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Plasma hormones in neotropical and domestic cats undergoing routine manipulations

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Abstract

Many neotropical felids are threatened with extinction and information on their physiology is required to assist in conservation. Their reproduction in captivity is poor, particularly for the smaller species. Several factors may be responsible, but stress is probably the most important. We assayed cortisol, LH, FSH, prolactin, testosterone, estradiol, and progesterone in single blood samples obtained under sedation from seven neotropical species and, for comparison, in stressed and unstressed domestic cats. Cortisol was also assayed in serial blood samples obtained after ACTH administration in *Leopardus tigrinus*, *L. wiedi* and domestic cats. While, in general, the results were fairly consistent, there were some statistically significant differences between species that were large enough to be of practical importance.

Keywords: Neotropical cats; Domestic cat; Hormones; Reproduction; Stress

1. Introduction

Most research on reproduction and stress in felids has been focused on the domestic cat (*Felis catus*) or large species such as lions (*Panthera leo*), tigers (*Panthera tigris*), leopards (*Panthera pardus*), and cheetahs (*Acinonyx jubatus*) (Brown et al., 1988; Wildt et al., 1988; Mellen, 1992; Lawler et al., 1993; Carlstead et al., 1993b; Brown and Wildt, 1997; Genaro et al., 2003). Endocrine studies on small neotropical cats are rare, even though they are threatened with extinction (Jackson, 1997). Further work is therefore required for conservation purposes (Brown et al., 2001; Swanson and Brown, 2004).

The reproductive success of Brazilian felids in captivity is poor (Brown and Wildt, 1997; Morais et al., 1997; Swanson and Wildt, 1997), especially for the so-called ''small felids'' such as the ocelot (*Leopardus pardalis*), margay (*L. wiedii*), Geoffroy's cat (*Oncifelis geoffroyi*), oncilla (*L. tigrinus*), jaguarondi (*Herpailurus yaguarondi*), and pampas cat (*O. colocolo*) (Genaro et al., 2001). This is probably caused by the constant stress of captivity (Swanson and Brown, 2004) including the proximity of larger predators, or inadequate housing, food, social conditioning or handling (Mellen, 1991; Carlstead et al., 1993a,b; Poweell, 1997; Mellen and Shepherdson, 1997). These conditions are reflected in the concentrations of the stress axis (pituitary-adrenal axis – HPA) hormones, especially cortisol, the most important glucocorticoid found in felids (Feldman, 1983). Cortisol influences metabolism, behaviour, and the immune system (Feldman, 1983; McCann et al., 2000) and interferes with gonadal function, inhibiting reproduction (Welsh and Johnson, 1981; Moberg, 1991; Fenske, 1997).

Steroid hormone secretion has been monitored in carnivores by non-invasive methods using saliva (Kobelt et al., 2003) or feces: >85% of steroid hormones appear as metabolites in feces from 12 to 24 h after secretion (Brown et al.,

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2001; for a review, see Brown and Wildt, 1997). However, some interference with animals, including restraint, is unavoidable in zoos and breeding colonies and it is important to understand the short-term effects of this on hormone concentrations. Only blood levels will suffice for this purpose because of the delay inherent in the non-invasive techniques mentioned above. Further, some knowledge of plasma hormone concentrations improves the interpretation of data obtained by non-invasive methods.

The aims of this study were three-fold: first, to compare the concentrations of pituitary and gonadal reproductive hormones in captive neotropical felids and the domestic cat (a possible experimental model for the family or, at least, for some of its species); second, to determine the normal cortisol levels of various neotropical cats during routine handling; and third, to examine the cortisol response of some of these species to ACTH stimulation, or stress, and compare it with that of the domestic cat.

2. Materials and methods

2.1. Animals

For Experiments 1 and 2 (pituitary and gonadal hormones, respectively), 22 male and 44 female randomly-bred domestic cats were kindly provided by non-governmental organizations (A.V.A., A.D.A.R.P., and GRU.A.BI., see Acknowledgments). They were kept under natural light and had free access to commercial dry feed and water. The following wild felids were studied (the number of males and females is given respectively in parentheses): oncilla (17 and 14), ocelots (12 and 5), margays (6 and 3), Geoffroy's cats (6 and 2), jaguarondis (12 and 10), jaguars (14 and 8), and pumas (12 and 7). All the animals were kept under similar conditions under natural light (the samples were taken between December and March) and were given raw meat, occasionally supplemented with vitamins, a commercial ration when available and water *ad libitum*. They were maintained in zoos or parks (see Acknowledgments) in groups of differing sizes. All were adults, and none of the females were pregnant or lactating, or had young them at the time of the study.

Experiment 3, like Experiments 1 and 2, involved both domestic cats and wild neotropical cats. These included 41 male and 42 female randomly-bred domestic cats. During the experiment they were housed indoors in individual 1 m^3 stainless-steel cages and had free access to commercial dry feed and water. The males and females were treated as separate experimental groups and these were further subdivided giving a total of four groups as follows: 24 males and 24 females were allowed to become habituated to the laboratory housing for 36 h before anaesthesia (habituated groups) and 17 males and 18 females were only introduced to the laboratory housing about 4 h before anaesthesia (non-habituated groups). The numbers of male and female neotropical cats used in the experiment were respectively: oncillas (17 and 14), ocelots (12 and 5), margays (6 and 3), Geoffroy's cats (6 and 2), jaguarondis (12 and 10), jaguars (*Panthera onca*) (14 and 8) and pumas (*Puma concolor*) (12 and 7). Blood samples were obtained from the cephalic vein immediately after the onset of anaesthesia and 30 min later.

In Experiment 4 the response to ACTH was measured in adult male domestic cats (4 experimental and 4 control), margays (2 experimental and 3 control) and oncillas (4 experimental and 3 control).

2.2. Anaesthesia

After a 12 h fast, both the domestic and wild cats were anesthetized with a combination of ketamine HCl (20 mg/kg, im, Francotar, Virbac do Brasil Ind. Com, Ltda, São Paulo, SP) and xylazine (1 mg/kg, im, Coopazine, Coopers Brasil, Ltda, Cotia, SP), and the anesthesia was augmented over time without agony to the animals. An attempt was made to minimize disturbance of the animal before injections. The anesthetic was successfully administered within a period of 2 min to both domestic and wild species. The small felids were captured with a conical net and then injected with the anesthetic; the large felids were anesthetized with darts.

2.3. Blood samples

The animals were anaesthetized between 09:00 AM and 11:00 AM. Blood samples were always taken immediately after the onset of anesthesia, heparinized and centrifuged at 2000*g* for 15 min at 4 °C within 60 min of collection. Plasma was separated and frozen at −20 °C until the time of radioimmunoassay.

Owing to the small amount of blood available it was not always possible to assay all six hormones in all samples: only in the domestic cat, jaguar and puma was it possible to compare the concentrations of all the hormones.

2.4. ACTH response

ACTH (10 μg/kg/100 μl, Cortrosina – Tetracosactida Akzo Nobel Ltda – Organon Division), or the same volume of 0.9% saline solution, was given into the right cephalic vein. Blood (0.5 ml) was collected immediately before and 5, 10, 20, 40, 60, 90 and 120 min after ACTH or saline administration.

2.5. Radioimmunoassay (RIA)

Plasma LH, FSH and Prl were determined by RIA using ovine kits provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK/NIH-USA). All results were expressed in terms of the NIH-RP_2 reference standards. The lower limits of detection were 0.08 ng/ml for LH, 0.2 ng/ml for FSH and 0.2 ng/ml for Prl. The intra-assay coefficients of variation were 5.5%, 4% and 4.5% for LH, FSH and Prl, respectively.

Testosterone was determined by RIA using ^{3}H-testosterone from NEN Life Science Products (Boston, MS, USA). The specific antibody was a generous gift from Dr. José Antunes-Rodrigues (Universidade de S. Paulo, Ribeirão Preto, Brazil). The lower limit of detection was 1.6 ng/ml. The intra-assay coefficient of variation was 5.4%. Plasma estradiol and progesterone were determined by an antibody RIA using specific kits provided by DPC-Diagnostic Products Corporation (Los Angeles, CA – USA). The lower limits of detection were 1.5 pg/ml and 0.2 ng/ml for estradiol and progesterone, respectively. The intra-assay coefficient of variation was 3% and 4.2% for both estradiol and progesterone. Plasma cortisol was determined by a double antibody RIA using specific kits provided by DPC – Diagnostic Products Corporation (Los Angeles, CA – USA). The lower limits of detection were 12.5 ng/ml. The intra-assay coefficient of variation was 2.7%. All samples for each hormone were analyzed in the same RIA so interassay variation is not an issue.

2.6. Statistical analysis

Plasma hormones values are expressed as the mean (±SEM) for each group (species, sex or treatment) and groups were compared by one-way ANOVA followed by the Tukey test when applicable, using Prism 2.01, (Graphpad Software, Inc, San Diego, California, USA) with the level of significance set at (*) $p < 0.05$ and (**) $p < 0.01$.

3. Results

3.1. Experiment 1: pituitary hormones

Plasma prolactin concentrations were similar in both sexes and in all the species studied (Fig. 1). FSH concentrations were more variable (Fig. 1), but no statistically significant differences could be demonstrated either between sexes or between species. No significant differences were demonstrable when the prolactin and FSH data were grouped according to genus in the wild species.

There were no significant differences in plasma LH concentrations, either between the sexes or between the six wild species studied (Fig. 1). However, LH concentrations were much higher ($p < 0.01$) in male Geoffroy's cats than male domestic cats.

3.2. Experiment 2: gonadal steroids

Plasma progesterone concentrations were determined in female domestic cats pumas, jaguarondis and jaguars. The only significant difference was between the domestic cat and the puma ($p < 0.01$) which was much higher (3.5 ng/ml; Fig. 2).

Plasma estradiol concentrations (Fig. 2) were similar in the wild species studied, except for the ocelot which had significantly higher ($p < 0.05$) values. The six wild species with similar plasma estradiol did not differ significantly from domestic cats.

Plasma testosterone concentrations (Fig. 2) were similar in the puma, ocelot, oncilla and domestic cat but significantly higher in the jaguar ($p < 0.01$) than in the other species.

3.3. Experiment 3: cortisol

In domestic cats (Fig. 3) mean plasma cortisol concentrations were very similar immediately after the onset of anaesthesia in habituated males, habituated females and non-habituated females. On the other hand, plasma cortisol was significantly ($p > 0.01$) higher in non-habituated than in habituated males.

Plasma cortisol was lower at 30 min than at time zero (immediately after capture and/or anesthesia) in both males and females of all species (Fig. 3). There were no difference between the sexes in the plasma cortisol concentrations of the wild felids. There were significant differences ($p > 0.05$) when species were compared, with pumas and jaguarondis having the highest levels.

3.4. Experiment 4: ACTH response

In the ACTH stimulation experiment, domestic cats had a lower basal plasma cortisol concentration at Time 0 than the two wild species studied (Fig. 3). This difference disappeared with time in the control groups, all species having similar low values after 20–40 min. Plasma cortisol increased significantly ($p > 0.01$) in response to ACTH in all the species studied. However, the response seemed to be slower in margays (40 min) than in domestic cats (5 min) or oncillas (5 min). Cortisol concentrations began to fall slightly between 90 and 120 min in domestic cats and oncillas but remained high at the end of the experiment in the margays.

4. Discussion and conclusions

Information on the endocrinology of domestic cats and the large wild felids is currently used as a model for other wild felids (Brown et al., 2001). Although this approach is useful, there is evidence of potentially important differences between species in the cat family including the poorly studied small species (Nogueira and Silva, 1997). Such small cats (<20 kg) represent about 80% of the 37 felid species in the world (Swanson and Wildt, 1997).

Among the gonadotrophins, LH has been more studied than FSH in felids (Genaro et al., 2003). We found no significant differences in FSH values between males and females (Fig. 1). It is important to record these values because FSH has been little studied in wild felids. Brown et al. (1988) pointed out that the basal FSH concentrations of leopards (*Panthera pardus japonensis*) and tigers (*P. tigris*) are not affected by sex or species, a result

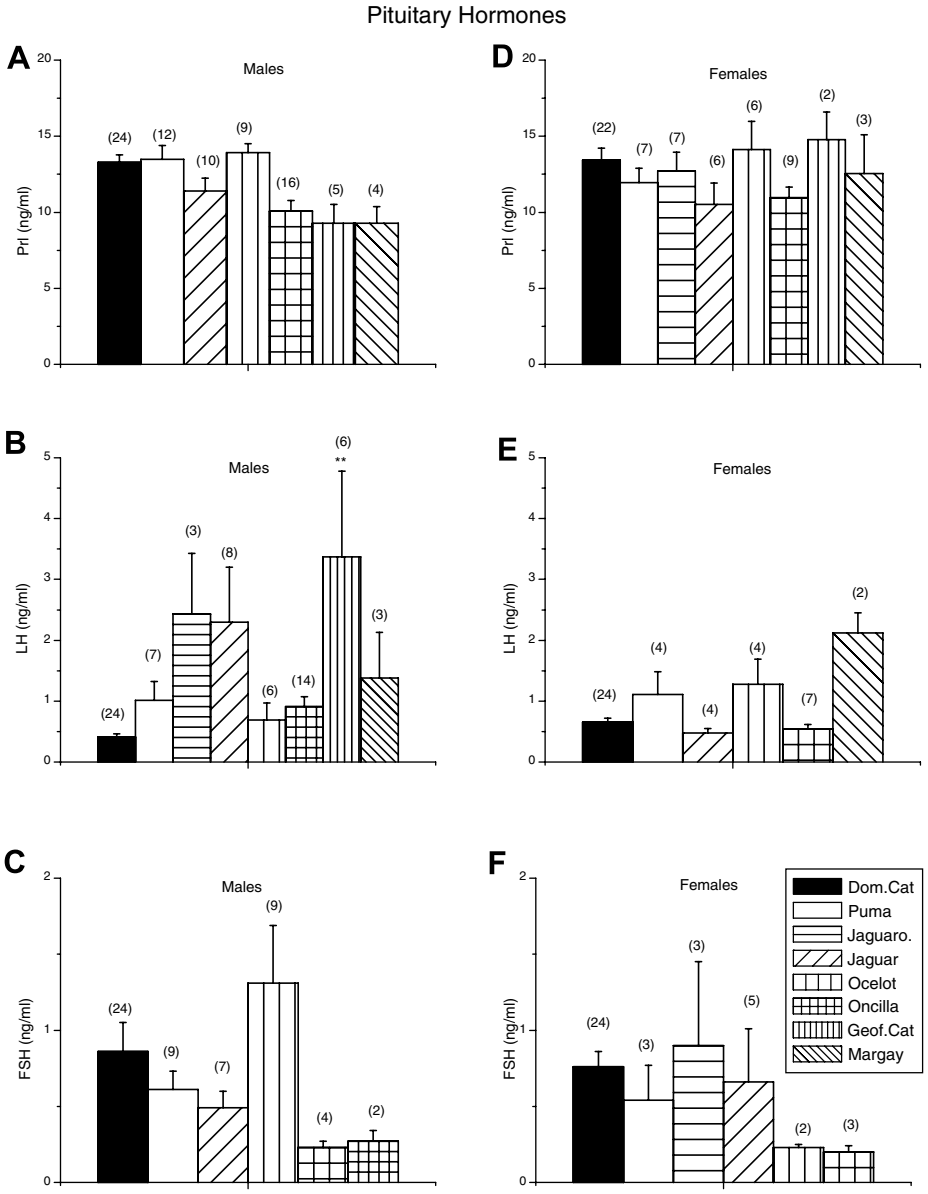

Fig. 1. Pituitary hormones in neotropical and domestic cats: Plasma prolactin in males (Panel A) and females (Panel D), luteinizing hormone in males (Panel B; $^{**}p < 0.01$ between Geoffroy's cat and domestic cat) and females (Panel E) and follicle stimulating hormone in males (Panel C) and females (Panel F). Blood samples were withdrawn immediately after anesthesia with ketamine and xylazine. The values are means ± SE. The number of animals is shown above each column.

confirmed in the present study on neotropical South American species.

The domestic cat, like most felids, has induced ovulation, with LH being released within minutes of coitus. Thus, this hormone, unlike FSH, shows wide variation in plasma concentrations. Johnson and Gay (1981a,b), studying LH in the domestic cat, demonstrated that plasma LH fluctuates episodically, with increases occurring at 20–30 min intervals, presumably reflecting pulsatile LH release.

Female ocelots had significantly higher estradiol concentrations than the other females studied. Since all animals were exposed to the natural photoperiod and since the collections were made at random, we may conclude that ocelots differ in this respect from other members of the family.

Progesterone concentrations were much higher in female pumas than female domestic cats, though these are distantly related members of the same family, illustrating another peculiarity of the group. We emphasize the values observed for female pumas because this specie, like the domestic cat, is a reflex ovulator, and therefore the high progesterone concentration observed in female pumas is relevant and peculiar.

Jaguars had significantly higher testosterone concentrations than domestic cats and the other felids studied. This point deserves attention since, in contrast to small felids,

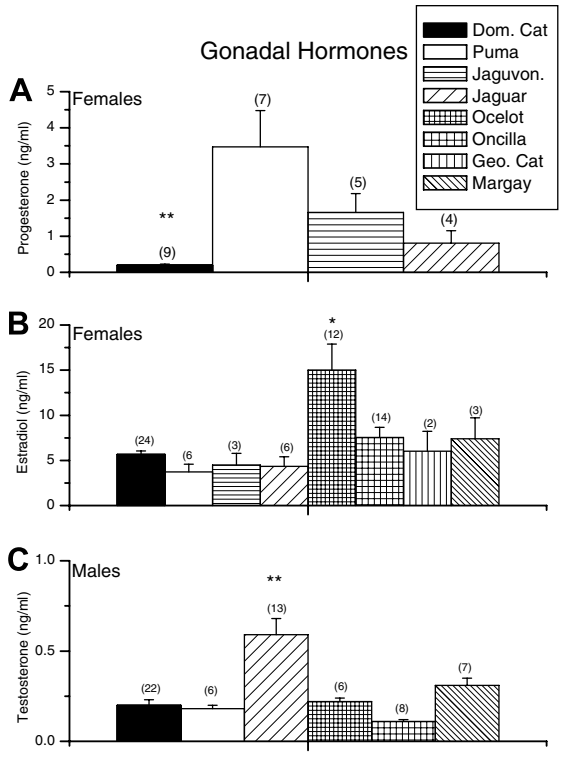

Fig. 2. Gonadal hormones in neotropical and domestic cats: Females: plasma progesterone (Panel A; $^{**}p < 0.01$ between puma and domestic cat) and estradiol (Panel B; $^{*}p < 0.05$ between ocelot and domestic cat, puma, jaguarondi, jaguar and oncilla) (progesterone; oestradiol). Males: plasma testosterone (Panel C; $^{**}p < 0.01$ between jaguar and domestic cat). Blood samples were withdrawn immediately after anesthesia with ketamine and xylazine. The values are means ± SE. The number of animals is shown above each column.

the jaguar has the best reproductive performance among neotropical felines (Oliveira, 1994).

Plasma Prl concentrations, a hormone related to reproduction and also a marker of animal stress, did not differ either between sexes or species. It is not known whether cats have a rhythm of Prl secretion (Jochle, 1997). The greatest daily variations in plasma Prl concentration occur in response to photoperiod with the highest levels occurring at night as is the case for melatonin (Leyva et al., 1984, 1989a,b).

Cortisol concentrations were higher in the puma and jaguarondi than in the oncilla, ocelot, jaguar, Geoffroy's cat, margay with the values being similar in the latter species. The pumas were anesthetized in what would seem to be a less stressful manner than the smaller species (darts as opposed to a conical net) but even so their plasma cortisol was higher. High plasma cortisol concentrations have been reported before in pumas but only in the male (Wildt et al., 1988). They have also been reported on other neotropical cats (jaguar, jaguarondi, oncilla and ocelot) (Nogueira and Silva, 1997), though without distinguishing between the sexes these results are similar that we showed.

The results shown in Fig. 3 support the view that, at least for the domestic cat, males and females respond differently to the same stress. This is probably related to the mechanisms of reproduction of the two sexes. Female cats had lower cortisol concentrations than males, especially after transport. This fact, together with the observation by Bolanos et al. (1997) that the adrenal can produce significant amounts of progesterone, supports the theory that the HPA axis may have different effects in males and females of the same species. This could be mediated either by sex differences in hormone concentration or differences in the sensitivity of the hypothalamus-pituitary-gonadal (HPG) axis of males and females to HPA axis hormones.

It should be also noted that the mechanism of ovulation in most members of the family is considered to be a reflex, in contrast to most mammals, which show spontaneous ovulation. However, in the case of the domestic cat, the cervical stimulation is not always required to induce ovulation (Lawler et al., 1993). There are also studies showing that some felids such as the bobcat (*Lynx rufus*) (Dukes, 1949; Crowe, 1975; Fritts and Sealander, 1978; Kitchener, 1991, apud IUCN site 1996 – The World Conservation Union) and the Canada lynx (*Lynx canadensis*) may ovulate spontaneously under conditions of altered prey availability (Kitchener, 1991, apud IUCN site 1996 – The World Conservation Union), i.e., a stress situation (prey availability) can alter the mechanism of ovulation in these two wild species. Therefore, considering that environmental variations alter the reproductive mechanisms of these animals, the study of cortisol in different handling situations (stress) can make important contributions not only to the understanding of the physiology of these species but also to the conservation of feline species. Stressful situations may influence reproduction in wild felids, for instance there is evidence that the *Canada lynx* ovulates spontaneously when prey is abundant but that ovulation is induced during times of nutritional stress. Likewise, there is some association between high cortisol concentrations and poor semen quality though this is by no means consistent. The low cortisol concentrations found in many of the wild felids we studied, including the highly fertile jaguar, may be somewhat reassuring but the high levels found in the puma, and the closely related jaguarundi, are a cause for concern.

Before administration of ACTH, plasma cortisol concentration was lower in the domestic cat than in the two wild species. It is perhaps not surprising that domestic cats would find handling less stressful than wild ones. An increase in cortisol secretion was observed for the domestic cat and oncilla 0–5 min after ACTH administration, while in the margay the increase occurred 20–40 min after ACTH. A reduction in serum cortisol was observed between 90 and 120 min after the stimulus for the domestic cat and oncilla but not for the margay. The control of basal cortisol secretion seems to be similar for the three species. However, the mechanisms for controlling cortisol secretion as a result of stressful stimuli may differ between species with specific differences in response to stressors such as handling, environment, feeding or the presence of other species. As emphasized by Wildt et al. (1988), most

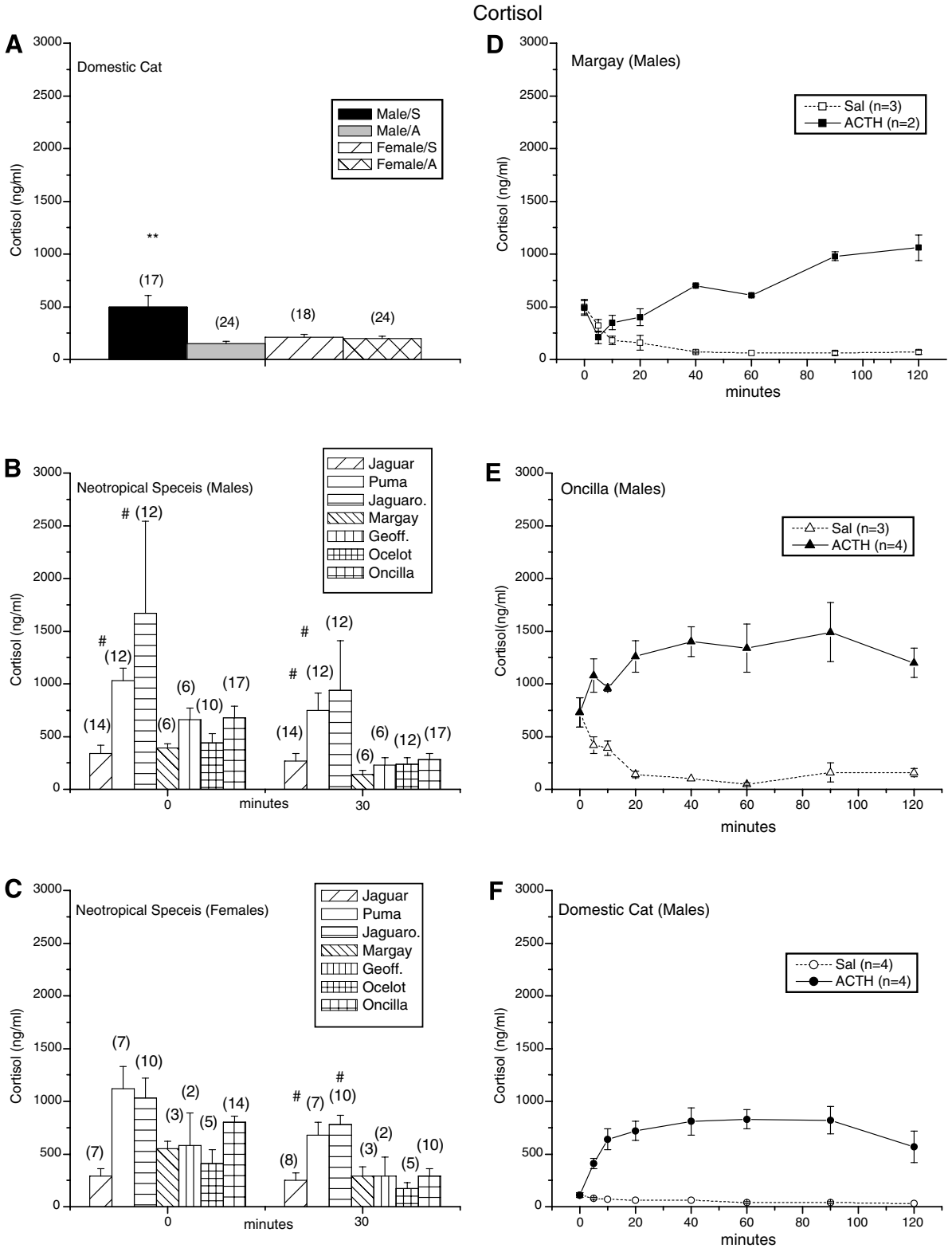

Fig. 3. Cortisol: Left panels. Panel A: Plasma cortisol concentrations in the plasma of domestic cats taken immediately after induction of anaesthesia with ketamine and rompun. The animals were grouped according to sex and habituation history. Some were allowed to become habituated to their laboratory cages for about 36 h (A) while others had been confined to their cages for less than 4 h (S). $^{**}p < 001$ Male (S) versus other groups. Panels B and C: Plasma cortisol concentrations in jaguars, pumas, jaguarondis, margays, Geoffroy's cats, ocelots and oncillas. Blood samples were taken immediately (0) after the onset of anaesthesia and 30 min later (30). #: $p < 0.05$ when pumas and jaguarondis were compared with other species. Right panels: Plasma cortisol concentrations in margays (Panel D, all male), oncillas (Panel E) and domestic cats (Panel F) given synthetic adrenocorticotropin hormone (ACTH 10 μg/kg/100 μl, iv) or the same volume of saline (Sal 0.9% NaCl). The horizontal axis shows the time of sample collection in minutes. The first sample (0) was taken immediately before the ACH or saline was given. Values are given as means ± SE. The number of animals is shown above each column.

zoos handle small felids in an identical manner without considering physiological and psychological differences, a fact also confirmed by Morais et al. (1997).

In summary, we have shown that

(1) basal Prl and FSH concentrations are similar in the domestic cat and the neotropical species studied and do not differ between males and females,
(2) basal LH and gonadal steroid concentrations in some neotropical felids differ from those of the domestic cat. This means that the domestic cat cannot be used as a general experimental model for all neotropical felids.

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