PPARα immunoreactivity translocated from the cytoplasm to the nucleus. 17β-HSD immunoreactivity was present in the spermatids and interstitial cells of control lizards and in secondary spermatocytes and spermatids of treated lizards. Leptin treatment had no statistically significant effect on testicular and circulating 17β-estradiol and testosterone levels. These observations indicate that leptin brings about a delay in testis summer regression in *Podarcis sicula*, playing a regulatory role in reproduction in this species as already hypothesized for mammals.

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1. Introduction

Leptin, the product of the Ob gene, is a 167 aminoacid secreted peptide, expressed mainly by adipose tissue and responsible for informing the brain about the status of the metabolic stores in adipose tissue (Zhang et al., 1994; Pelleymounter et al., 1995; Halaas et al., 1995). In the brain, leptin suppresses neuropeptide Y (NPY) expression and secretion in the arcuate nucleus with a consequent inhibition of food intake (Auwerx and Staels, 1998). Leptin travels through the blood stream both as a free protein and in a form that is bound to the soluble isoform of its receptor (Sinha, 1996). Leptin acts at the level of the central nervous system and on the peripheral tissues and organs as well, by binding to a membrane receptor (Ob-R) (Tartaglia et al., 1995). Ob-R localization in the hypothalamic-pituitary axis has led to the suggestion that leptin can control reproductive processes through hypothalamic gonadotropin-releasing hormone and hypophyseal gonadotropins (Yu et al., 1997; Zieba et al., 2005; Dridi et al., 2005a). Leptin may act directly on GnRH neurons or indirectly through interneurons to influence GnRH release. Moreover, the occurrence of Ob-R in the peripheral structures of the reproductive system suggests that leptin may have also a direct effect on the downstream endocrine targets of the reproductive axis such as anterior pituitary, ovary, testis, uterus, placenta, adrenal glands (Caprio et al., 2001; Brann et al, 2002; Cassy et al., 2004; Paczoska-Eliasiewicz et al., 2006).

Studies carried out on genetically obese (ob/ob) mice indicate that their reproductive function is impaired and that leptin is able to restore spermatogenesis and reproductive function (Bhat et al., 2006). However, the role of leptin is primarily inhibitory on androgen production in rats, while studies in primates and mice suggest leptin may not affect testicular steroidogenesis (Tena-Sempere et al., 1999; Lado-Abeal et al., 1999). Moreover, leptin shows a biphasic modulation of testosterone in immature sheep testis, being inhibitory at low concentrations but almost ineffective at both very low and high concentrations (Herrid et al., 2008). Thus, species differences may exist in terms of the specific role leptin plays within the testis.

Among reptiles, the fence lizard *Sceloporus undulatus* expresses a leptin-like protein, responds to injections of recombinant murine

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leptin (Niewiarowski et al., 2000) and shows leptin circulating levels consistent with the seasonal variations in free-ranging mammals (Spanovich et al., 2006). In *Podarcis sicula* leptin levels in the plasma, liver and fat bodies fluctuate during the reproductive cycle, in a way consistent with a possible role in reproduction (Paolucci et al., 2001).

Podarcis sicula at this latitude is a seasonal breeder which actively reproduces from March to June (Angelini and Botte, 1980). In the spring, males leave their winter shelters and their gonads and secondary sexual characteristics start developing. Courtship starts at the beginning of March–April and is followed by several weeks of breeding activity. Males show a discontinuous spermatogenetic cycle with two waves of sperm production: in fall and early spring, although only the latter is of physiological relevance. In early summer the testes start regressing and enter the so-called "summer regression". At the end of this period the tubules regress, their lumen disappears, germ cells degenerate except for spermatogonia, but the Sertoli cells are still intact. Leydig cells fill with lipid drops and soon shrink and become indistinguishable from intertubular fibroblasts (Varano et al., 1973).

The objectives of the current study were to investigate the potential actions of leptin on *P. sicula* testis and on testosterone and 17β-estradiol levels in the blood and testis. To reach these goals we employed adult males at the end of their breeding activity, when testes are about to enter "summer regression". This period of the reproductive cycle was chosen for the following reason. It is well known that at beginning of summer regression *P. sicula* circulating androgen concentration is low but not undetectable. However, their testis is potentially active (Andò et al., 1992) before entering into the refractory period, when testis and secondary sexual organs regress and no longer respond to hormonal and environmental stimuli. (Angelini and Botte, 1992). The testis of *P. sicula* in this period may be assimilated to that of *ob/ob* mice which show sterility defects that are reversed by leptin treatment (Mounzih et al., 1997). In this study lizards were treated with mouse recombinant leptin. Thereafter testis morphology, immunolocalization of leptin receptor (Ob-R), 17β-hydroxysteroid dehydrogenase (17β-HSD), and PPARα one of the peroxisome proliferator-activated receptors (PPARs). The 17β-HSD enzymes play essential roles in steroid hormone formation by catalyzing the final steps in androgen and estrogen biosynthesis (Labrie et al., 2000, 2003; Luu-The 2001; Mindnich et al., 2004). PPARs are a family of transcription factors activated by peroxisome proliferators, regulating the expression of genes (Michalik et al., 2006) and play essential roles in the regulation of cellular differentiation, development, and metabolism (carbohydrate, lipid, and protein) of higher organisms (Berger and Moller, 2002; Feige et al., 2006). Testosterone and 17β-estradiol levels in blood and testis were analyzed by RIA method.

2. Materials and methods

2.1. Animals

Eighty adult males of *P. sicula* were captured from the outskirts of Naples in June–July 2005–2006 during the "summer regression". Lizards weighed between 10 and 11 g. They were transferred into laboratory terraria with a photoperiod corresponding to the season and allowed to acclimatize for 10 days, before beginning the experiments. Meal worms and tap water were furnished *ad libitum*. Lizards appeared to be in perfect health since they were metabolically active and fed with appetite. We repeated the experiments over a period of 2 years with reproducible results.

Lizards were caught under permission of the Department of the Environment 1/06/2000 n.SCN/2D/2000/9213.

2.2. Experimental design

Leptin treatment was carried out by injecting peritoneally, between 9:00 and 10:00 a.m., mouse recombinant leptin (Alexis, Switzerland) (0.1 μg) in 0.1 ml of saline solution (NaCl 0.64%). Lizards were treated as follows:

Group 1: eight lizards injected with 0.1 ml of saline solution (saline group);
Group 2: eight lizards injected with five doses of leptin on alternate days, and sacrificed 24 h after the last injection (leptin-treated 5 inj);
Group 3: eight lizards injected with 10 doses of leptin and sacrificed 24 h after the last injection (leptin-treated10 inj);
Group 4: eight lizards injected with 10 doses of leptin and sacrificed 20 days after the last injection (leptin-treated 10 inj plus 20 days).

Each injection contained leptin concentration 250 times higher than the physiological concentration, based on the circulating leptin concentration in the same species (Paolucci et al., 2001). This experiment was repeated twice, (one experiment was performed in 2005 and one experiment was performed in 2006). A total of forty animals for each year were analysed. All animals were sacrificed during the first half of July. Blood was withdrawn through a heparinized glass capillary inserted into the heart. Blood samples were centrifuged at 800*g* for 15 min and the resulting plasma was stored at −80 °C for testosterone and 17β-estradiol analysis by RIA method. Testes and epididymes were removed and treated as reported in the following section.

2.3. Tissue sampling

For each lizard the testes and epididymes were removed, weighed and differently treated. One testis and epididymis were quickly fixed in Bouin's fluid for 3–5 h, dehydrated, embedded in Paraplast, and sectioned at 5 μm. Some sections were stained with hematoxylin-eosin and Mallory trichrome stain. The other testis was stored at −80 °C until 17β-estradiol and testosterone measurement by RIA method was taken.

2.4. Immunohistochemistry

Immunohistochemical PAP reactions were performed using the following primary antisera: rabbit anti-mouse (synthetic peptide derived from amino acids 577–594 of the extracellular domain of the leptin receptor) leptin receptor (Alexis, Switzerland, Europe) (1:1000); PPARα (Santa Cruz Biotechnology, CA, USA) (1:400), 17β-hydroxysteroid dehydrogenase (17β-HSD), isoform 1, (Santa Cruz Biotechnology) (1:300). DAB (3,3′-diaminobenzidine) was used as chromogens to produce brown precipitate. To test the specificity of the reagents, the following controls were performed (a) omission of the primary antiserum and incubation of the sections with swine non-immune serum or the bovine serum albumin (BSA; Sigma, St. Louis, MO, USA); (b) preabsorption of the primary antiserum with 10 or 20 nmol/ml of the corresponding peptide for 24 h at 4 °C. When corresponding peptides were used, the staining was abolished. The light microscopic observations were performed using a Zeiss Axioskop microscope equipped for bright field and fluorescence microscopy. Images were captured either on photographic film (Ilford Pan F) or using a TV camera attached to the microscope.

2.5. Hormone assays

Testosterone and 17β-estradiol were determined in plasma and testicular extracts by RIA, using commercial antibodies obtained

from Sigma. Testicular extracts were prepared according to Paolucci and Di Fiore (1992). Briefly, testes were weighed and homogenized with 1:2 (w/v) methanol (diluted 1:2 with distilled H_2O), vortexed and centrifuged. Testicular supernatants and plasma were extracted twice with seven ml of ethyl ether, evaporated under a nitrogen stream, dissolved in 1 ml RIA buffer (Sigma), and used for testosterone and 17β-estradiol determinations. The lowest sensitivity value was 15 pg/0.1 ml. Testosterone antibodies showed a cross-reactivity of 23% with 5α-dihydrotestosterone, 2.8% with 11β-hydroxytestosterone and 2% with 5β-dihydrotestosterone, the other cross-reactivity values were under 1.7%. The intra-assay coefficient of variation was 9.5% and the inter-assay coefficient of variation was 12.5%. 17β-estradiol antibodies showed a cross-reactivity of less than 10% with estrone and less than 5% with 17α-estradiol and estriol. The intra-assay coefficient of variation was 8.5% and the inter-assay coefficient of variation was 10.5%. The number of analyzed animals for each experimental group (n) is reported in the legends. The experiments were carried out in 2005 and 2006. All experimental groups were analyzed.

2.6. Statistics

Numerical data were analyzed by one-way ANOVA method, followed by Duncan's multiple range test. Values were expressed as means ± SD. Statistics was used only for the analysis of the RIA data.

3. Results

3.1. Leptin effects on spermatogenesis and epididymis morphology

The histological morphology of the testis and epididymis of saline group lizards was typical of free-living lizards during the summer regression (data not shown). In the saline group the majority of the seminiferous tubules had extremely narrow or no lumina. Primary spermatocytes and secondary spermatocytes were present. Few round and rare elongated spermatids could be detected. In the narrow lumina round masses containing elongated spermatids/spermatozoa were present. Few Leydig cells, often indistinguishable from peritubular fibrocytes were present. The epididymis appeared regressed and devoid of spermatozoa and secretion, the epithelium was flat (Fig. 1(1 and 2)).

In the leptin-treated 5 inj group the majority of seminiferous tubules had lumina, the seminiferous epithelium showed germinal cells at all stages, including spermatozoa attached to the tubular wall and some free within lumina. The epididymis epithelium was higher than the control, with evident signs of secretory activity and with binucleated cells. Spermatozoa and the content of secretory granules were present in the lumina of the ducts (Fig. 1(3 and 4)).

In leptin-treated 10 inj group seminiferous tubules had the diameter of the lumina increased with respect to the saline and leptin-treated 5 inj group. The seminiferous epithelium showed germinal cells at all stages. In particular some spermatogonial mitosis and meiotic metaphases could be detected. Spermatozoa were more abundant with respect to the leptin-treated 5 inj group. They were both free in the lumen and binding to the tubular wall. The epididymis epithelium was higher than the control, but similar to the leptin-treated 5 inj group with mitosis and binucleated cells (Fig. 1(5 and 6)).

In leptin-treated lizards 10 inj plus 20 days the seminiferous tubules with open lumina and seminiferous epithelium with germinal cells at all stages, including spermatozoa were present although less abundant than in the leptin-treated 5 inj and 10 inj groups. Moreover, the lumina diameter was narrower than in the leptin-treated 10 inj group. The epididymis epithelium was higher than the control with signs of secretory activity and binucleated cells as in the leptin-treated groups. Some spermatozoa were present in the lumen of the ducts (Fig. 1(7 and 8)).

3.2. Leptin effects on plasma and testicular testosterone levels

Testosterone and 17β-estradiol concentrations in the blood and testis of *P. sicula* are reported in Table 1. Leptin treatment did not have any statistically significant effect, neither on blood nor on testicular sex steroids.

3.3. Immunolocalization

All leptin-treated groups were analyzed and gave similar results therefore in the following paragraphs we will refer to them as "leptin-treated groups".

3.4. Leptin receptor

In the saline group leptin receptor immunostaining was detected in secondary spermatocytes and elongated spermatids/spermatozoa. In leptin-treated groups secondary spermatocytes, spermatids/spermatozoa, rare spermatogonia, few Leydig cells and peritubular fibrocytes were immunostained (Fig. 2(9–11)).

3.5. PPARα

In the saline group PPARα immunostaining was detected in the cytoplasm of secondary spermatocytes and spermatids. In leptin-treated groups, PPARα immunostaining was evident in the nuclear compartment of secondary spermatocytes and spermatids (Fig. 3(12–15)).

3.6. 17β-HSD

In the saline group immunoreactivity was detected in the cytoplasm of spermatids and interstitial cells. In leptin-treated groups, 17β-HSD immunoreactivity was present mainly in the cytoplasm of secondary spermatocytes, round and elongated spermatids and to a lesser extent in interstitial cells (Fig. 3(16 and 17)). PPARα and 17β-HSD immunoreactivity in leptin-treated animals partially overlapped (Fig. 3(18 and 19)).

4. Discussion

The aim of this paper was to evaluate the effect(s) of leptin administration on testicular and circulating sex steroids and testicular and epididymis function in the lizard *P. sicula* at the beginning of summer regression. In this phase of the reproductive cycle circulating androgen concentration is low but not undetectable. However, the testis is still potentially active (Andò et al., 1992, present data), before entering into the refractory period when testis and secondary sexual organs regress and no longer respond to hormonal and environmental stimuli. (Angelini and Botte, 1992). In this period the testis of *P. sicula* may be assimilated to that of *ob*/*ob* mice which show sterility defects but are reported to occasionally reproduce. Indeed, several works have demonstrated that leptin treatment treats the sterility of these genetically obese ob/ob males (Mounzih et al., 1997).

The present study shows evidence, for the first time, of leptin involvement in testicular activity in a lower vertebrate. Leptin receptor immunoreactivity has been detected in both the germ cells and the interstitial cells of *P. sicula* testis. In other species, transcripts encoding leptin receptor isoforms are generated by

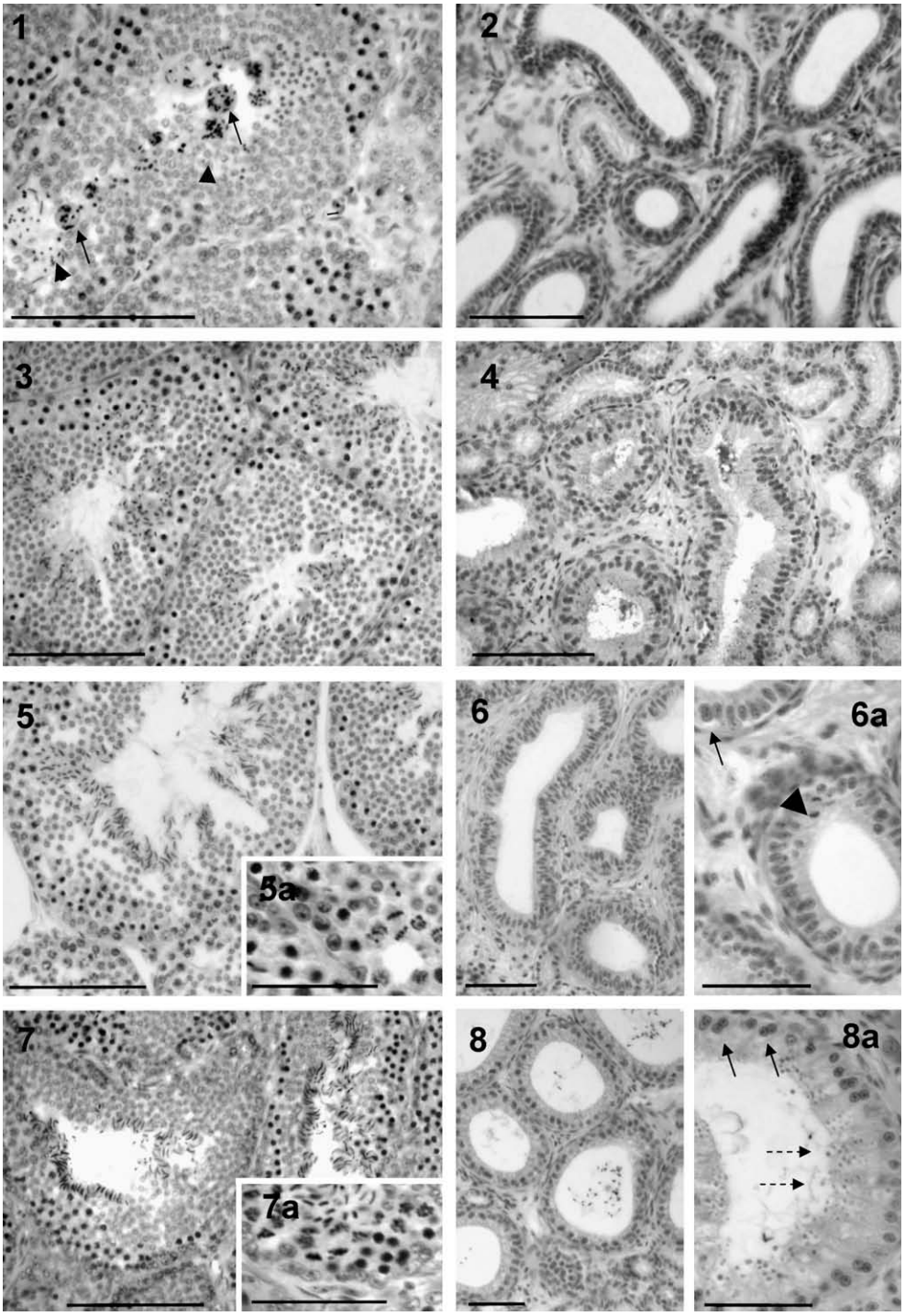

Fig. 1. (1 and 2) Morphology of the testis (1) and epididymis (2) of a saline control lizard. Tubules show greatly reduced or no lumen. The seminiferous epithelium shows germinal cells at all stages, although spermatids and spermatozoa are scarce. Note round masses containing elongated spermatids/spermatozoa. The epididymis appears regressed and devoid of spermatozoa and secretion. Black arrows indicate round masses, arrowheads secondary spermatocytes and spermatozoa. Hematoxylin/eosin stain. Bars 100 μm. (3 and 4) Morphology of the testis (3) and epididymis (4) of a lizard treated with 5 injections of leptin. Note the enlarged lumen of tubules and the seminiferous epithelium with secondary spermatocytes and spermatids, numerous spermatozoa either bound to tubular wall or released into the lumen ((3), bar 100 μm). An increase in the height of the epididymal channel cells is evident ((4), bar 50 μm). (5 and 6) Morphology of the testis (5) and epididymis (6) of a lizard treated with 10 injections of leptin. Many tubules show evident lumina and seminiferous epithelium with germinal cells at all stages. Spermatozoa are abundant and are either bound to the tubular wall or free in the lumen. In the insert some meiotic figures ((5) and (5a), bar 100 and 25 μm, respectively). An increase in the height of the cells of the epididymal channel is evident. The epididymal channel cells are frequently binucleated (arrows) and show signs of secretory activity (arrowhead (6) and (6a) bars 50 μm). (7 and 8) Morphology of the testis (7) and epididymis (8) of a lizard treated with 10 injections of leptin and sacrificed 20 days after the last injection. Tubules show lumina and seminiferous epithelium with germinal cells at all stages, including numerous spermatozoa. The epididymis shows tall cells, binucleated (arrow) with evident secretory activity (dashed arrows (8) and (8a) bars 100 μm).

alternative splicing and result in the expression of receptors with short or long intracellular domains (Tartaglia, 1997; Tartaglia et al., 1995). To date, six isoforms have been identified in mammals (Lollmann et al., 1997), the long forms believed to be responsible

Table 1
Changes in testosterone and 17β-estradiol in blood and testis of the lizard *Podarcis sicula* treated with saline solution (control) and recombinant mouse leptin. Values are expressed as means ± SD (n = 6 for each group).

	Saline	Leptin (5 inj)	Leptin (10 inj)	Leptin (10 inj plus 20 days before sacrifice)
Testosterone (blood) (ng/ml)	3.2 ± 0.5	4.1 ± 0.6	5.3 ± 0.8	3.4 ± 0.5
17β-Estradiol (blood) (ng/ml)	1.1 ± 0.5	1.0 ± 0.4	1.2 ± 0.5	1.1 ± 0.6
Testosterone (testis) (ng/0.1 g tissue)	6.4 ± 0.7	4.1 ± 0.6	6.7 ± 0.8	6.8 ± 0.8
17β-Estradiol (testis) (ng/0.1 g tissue)	3.3 ± 0.5	3.3 ± 0.5	3.3 ± 0.4	3.3 ± 0.5

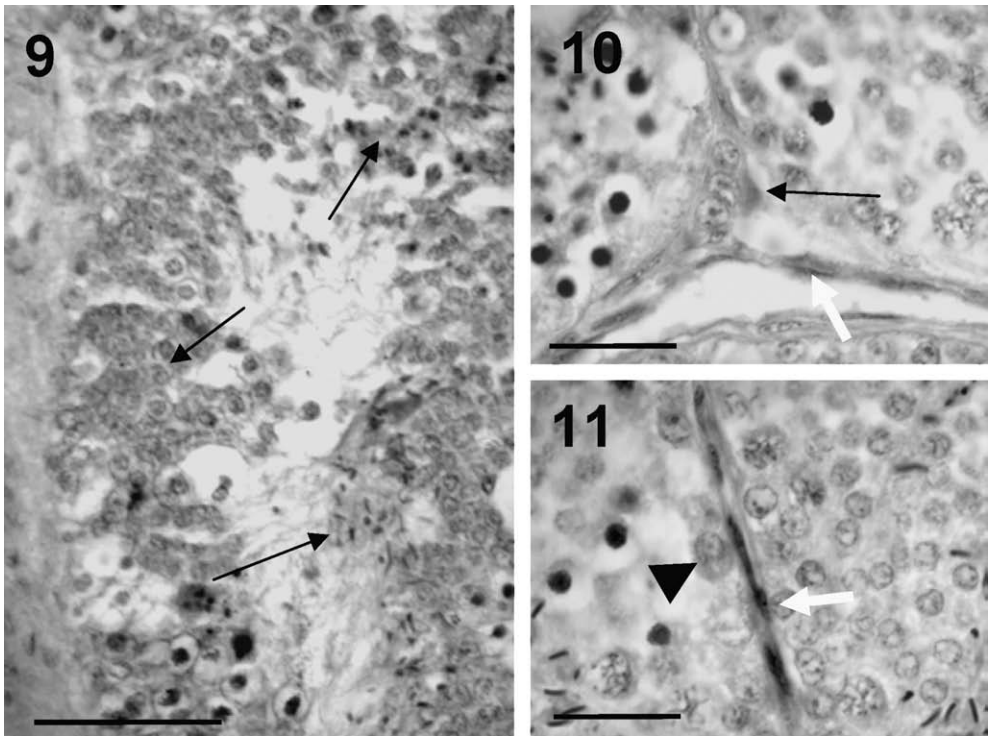

Fig. 2. (9) Cross section of the testis of a saline control lizard immunostained for leptin receptor. Note that germinal cells at the last stage are immunoreactive. The arrows point to secondary spermatocytes and spermatids/spermatozoa. Bar 50 μm. (10 and 11) Cross section of the testis of a leptin-treated lizard immunostained for leptin receptor. Leptin receptor immunoreactivity is localized in the interstitium, but only some cells are immunolabeled, Leydig cells (black arrows) and peritubular fibrocytes (white arrow). Rare leptin receptor immunoreactive spermatogonia were present (arrowhead). Bars 25 μm.

for most of the biological effects of leptin (Bjorbaek et al., 1997). The leptin receptor antibody employed here was a polyclonal antibody against the mammalian leptin receptor long form, therefore, it seems that also in *P. sicula*, leptin may be able to exert its biological action in the testis. The presence of leptin receptor immunoreactivity in both germ cells and interstitial cells in *P. sicula*, is in agreement with a direct leptin action on testicular function, through its specific receptor, widely demonstrated in mammals (Spicer, 2001; Blüher and Mantzoros, 2007; for reviews), although Caprio et al. (2003) failed to detect leptin receptor immunoreactivity within the tubules of rat testis in any developmental stage and leptin immunoreactivity was exclusively confined to Leydig cells. However, leptin receptor immunoreactivity within germ cells is stage-specific in mouse testis (El-Hefnaway et al., 2000). In *P. sicula* leptin receptor immunoreactivity is present in specific stages of germ cells sustaining that the pattern of leptin receptor in the testis is species specific.

Although not statistically significant, we detected small changes in sex steroid concentration. The measured values in this study do not differ from those reported by Andò et al. (1992) in *P. sicula*, but are lower than the average values measured in the collared lizard *Crotaphytus collaris* and in the horned lizards, *Phrynosoma cornutum* (Baird and Hews, 2007; Wack et al., 2008). Studies carried out in mammals indicate that species differences may exist in terms of the specific role leptin plays within the testis. Leptin inhibits testosterone secretion from rat testis (Tena-Sempere et al., 1999), has a direct inhibitory effect on hCG-induced testosterone production in adult rat Leydig cells but has no effect on basal androgen release (Caprio et al., 1999). In contrast, leptin infusion has no effects on the circulating levels of testosterone in male rhesus monkeys (Lado-Abeal et al., 1999), or Siberian hamsters (Atcha et al., 2000). Moreover, leptin ability to suppress testosterone secretion directly at testicular level is restricted to the adult period in rat testis providing additional evidence that leptin effect on testicular function is developmentally regulated (Tena-Sempere et al., 2001).

Testosterone is required to maintain or restore spermatogenesis. In adult rats, spermatogenesis can be quantitatively maintained or restored at testosterone concentrations far lower than those normally present within the testis but greater than that present in serum (Zirkin, 1999). In the testis of leptin-treated *P. sicula* seminiferous tubules had open lumina with respect to the controls and germ cells at all stages. Epididymis epithelium was thicker than the control, with signs of secretory activity and spermatozoa in the lumen of the ducts. This is a description in agreement with a picture of delayed regression, showing signs of spermatogenesis

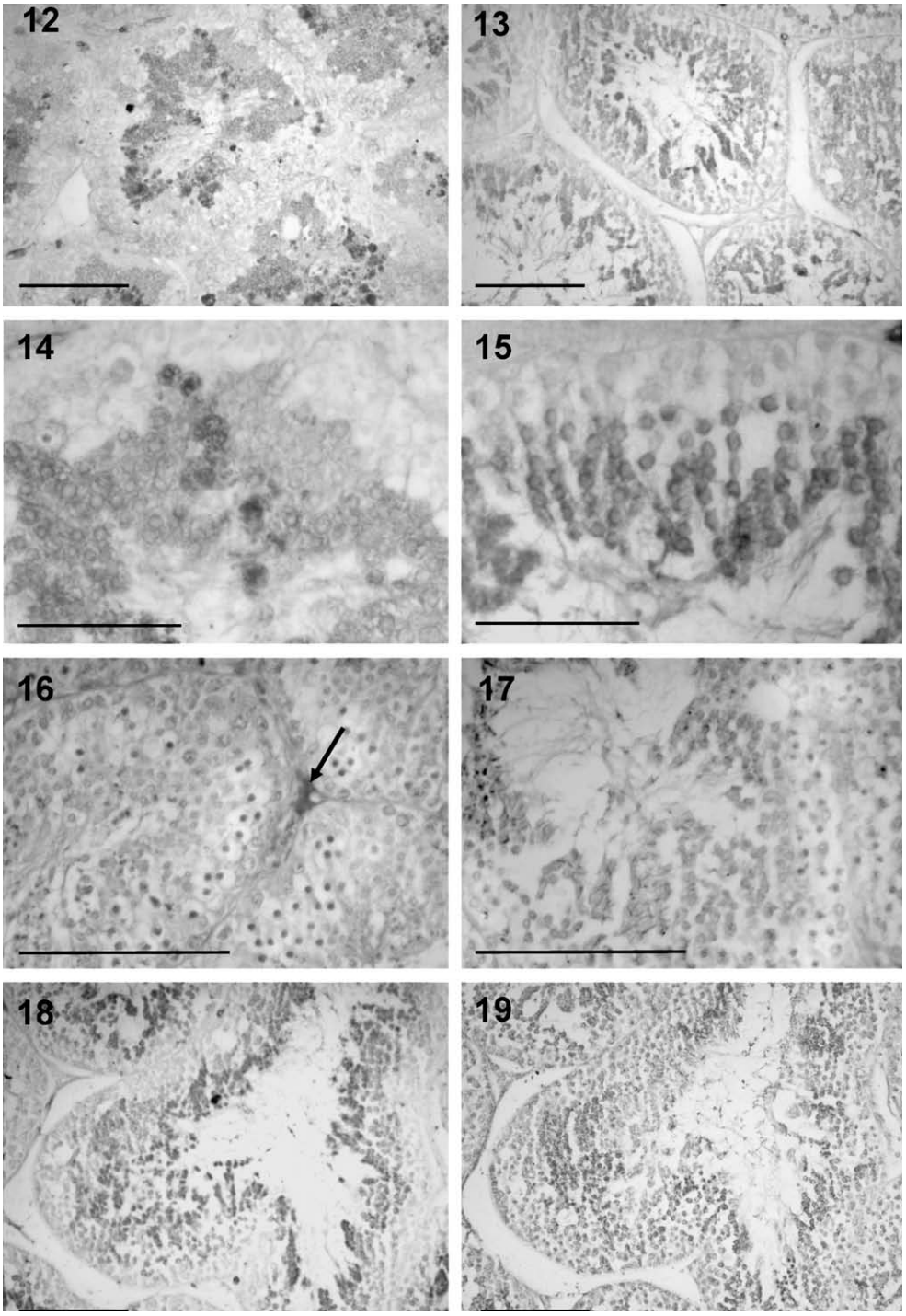

Fig. 3. (12 and 14) Cross sections of the testis of control lizard immunostained for PPARα. Note that the immunoreactivity is localized in the last stage of germinal cells, mainly secondary spermatocytes, spermatids and when present, spermatozoa (12). (14) An higher magnification of (12) showing PPARα immunoreactivity into the cytoplasm and perinuclear area. Bars 100 and 50 μm, respectively. (13 and 15) Cross section of the testis of leptin-treated lizard immunostained for PPARα. Immunoreactivity is present in the last stages of germinal cells (13). (15) An higher magnification of (13) showing PPARα immunoreactivity localized into the nuclei of the immunoreactive cells. Bars 100 and 50 μm, respectively. (16) Cross sections of the testis of a saline control lizard immunostained for 17β-HSD. Immunoreactivity is present in the interstitium and in few spermatids. Bar 100 μm. (17) Cross sections of the testis of leptin-treated lizard immunostained for 17β-HSD. Immunoreactivity is localized mainly in secondary spermatocytes and spermatids. Bar 100 μm. (18 and 19) Two serial sections of leptin-treated lizard immunostained for PPARα and 17β-HSD to show the partially overlapping immunoreactive cells. Bars 100 μm.

restoration in spite of any increase in testosterone. Such a discrepancy might be explained in light of the consideration that the increase in sex steroid levels, if any, could have occurred in the first days after leptin treatment and it was no longer detectable at the end of the treatment. On the other hand, *in vitro* leptin treatment of rat testis (Tena-Sempere et al., 1999) and *P. sicula* testis (data not shown) reveals that leptin effects on testosterone levels were observed within 3 h.

In leptin-treated *P. sicula*, PPARα immunoreactivity shifted from the cytoplasm to the nucleus of secondary spermatocytes and sper-

matids. In the mouse myoblastoma cell line, leptin upregulates PPARα gene expression by rapid translocation to the nucleus (Suzuki et al., 2007). PPARs are a family of nuclear hormone receptors belonging to the steroid receptor superfamily (Issemann and Green, 1990). PPARs have been identified in a variety of species from chickens (Meng et al., 2005) and fish (Ibabe et al., 2005), to humans (Desvergne and Wahli, 1999; Escher and Wahli, 2000). Although a great deal has been learned about PPARs since their discovery, very little is known regarding how these factors impact gonadal function. All three PPAR family members – alpha, delta, and gamma, are expressed in the ovary (Komar, 2005). PPARα in mammalian testis seems to regulate both tubule and interstitial cells differentiation (Schultz et al., 1999; Froment et al., 2006) and are expressed in both somatic and germ cells (Bhattacharya et al., 2005). Moreover, it seems that the presence of these nuclear receptors is required for normal steroid formation (Ward et al., 1998). Bendinelli et al. (2005) report PPARα activation after *in vitro* leptin treatment of muscle cells. Once activated, PPARα enters the nuclei where it turns target genes on and off. Genes responsible for lipid metabolism and therefore steroidogenesis are among them (Staels et al., 1998).

In rat and mouse liver and intestine PPARα ligands induce the expression of 17β-HSD IV, through a PPARα-dependent mechanism (Corton et al., 1997; Fan et al., 2005), and 17β-HSD XI (Motojima, 2004). The 17β-hydroxysteroid dehydrogenase enzymes play essential roles in steroid hormone formation by catalyzing the final steps in androgen and estrogen biosynthesis (Labrie et al., 2000; Mindnich et al., 2004). The antibody employed here was against 17β-HSD type I in mammals. The 17β-HSD type I is found in the ovary, placenta and mammary gland where it catalyzes the conversion of estrone to 17β-estradiol also in male germ cells (Pelletier et al., 2004). It also catalyzes the conversion of androstenedione to testosterone *in vitro* (Nokelainen et al., 1996). We do not know which type of 17β-HSD is expressed in reptiles and whether or not there are homologies with the 17β-HSD isoforms present in mammals. Thus we employed antibodies against the 17β-HSD isoform with a wide range of actions. In the ovary of *P. sicula* hydroxysteroid dehydrogenase activity has been demonstrated, among which a 17β-hydroxysteroid dehydrogenase with testosterone and 17β-estradiol as substrates (Rastogi et al., 1976). In leptin-treated *P. sicula*, 17β-HSD immunoreactivity was found in secondary spermatocytes and spermatids where it partially overlapped with PPARα immunoreactivity. In mouse testis 17β-HSD type I is expressed in germ cells, but not in Leydig cells, suggesting that the enzyme may contribute to the local production of steroids where it may exert either autocrine or paracrine activity in the seminiferous tubules (Pelletier et al., 2004). This would explain why the observed leptin-induced changes in 17β-HSD localization in *P. sicula* may not be associated with steroid accumulation and release from the testis. No significant increase in testicular 17β-estradiol was found in *P. sicula*, suggesting that, also in the case of testosterone, the 17β-estradiol action, if any, is limited to the cells involved in its formation.

Regarding the differences observed between the 5 and 10 injection treated groups in the morphology of the testis, they seemed to be dose-dependent with higher doses corresponding to more testicular activity. We can hypothesize that leptin administered on alternate days (present study) did not downregulate its receptor as it has been shown to occur in birds undergone to continuous infusion of leptin (Dridi et al., 2005b).

One possibility that cannot be overlooked is that the effects of leptin on reproductive function in *P. sicula* were not only direct at testicular level but also indirect, probably through the hypothalamus-pituitary axis. Leptin has a very potent effect on the anterior pituitary in vitro. It stimulates FSH and LH release in male and female rats, although the leptin dose needed to stimulate FSH release was two orders of magnitude higher than the one required to stimulate LH (Yu et al., 1997). In mammals PPARα expression was increased in cultured seminiferous tubules after FSH treatment during all stages of the cycle (Schultz et al., 1999). These results indicate that PPARα expression is controlled in part by FSH, and that PPARα carries out a specific functional role during different stages of the differentiation cycle (Corton and Lapinskas, 2005). Although in *P. sicula* testosterone level did not increase after leptin treatment, the recovery of spermatogenesis could be the result of the interaction between FSH with the "baseline" testosterone concentration. Normally, FSH stimulates testosterone production, but not in this case, where the *P. sicula* Leydig cells are likely to be exhausted at the end of the breeding period and do not respond to further stimulation, a possibility also sustained by their morphological appearance (present data; Varano et al., 1973). In the wall lizard, *Hemidactilus flaviviridis* FSH treatment, but not testosterone treatment, which resulted in the inhibition of spermatogenesis, induced spermatogenesis activity and epididymis hypertrophy (Rai and Haider, 1995).

In conclusion, using the lizard model, it has been shown that in a lower vertebrate species leptin acts on testicular function and may play both a direct and indirect regulatory role in reproduction also in this species as already proposed in mammals. After leptin treatment of lizards at the end of the breeding period, testis morphology indicated that leptin may bring about a delay in testis regression in *P. sicula*. The presence of PPARα and 17β-HSD immunoreactivity in germ cells after leptin treatment lets us suppose that leptin may act through a multi-signalling pathway in this species also.

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