Research in Veterinary Science xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

A comparative study by age and gender of the pituitary adenoma and ACTH and α-MSH secretion in dogs with pituitary-dependent hyperadrenocorticism

M.F. Gallelli [a], M.F. Cabrera Blatter [b], V. Castillo [b,*]

[a] U. Endocrinología, Htal. Escuela de Medicina Veterinaria, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Av. Chorroarín 280, 1427 Ciudad, Autónoma de Buenos Aires, Argentina

[b] Área Clínica Médica Pequeños Animales, U. Endocrinología, Htal. Escuela de Medicina Veterinaria, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Av. Chorroarín 280, 1427 Ciudad, Autónoma de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Accepted 28 June 2009
Available online xxxx

Keywords:
Cushing's disease
Pituitary-dependent hyperadrenocorticism
Hypercortisolism
Corticotrophinoma
Pituitary adenoma

ABSTRACT

Pituitary-dependent hyperadrenocorticism (PDH) is frequent in dogs. Little is known about its presentation in different age groups and its characteristics. Dividing the population under study ($n = 107$) into three age groups we observed that 11.2% were young, 51.4% adults and 37.4% aged. Using magnetic resonance, pituitary tumours were intra-sellar (IS) in 30.8% and extra-sellar (ES) in 62.6% and the pars intermedia (PI) was affected in 6.5%. ES are predominant in females and IS in males ($p < 0.0001$). In the adult-aged population, the ES and PI are predominant, while in the young, the IS predominate ($p < 0.0001$). ACTH concentration was greater in the ES vs. IS ($p < 0.05$). α-MSH did not present significant differences according to tumour size, showing a negative correlation ($r = -0.47$; $p < 0.01$) vs. ACTH. Differences in adenoma size according to gender and their age-related frequency of apparition could be because of different origins of the corticotrophinoma.

1. Introduction

Cushing's syndrome (CS) represents 23% (129/560) of the endocrinological cases seen in the Endocrinology Unit of the School Hospital of the Faculty of Veterinary Sciences of the University of Buenos Aires (UBA) in 2003–2008 period. According to our statistics, 83% (107/129) of them are produced by a pituitary adenoma that secretes ACTH or corticotrophinoma; therefore receiving the name of pituitary-dependent hyperadrenocorticism (PDH) or Cushing's disease (CD). It is known that in general CS affects females more than males and that it is more frequent in adult dogs (Ling et al., 1979). Nevertheless, when it is specifically produced by a corticotrophinoma or dysfunctional pars intermedia, there are insufficient reports on its presentation and on its functional and morphological characteristics (evaluated by images) according to gender and age group. What have been extensively reported are the changes in activity of the adrenal axis as dogs get older, both in regard to modifications of glucocorticoid inhibitory actions on the hypothalamus–hypophysis (Reul et al., 1991; Rothuizen, 1991; Rothuizen et al., 1993), and a greater secretion of cortisol by the adrenal gland (Goy-Thollot et al., 2007).

Corticotroph cells in dog hypophysis can be found in the pars distalis (PD) and in the pars intermedia (PI), both synthesizing pro-opiomelanocortin (POMC) but differing in the presence of convertase 1 and 2 (C1, C2). In the former, presence of C1 determines synthesis and release of ACTH; whereas in the PI, C2 produces cleavage of ACTH resulting in α-MSH and corticotropin-like intermediate lobe peptide (Zhou et al., 1993; Don and Day, 2002; Tanaka, 2003). The factors that regulate (stimulating and inhibiting) corticotroph cells (adrenalin, vasopressin, corticotroph releasing hormone, glucocorticoids; among others) are widely known (Antoni, 1986; Bosje et al., 2002). What is under discussion is whether dopamine (DA) should be included with cortisol among the inhibitors, because D2 dopaminergic receptors are found in corticotroph cells of the PD, both in normal cells (Munemura et al., 1980; Colao et al., 2000; Pivonello et al., 2004) and tumoural ones (de Bruin et al., 2008). Also, in PI without irrigation, regulation is carried out by dopaminergic neurons, which maintain a strong and permanent inhibition on it (Peterson et al., 1986; Kemppainen and Sartin, 1988; Meij et al., 1997a; Saiardi et al., 1997). Both in humans and dogs it has been described that aged individuals show dopaminergic neurodegeneration with a higher stimulation of the corticotroph area because of the loss of the DA inhibitory effect (Rothuizen, 1991; Hereñú et al., 2006). This could determine a difference in the origin of PDH according to the age of the animal.

Regarding gender, sexual dimorphism of the corticotroph area of the hypophysis has been described in various species

* Corresponding author. Tel.: +54 11 4902 0170; fax: +54 11 4524 8496.
E-mail address: vcastill@fvet.uba.ar (V. Castillo).

0034-5288/$ - see front matter
doi:10.1016/j.rvsc.2009.06.011

(Oishi et al., 1993; Speert et al., 2002; Vidal et al., 1995; Filippa and Mohamed, 2006) as has the effect that estrogens (E2) exert by diverse proposed mechanisms over the corticotroph cells (Burgess and Handa, 1992; Carey et al., 1995; Atkinson and Waddell, 1997). Kipp et al. (2006) have recently shown that E2 have a neuroprotective effect on dopaminergic neurons. Thus, variations of E2 due to the sexual cycle or their decrease because of ageing or after castration could be related to the development of PDH. According to all of the above, appearance of this disease in the dog could present differences according to age and gender. In humans, pituitary adenoma has been classified into micro and macro adenoma according to its size evaluated by computed tomography (CT) (Asa and Ezzat, 1998; Selvais et al., 1998). More recently, the CT features of pituitary adenoma have been described also in canine literature (Bosje et al., 2002; Ohlerth and Scharf, 2007; Van der Vlugt-Meijer et al., 2002, 2003). Nevertheless, in human, nuclear magnetic resonance imaging (NMRI) would be the most precise image diagnosis method for detecting and evaluating hypophyseal tumours (Lambrecht and Rubens, 1989; Korogi and Mutsumasa, 1995). There are also studies that describe the use of NMRI in dogs with PDH with good results (Bertoy et al., 1995; Duesberg et al., 1995). Using NMRI mid-line sagital sections, we have classified a pituitary tumour as intra-sellar or extra-sellar, which allows us to become independent of the difference in size between breeds (Castillo et al., 2006, 2008; Hullinger, 1993).

The objective of the following study was to analyze PDH presentation according to its distribution by age and gender, and to analyze morphological (according to images) and functional differences of the corticotrophinoma.

2. Materials and methods

2.1. Population under study and diagnosis of PDH

A retrospective study using clinical histories from 2003–2008 was carried out on 107 dogs with PDH, involving various different breeds (56%) and mongrels (44%). The animals were divided into three groups: young: ⩽ 6 years old; adults: ⩾ 7 ⩽10 years old and aged, as from 11 years. All cases were attended to at the Endocrinology Unit of the School Hospital of the Faculty of Veterinary Sciences-UBA. The following diagnostic criteria was used: urine cortisol:creatinine ratio (C/CR) >70 nmol/L; >50% reduction with respect to the basal value of C/CR, after administration of 0.1 mg/kg dexametasone every 8 h, according to that described by Galac et al. (1997) and Rijnberk et al. (1988) and an increase in ACTH and α-MSH. Presence of pituitary adenoma or alteration of PI was confirmed by NMRI.

2.2. Diagnostic imaging

The sellar region was assessed using a closed NMR gear General Electric 1T and the same operator, evaluating sagital, axial and coronal sections every 2 mm, with gadolinium to contrast.

To evaluate tumour aspect and size, mid-line sagital sections were used in accordance with Voutetakis et al. (2004) taking into account the variability described in the size of the pituitary gland in dogs (Hullinger, 1993). Those tumours that overlapped the imaginary line that joins both ends of the sella turcica (cranial–caudal) were classified as extra-sellar (ES); while those that remained within the boundaries of the sella turcica were classified as intra-sellar (IS) as described in human by Asa and Ezzat (1998). Tumour size was evaluated measuring the maximum height of the adenoma (Fig. 1) and the appearance of the PI was also studied. According to our previous observations in healthy dogs, PI was considered abnormal when its width was >0.5 mm in the sagital section (Castillo et al., 2008).

2.3. Hormone measurements

Plasma ACTH concentration was measured by means of the immunoradiometric assay (IRMA) using a commercial kit (Nichols Advantage ACTH Assay, Nichols Institute Diagnostics, Bad Vilbel, Germany). α-MSH (Euro-Diagnostica AB, Malmö, Sweden) was measured by means of the radioimmuno assay (RIA).The intra-assay and inter-assay coefficients of variation for ACTH were 3% and 6.8% respectively; and 2.9% and 4.0% respectively for α-MSH.

Urine cortisol was measured by means of RIA, using a commercial kit (DPC Corporation, San Diego, California, USA).

The urine cortisol was expressed as a ratio of urine cortisol to creatinine (measured in Metrolab Autoanalizer Merck, Germany). The inter- and intra-assay coefficients of variation for cortisol were 8% and 5%, respectively.

2.4. Statistical–epidemiological analysis

A transversal retrospective study, based on clinical histories of patients attended to during the 2003–2008 periods, was carried out. A contingency table followed by Fischer's exact test and a calculation of the relative risk were used to compare qualitative results between groups. To be able to use parametric tests, hormone values were previously normalized by logarithmic transformation and were then expressed as mean ± standard deviation (SD). Comparison between means (ACTH and α-MSH) of the three groups was carried out using ANOVA followed by Bonferroni's test. Comparison by gender and by IS and ES projection was carried out using an unpaired t test. A multiple linear regression was used to evaluate if ACTH and α-MSH secretion was related to the size of the adenoma and its IS and ES projection; Pearson's correlation was also carried out between ACTH and α-MSH. $p < 0.05$ was considered significant and the values were expressed as mean ± SD.

3. Results

3.1. Population under study

Out of the total number of patients studied, a greater frequency of presentation was observed in females 74.8% (80/107) than in males 25.2% (27/107).

Regarding age distribution, the median for the whole female population was 10 years (range: 4–16 years) and 9.5 in males (range 4–15 years) showing no significant differences between sexes; therefore the average age of presentation (both sexes) was 8.64 ± 3.2 years (mean ± SD). Analyzing the different groups, 11.2% were young dogs, the majority being females; 51.4% were adults, 67.3% being female and 32.3% male; finally, 37.4% were aged dogs, out of which, 80% were female (Fig. 1a). Regarding the proportion of case presentations in females and males, no significant differences were found between young, adult and aged dogs. Average age for each group (both sexes) was: 4.5 ± 1.6 years in young: 9.2 ± 1.1 years in adults and 12.6 ± 1.3 years in aged.

A progressive increase with age in case presentation was observed in the females, being maximum between 7 and 10 years, to then decline and increase once again between 12 and 14 years of age (Fig. 1b). On the other hand, a different behavior was observed in males: the increase in case presentation was in peaks with maximum frequencies at 6, 8 and 10 years (Fig. 1b).

3.2. Diagnostic imaging

Using NMRI (Fig. 2) in both sexes, 62.6% (67/107) presented adenomas of extra-sellar projection; 30.8% (33/107) intra-sellar

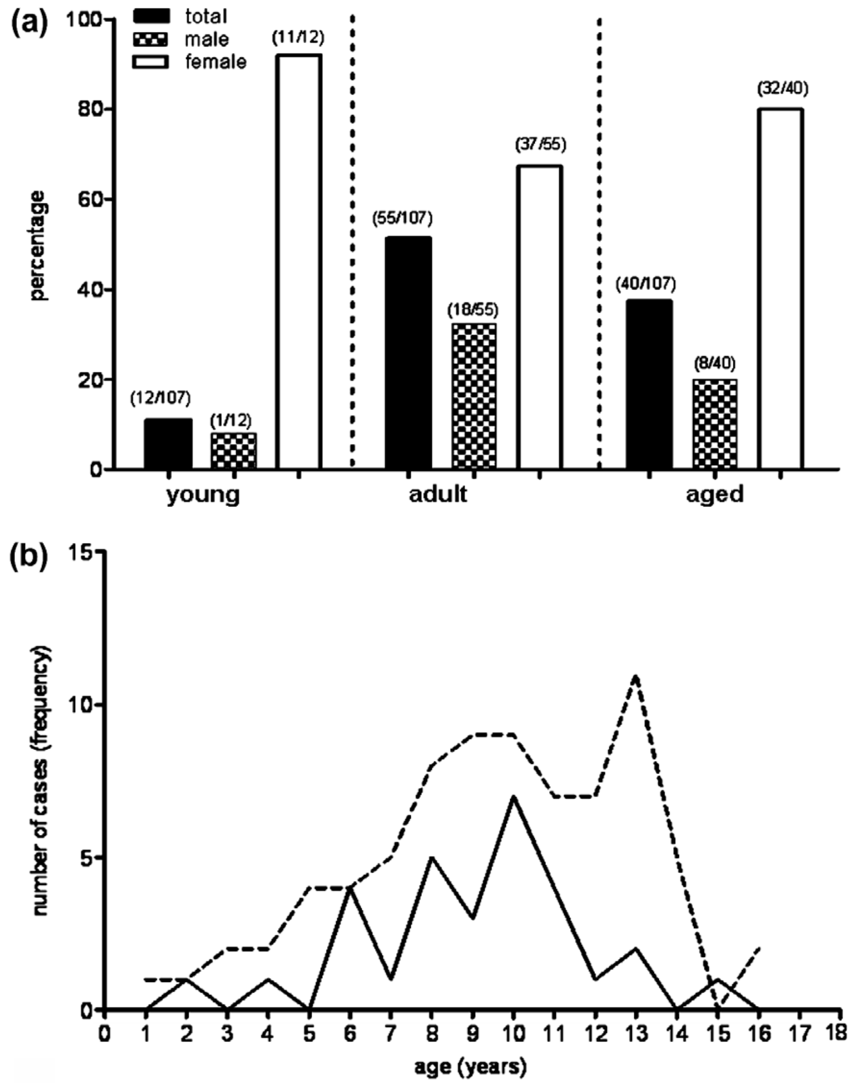

Fig. 1. (a) Proportional distribution of dogs with PDH by groups (young, adults and aged) and by gender and (b) age presentation frequency according to gender. Note the progressive way cases increase in females, while in males the increase is acute; although an age-related increase is also evident. Full line: males, dotted line: females. Absolute numbers are expressed between parenthesis (number cases/total number).

and only 6.5% (7/107) presented PI alterations (Fig. 3a). Related to the gender of the individuals (Fig. 3a), tumours with extra-sellar projection were predominant in females (91%) as were PI alterations. In males on the other hand, intra-sellar tumours were predominant (60.6%). This different presentation between genders was significant ($p < 0.0001$) with a relative risk of 0.15 (CI 95% = 0.06–0.33). When analyzing adenoma presentation by age group, significant differences were found ($p < 0.0001$) with IS being predominant in young dogs (53.8%) and ES in adults and aged dogs (67.8% and 94.4% respectively) (Fig. 3b). PI alteration in NMRI was observed in the aged group, except for one case seen in a 10 year old dog, but which was very close to being 11 years old (Fig. 3b); and the majority were aged females (6/7).

3.3. Hormone measurements

In those dogs with visible adenoma, an equimolar secretion of ACTH and α-MSH was observed in 47% (IS + ES: 47/100, IS 16, ES:31) of the cases with adenomas. ACTH secretion was greater than that of α-MSH in 18% (IS + ES: 18/100, IS: 4, ES: 14) of the individuals evaluated, with the remaining 35% (IS + ES: 35/100, IS: 13, ES: 22) secreting α-MSH predominantly. When considering the adenoma projection and PI, no significant differences were shown when analyzing these hormone proportions between IS and ES projections (Fig. 4a). ACTH was the predominant hormone in dogs with the PI affected (7/7). Neither was significant differences found between young, adult or aged dogs with respect to the ACTH and α-MSH secretion.

Analyzing ACTH and α-MSH concentrations in the three age groups, in young dogs the mean and SD was 18.7 ± 10.7 and 34.5 ± 29 pmol/L respectively; in adults 16.4 ± 11 and 30 ± 31.2 pmol/L and in aged dogs 14 ± 6.2 and 31.4 ± 30 pmol/L respectively. No significant differences were found between these hormone concentrations and the age groups or sexes.

Regarding the adenoma's projection, ACTH concentrations were significantly higher in ES vs. IS ($p < 0.05$), whereas α-MSH concentrations showed no significant differences (Fig. 4b).

The multiple regression and correlation analyses showed that only ACTH correlates individually with adenoma size and projection ($r = 0.37$, $p < 0.05$) (Fig. 5a). No correlation was found between adenoma size and projection and α-MSH. On the contrary, this hormone showed a negative correlation with ACTH ($r = -0.47$;

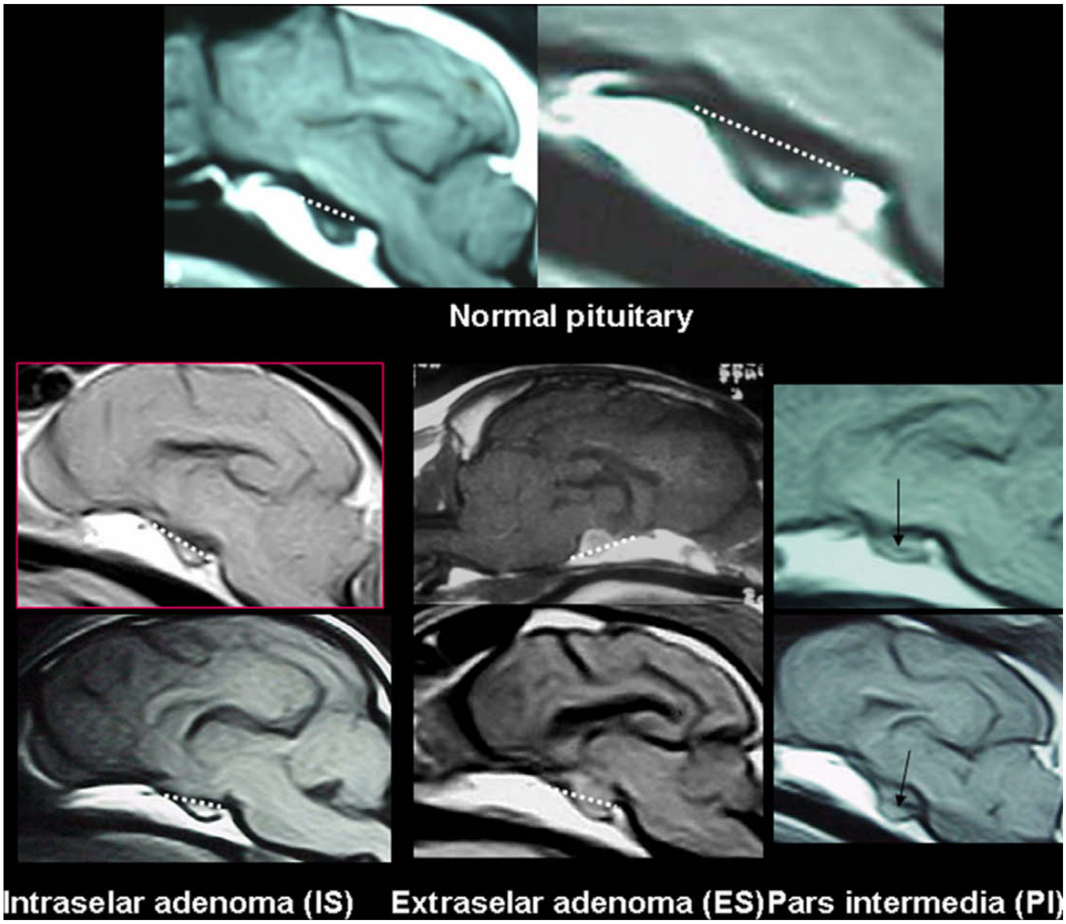

Fig. 2. NMRI sagital sections, contrasted with gladolinium, in dogs with PDH. The dotted line indicates the upper limit of the sella turcica. IS do not surpass the line, conversely ES projecting adenomas exceed that one. The pars intermedia (arrow) can be seen showing a greater width than normal pituitary.

$p < 0.01$), observing lower concentrations of α-MSH as ACTH increased (Fig. 5b).

4. Discussion

PDH is a frequent pathology in dogs that presents differences between sexes and age groups, both in frequency of corticotrophinoma apparition and in morphology and hormone secretion. We think that those differences could be due to different factors that operate on the corticotroph area of the PD and PI of the pituitary gland. Considering a decrease in dopaminergic tone due to neuron degeneration (Rothuizen, 1991; Reul et al., 1991; Hereñú et al., 2006) and the sexual dimorphism of the corticotroph cells (Carey et al., 1995; Atkinson and Waddell, 1997; Kipp et al., 2006), said factors can be: a different molecular origin of the ACTH producing adenoma according to gender and age, and/or the effect of the sexual hormones on the corticotroph cells. The highest prevalence of PDH is in animals over 7 years of age, with only an 11.2% in young dogs. These results coincide with reports in humans, where a low prevalence was found in children and adolescents (Kane et al., 1994; Leinung et al., 1995). The occurrence of cases increases progressively in females as from 7 years of age. On the other hand in the male, presentation is in peaks, which also increase with age. This different expression of PDH according to gender and age suggests, on the one hand a probable effect of the sexual hormones on the corticotroph, perhaps either unleashing the corticotrophinoma or aiding its development. On the other hand, the fact that in the healthy aged dog cortisol regulation of the hypothalamus and hypophysis is altered (Rothuizen et al., 1993; Reul et al., 1991) could cause an increased activity of the corticotroph area, possibly leading to the formation of the ACTH producing adenoma.

With regards to the effect of the sexual hormones, different mechanisms by which E2 and progesterone (P4) operate on the hypothalamus–hypophysis axis (H–H-A) have been described. It has been postulated in rats and sheep (Keller-Wood, 1998; Carey et al., 1995; Atkinson and Waddell, 1997) that they could exert their effect at the mineralocorticoid and glucocorticoid receptor level in hypothalamus and hypophysis, interfering with the endogenous corticoid negative feedback. The corticotroph area presents sexual dimorphism in various species, with increases in cortisol plasma concentrations being observed in females during the proestrus (Atkinson and Waddell, 1997), as well as significant increases in corticotroph cells as from puberty (Vidal et al., 1995) and a greater number of corticotroph cells with respect to the males (Filippa and Mohamed, 2006). These same authors point out that as the animal gets older; there is a progressive increase in the size of the area occupied by ACTH producing cells. Although progesterone action on the corticotroph cell is still under discussion, with studies reporting no observable effects on ACTH and cortisol secretion after administration of medroxiprogesterone (Beijerink et al., 2007), we observed that in five females that returned to oestrus while receiving treatment with isotretinoin 9-cis (Castillo et al., 2006) α-MSH concentration increased notably (data not shown). It is probable that the natural or endogenous progesterone exerts a direct effect at least on the PI (site of α-MSH synthesis in the dog), whereas the synthetic forms do not have this effect. An analysis of α-MSH and ACTH variations during the female sexual cycle

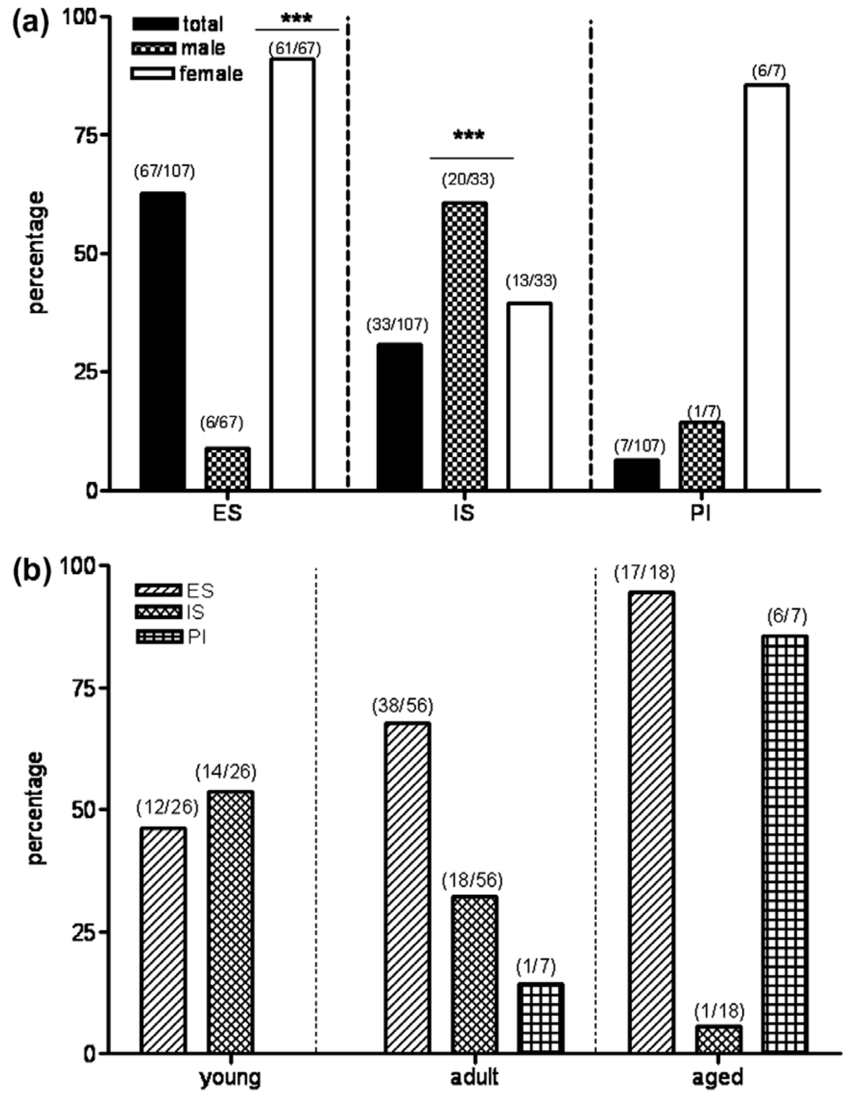

Fig. 3. Proportional distribution of the adenoma projection and PI according to gender (a) and (b) according to age group. (a) Note the greater proportion of ES adenomas and PI affected in females. On the contrary, in males IS predominate. (b) IS projection predominates in the young. ES predominate in adults and in aged, where the PI appears affected. (a) $^{***}p < 0.0001$ ES vs. IS between females and males (X^2, Fisher's exact test). (b) $^{***}p < 0.0001$ ES vs. IS between young, adults and aged (X^2, test and tendency). Absolute numbers are expressed between parenthesis (number of cases/total number).

remains for future studies. On the one hand, the cyclic action of estrogens and progesterone in the female, which is different to the secretion mode of androgens, could be a stimulating factor for the development of the corticotrophinoma and would explain why PDH is more frequent in females and also the higher risk of suffering macro adenomas. On the other hand, if the neuroprotective action of E2, described by Kipp et al. (2006) is taken into account, the process of dopaminergic neuron degeneration could be even more affected when E2 decline because of age or due to castration of the female, resulting in a lower inhibition both of the PD corticotroph and of the PI. Therefore, if both the above and that described by Rothuizen et al. (1993) and Reul et al. (1991) are taken into account, the HHA alteration in aged dogs and dopaminergic neurodegeneration would cause the differences observed in age and gender in PDH.

Regarding the morphological characteristics of the adenoma (evaluated by NMRI) the extra-sellar projection is clearly predominant in adult and aged dogs and the IS predominates in young dogs. This is similar to reports in man, where micro adenomas are more frequent in children (Cannavó et al., 2003). When analyzing gender differences we observed that, contrary to females, intrasellar tumours predominate in males. This reinforces the concept of sexual dimorphism previously discussed in relation to the development of the corticotrophinoma and its probable different molecular origin according to gender and ages. The PI was only affected in aged animals, a possible explanation being the above mentioned dopaminergic neurodegeneration (Rothuizen, 1991; Hereñú et al., 2006). It is evident that as the number of dopamine producing neurons decreases, the inhibitory effect of this peptide on dopaminergic receptors on the corticotroph of the PD and PI will be affected (Munemura et al., 1980; Colao et al., 2000; Pivonello et al., 2004) resulting in a greater stimulatory action on those cells; thus increasing POMC and ACTH synthesis accompanied by hypertrophy of the area. The fact that no increase in α-MSH is observed, despite the PI being more stimulated, would be due to a less significant action of the convertase 2 enzyme (that cleaves ACTH α-MSH) which is dependent on dopamine in aged individuals (Tanaka, 2003). On the contrary, in dogs with a predominance of α-MSH and an extra-sellar adenoma, its origin would be the PI (melanocorticotrophinoma), as has already been proposed by Hanson et al. (2006) and Peterson et al. (1986).

Regarding ACTH and α-MSH concentrations, our results show that they are not significantly different between gender and age groups of animals with PDH in agreement with Meij et al.

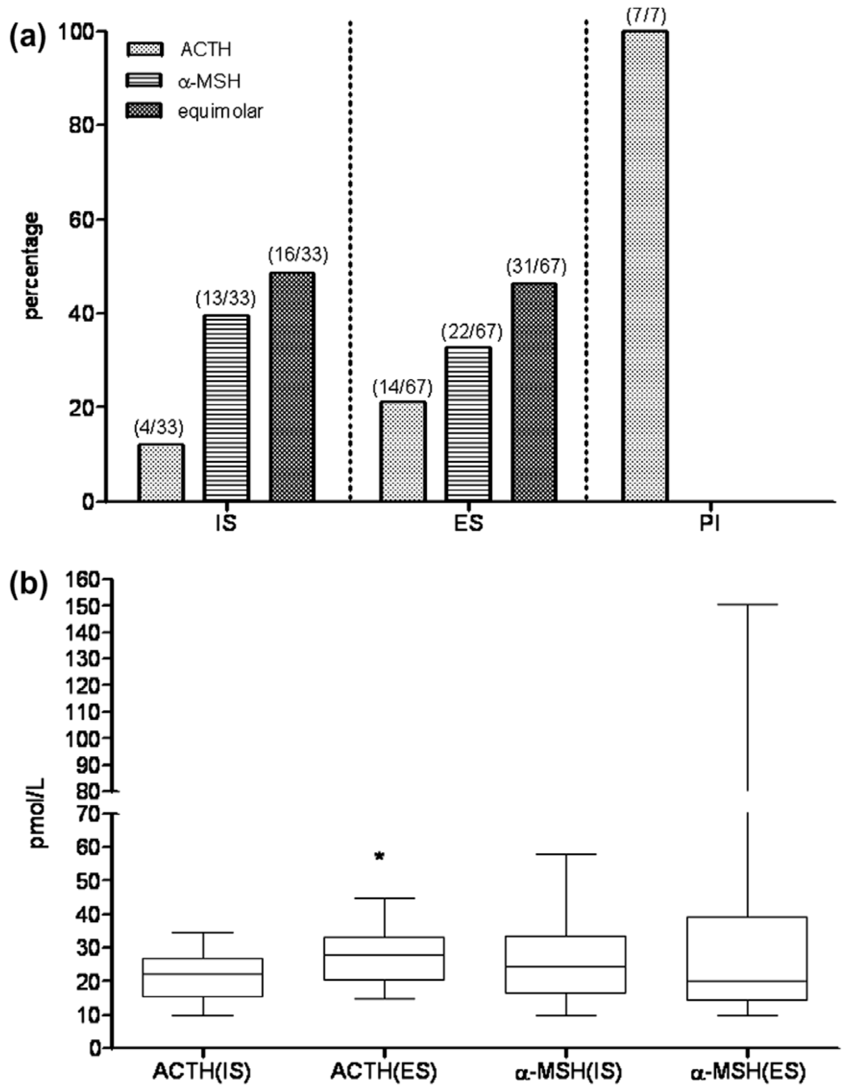

Fig. 4. Proportional distribution of ACTH and α-MSH according to whether their secretion is equimolar or one or other of them predominates according to the adenoma projection (IS or ES) or PI (a) and (b) hormone concentration according to ES or IS adenoma projection in dogs with PDH. No significant differences were found between IS and ES according to hormone secretion ($X^{2'}$ test). In all cases, ACTH was predominant in PI (a). In (b), ACTH concentration was significantly greater in ES vs. IS (*$p < 0.05$); on the contrary, α-MSH did not present significant differences (Unpaired *t* test). (IS): intra-sellar; (ES): extra-sellar.

(1997b) with respect to the gender. As has been described by other authors (Losa et al., 2000; Bosje et al., 2002; Hanson et al., 2006), we also found a correlation between ACTH and adenoma size. In most cases equimolar secretion of ACTH and α-MSH is maintained, both in intra-sellar and extra-sellar projecting tumours. Nevertheless, we noticed that equimolarity was lost the larger the adenoma; with ACTH predominating in extra-sellar tumours. Noticeably, α-MSH did not present differences between IS and ES, with a decrease in concentration being noted in the latter as well as a negative correlation with ACTH. This could be explained if either different molecular origins are considered or different mutations which lead to development of the adenoma, giving as a result a greater corticotroph cell proliferation index in the PD (Kooistra et al., 1997; Katznelson et al., 1998; Mu et al., 1998). It is known that corticotrophinoma growth is self-limiting and that those that progress to a larger size are the result of a greater number of mutations or of the action of tumour growth stimulating cytokines present in the hypophysis (Dahia and Grossman, 1999; Levy and Lightman, 2003; Melmed, 2003). Therefore in extra-sellar projecting adenomas, particularly the larger ones, the change to α-MSH could be lost, perhaps because of a decrease in C2 activity or due to a decrease in dopaminergic receptors; or the greater number of tumoral corticotroph cells coming from the PD, which would make ACTH secretion predominate over α-MSH in this type of adenomas (Laurent et al., 2002; Westphal et al., 1999; Pivonello et al., 2004) and would explain the negative correlation observed between both hormones.

In conclusion, presentation of corticotrophinomas is more frequent in adult and aged dogs; with a higher prevalence in females. Tumoral size was associated with ACTH secretion and showed age and gender related differences. Taking these results into account can guide both clinical and surgical treatment of dogs with PDH. This is important considering that the larger adenomas are those displaying most difficulties when treated, are the most frequently recurrent ones after surgery (Losa et al., 2000; Hanson et al., 2006) and are also the most refractory to medical treatments (Castillo et al., 2008).

Awareness of these differences opens up a way to studying the probable action of other variables related to age and gender that could influence the development of PDH.

Please cite this article in press as: Gallelli, M.F., et al. A comparative study by age and gender of the pituitary adenoma and ACTH and α-MSH secretion in dogs with pituitary-dependent hyperadrenocorticism. Res. Vet. Sci. (2009), doi:10.1016/j.rvsc.2009.06.011

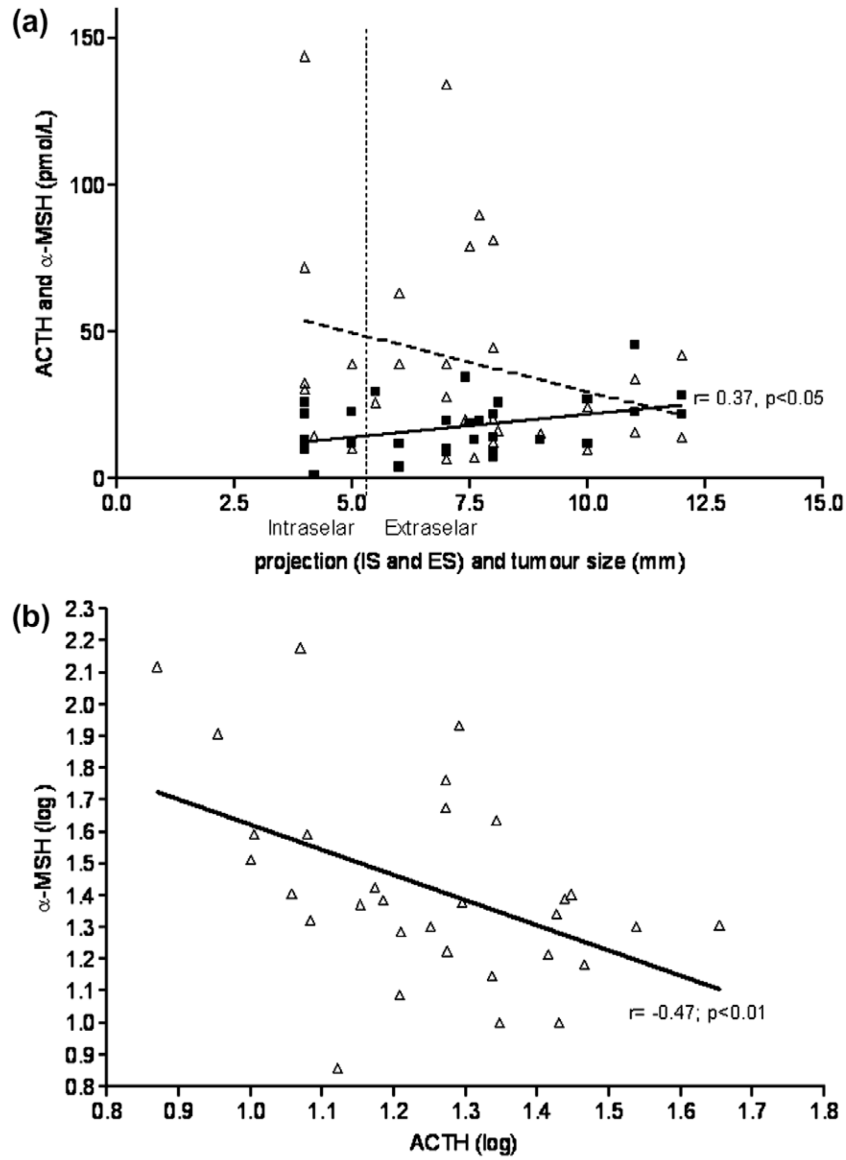

Fig. 5. Multiple linear regression: (a) between adenoma size and projection vs. ACTH and α-MSH and (b) Correlation between ACTH vs. α-MSH. (a) Note that ACTH concentrations (solid line) increase with adenoma size, while α-MSH (doted line) values decrease. (b) It can be seen that α-MSH correlates negatively with ACTH. Full squares: ACTH; open triangles: α-MSH. Multiple linear regression (a); linear regression and Pearson's correlation (b).

Acknowledgments

We would like to thank Dr E. Arzt and Dr. G. Stalla for measuring the ACTH and α- MSH. This project received funding from UBACyT, University of Buenos Aires (V045 and V006).

References

Antoni, F.A., 1986. Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor. Endocrine Reviews 7, 351–378.

Asa, S.L., Ezzat, S., 1998. The cytogenesis and pathogenesis of pituitary adenomas. Endocrine Reviews 19, 798–827.

Atkinson, H.C., Waddell, B.J., 1997. Circadian variation in basal plasma corticosterone and adrenocorticotropin in the rat: sexual dimorphism and changes across the estrous cycle. Endocrinology 138, 3842–3848.

Beijerink, N.J., Bhatti, S.F.M., Okkens, A.C., Dieleman, S.J., Mol, J.A., Duchateau, L., Van Hamb, L.M.L., Kooistra, H.S., 2007. Adenohypophyseal function in bitches treated with medroxyprogesterone acetate. Domestic Animal Endocrinology 32, 63–78.

Bertoy, E.H., Feldman, E.C., Nelson, R.W., Duesberg, C.A., Kass, P.H., Reid, M.H., Dublin, A.B., 1995. Magnetic resonance imaging of the brain in dogs with recently diagnosed but untreated pituitary-dependent hyperadrenocorticism. Journal of the American Veterinary Medical Association 206, 651–656.

Bosje, J., Rijnberk, A., Mol, J., Voorhout, G., Kooistra, H., 2002. Plasma concentrations of ACTH precursors correlate with pituitary size and resistance to dexamethasone in dogs with pituitary-dependent hyperadrenocorticism. Domestic Animal Endocrinology 22, 201–210.

Burgess, L.H., Handa, R.J., 1992. Chronic estrogen-induced alterations in adrenocorticotropin and corticosterone secretion, and glucocorticoid receptor-mediated functions in female rats. Endocrinology 131, 1261–1269.

Cannavó, S., Almota, B., Dall'Asta, C., Corsello, S., Lovicu, RM., De Menis, E., Trimarchi, F., Ambrosi, B., 2003. Long-term results of treatment in patients with ACTH-secreting pituitary macroadenomas. European Journal of Endocrinology 149, 195–200.

Carey, M.P., Deterd, C.H., deKoning, J., Helmerhorst, F., deKloe, E.R., 1995. The influence of ovarian steroids on hypothalamic-pituitary–adrenal regulation in the rat. Journal of Endocrinology 144, 311–321.

Castillo, V.A., Giacomini, D.P., Paez-Pered, M., Stalla, J., Labeur, M., Theodoropoulou, M., Holsboer, F., Grossman, A., Stalla, G., Arzt, E., 2006. Retinoic acid as a novel medical therapy for Cushing's disease in dogs. Endocrinology 174, 4438–4444.

Castillo, V.A., Gomez, N.V., Lalia, J.C., Cabrera Blatter, M.F., Garcia, J.D., 2008. Cushinǵs disease in dogs: Cabergoline treatment. Research in Veterinary Science 85, 26–34.

Colao, A., Lombardi, G., Annunziato, L., 2000. Cabergoline. Experimental Opinion Pharmacotherapy 1, 555–574.

Dahia, P.L., Grossman, A.B., 1999. The molecular pathogenesis of corticotroph tumors. Endocrinology Review 20, 136–155.

de Bruin, C., Hanson, J.M., Meij, B.P., Kooistra, H.S., Waaijers, A.M., Uitterlinden, P., Lamberts, S.W.J., Hofland, L.J., 2008. Expression and functional analysis of dopamine receptor subtype 2 and somatostatin receptor subtypes in canine Cushing's disease. Endocrinology 149, 4357–4366.

Don, W., Day, R., 2002. Gene expression of proprotein convertases in individual rat anterior pituitary cells and their regulation in corticotrophs mediated by glucocorticoids. Endocrinology 143, 254–262.

Duesberg, C.A., Feldman, E.C., Nelson, R.W., Bertoy, E.H., Dublin, A.B., Reid, M.H., 1995. Magnetic resonance imaging for diagnosis of pituitary macrotumors in dogs. Journal of the American Veterinary Medical Association 206, 657–662.

Filippa, V., Mohamed, F., 2006. ACTH cells of pituitary pars distalis of viscacha (*Lagostomus maximus maximus*): Immunohistochemical study in relation to season, sex, and growth. General and Comparative Endocrinology 146, 217–225.

Galac, S., Kooistra, H., Teske, E., Rijnberk, A., 1997. Urinary corticoid/creatinine ratios in the differentiation between pituitary-dependent hyperadrenocorticism and hyperadrenocorticism due to adrenocortical tumour in the dog. Veterinary Quarterly 19, 17–20.

Goy-Thollot, I., Decosne-Junot, C.h., Bonnet, J.M., 2007. Influence of aging on adrenal responsiveness in a population of eleven healthy beagles. Research in Veterinary Science 82, 195–201.

Hanson, J.M., Kooistra, H.S., Mol, J.A., Teske, E., Meij, B.P., 2006. Plasma profiles of adrenocorticotropic hormone, cortisol, α-melanocyte-stimulating hormone, and growth hormone in dogs with pituitary-dependent hyperadrenocorticism before and alter hypophysectomy. Journal of Endocrinology 190, 601–609.

Hereñú, C., Cristina, C., Rimoldi, O., Becú-Villalobos, D., Cambiaggi, D., Portiansky, E., Goya, R., 2006. Restorative effect of insulin-like growth factor-I gene therapy in the hypothalamus of senile rats with dopaminergic dysfunction. Gene Therapy 13, 1–9.

Hullinger, R.L., 1993. The endocrine system. In: Evans, H.E. (Ed.), Miller's Anatomy of the Dog, third ed. WB Saunders, Philadelphia, pp. 559–585.

Kane, L., Leinung, M., Scheithauer, BW., Bergstralh, EJ., Laws, E.R., Groover Jr., R.B., Kovacs, K., Horvath, E., Zimmerman, D., 1994. Pituitary adenomas in childhood and adolescence. Journal of Clinical Endocrinology and Metabolism 79, 1135–1140.

Katznelson, L., Bogan, J.S., Trob, J.R., Schoenfeld, D.A., Hedley-White, E.T., Hsu, D.W., Zervas, N.T., Swearingen, B., Sleeper, M., Klibanski, A., 1998. Biochemical assessment of Cushing's disease in patients with corticotroph macroadenomas. Journal of Clinical Endocrinology and Metabolism 83, 1619–1623.

Keller-Wood, M., 1998. Evidence for reset of regulated cortisol in pregnancy: studies in adrenalectomized ewes. American Journal of Physiology 274, 145–151.

Kemppainen, RJ., Sartin, JL., 1988. Differential secretion of proopiomelanocortin peptides by the pars distalis and pars intermedia of beagle dogs. Journal of Endocrinology 117, 91–96.

Kipp, M., Karakaya, S., Pawlak, J., Araujo-Wright, G., Arnold, S., Beber, C., 2006. Estrogen and the development and protection of nigrostriatal dopaminergic neurons: concerted action of a multitude of signals, protective molecules. And growth factors Frontiers in Neuroendocrinology 27, 376–390.

Kooistra, H., Voorhout, G., Mol, J., Rijnberk, A., 1997. Correlation between impairment of glucocorticoid feedback and the size of the pituitary gland in dogs with pituitary-dependent hyperadrenocorticism. Journal of Endocrinology 152, 387–394.

Korogi, Y., Mutsumasa, T., 1995. Current concepts of imaging in patients with pituitary/hypothalamic dysfunction. Seminars in Ultrasound, CT, and MRI 16, 270–278.

Lambrecht, L., Rubens, R., 1989. MRI signal intensity profiles of sellar and perisellar lesions. Acta Clinica Bélgica Journal 44, 303–310.

Laurent, V., Kimble, A., Peng, B., Zhu, P., Pintar, JE., Steiner, DF., Lindberg, I., 2002. Mortality in 7B2 null mice can be rescued by adrenalectomy: involvement of dopamine in ACTH hypersecretion. Proceedings of the National Academy of Sciences USA 99, 3087–3092.

Leinung, M.C., Kane, L.A., Scheithauer, B.W., Carpenter, P.C., Laws Jr., E.R., Zimmerman, D., 1995. Long term follow-up of transsphenoidal surgery for the treatment of Cushing's disease in childhood. Journal of Clinical Endocrinology and Metabolism 80, 2475–2479.

Levy, A., Lightman, S., 2003. Molecular defects in the pathogenesis of pituitary tumours. Frontiers in Neuroendocrinology 24, 94–127.

Ling, G., Stabenfeldt, G., Comer, K., Gribble, D., Schechter, R., 1979. Canine hyperadrenocorticism: pre-treatment clinical and laboratory evaluation of 117 cases. Journal of the American Veterinary Medical Association 174, 1211–1215.

Losa, M., Barzaghi, R., Mortini, P., Franzin, A., Mangili, F., Terreni, MR., Giovanelli, M., 2000. Determination of the proliferation and apoptotic index in adrenocorticotropin-secreting pituitary tumors. Comparison between micro and macroadenomas. American Journal of Pathology 156, 245–251.

Meij, B.P., Mol, J.A., Van den Ingh, T.S.G.A.M., Bevers, M.M., Hazewinkel, H.A., Rijnberk, A., 1997a. Assessment of pituitary function after transsphenoidal hypophysectomy in beagle dogs. Domestic Animal Endocrinology 14, 81–97.

Meij, B.P., Mol, J.A., Bevers, M.M., Rijnberk, A., 1997b. Alterations in anterior pituitary function of dogs with pituitary-dependent hyperadrenocorticism. Journal of Endocrinology 154, 505–512.

Melmed, S., 2003. Mechanisms for pituitary tumorigenesis: the plastic pituitary. Journal of Clinical Investigation 112, 1603–1618.

Mu, Y-M., Takayanagi, R., Imasaki, K., Ohe, K., Ikuyama, S., Yanase, T., Nawata, H., 1998. Low level of glucocorticoid receptor messenger ribonucleic acid in pituitary adenomas manifesting Cushing's disease with resistance to a high dose dexamethasone suppression test. Clinical Endocrinology (Oxf) 49, 301–306.

Munemura, M., Cote, T., Tsuruta, K., Eskay, R., Kebabian, J., 1980. The dopamine receptor in the intermediate lobe of the rat anterior pituitary gland: pharmacological characterization. Endocrinology 106, 1676–1683.

Ohlerth, S., Scharf, G., 2007. Computed tomography in small animals – basic principles and state of the art applications. The Veterinary Journal 173, 254–271.

Oishi, Y., Okuda, M., Takahashi, H., Fujii, T., Morii, S., 1993. Cellular proliferation in the anterior pituitary gland of normal adult rats: influences of sex, estrous cycle and circadian change. Anatomical Record 235, 111–120.

Peterson, M.E., Orth, D.N., Halmi, N.S., Zielinski, A.C., Davis, D.R., Chavez, F.T., Drucker, W.D., 1986. Plasma immunoreactive proopiomelanocortin peptides and cortisol in normal dogs and dogs with Addison's disease and Cushing's syndrome: basal concentrations. Endocrinology 119, 720–730.

Pivonello, R., Ferone, D., de Herder, W., Kros, J., del basso de Caro, M., Arvigo, M., Annunziato, L., Lombardi, G., Colao, A., Hofland, L., Lamberts, S., 2004. Dopamine receptor expression and function in corticotroph pituitary tumors. Journal of Clinical Endocrinology and Metabolism 89, 2452–2462.

Reul, J.M., Rothuizen, J., de Kloet, E.R., 1991. Age-related changes in the dog hypothalamic–pituitary–adrenocortical system: neuroendocrine activity and corticosteroid receptors. The Journal of Steroid Biochemistry and Molecular Biology 40, 63–69.

Rijnberk, A., Van Wees, A., Mol, J., 1988. Assessment of two tests for the diagnosis of canine hyperadrenocorticism. Veterinary Research 122, 178–180.

Rothuizen, J., 1991. Aging and the hypothalamus–pituitary–adrenocortical axis, with special reference to the dog. Acta Endocrinologica 125, 73–76.

Rothuizen, J., Reul, J.M., Van Sluijs, F.J., Mol, J.A., Rijnberk, A., de Kloet, E.R., 1993. Increased neuroendocrine reactivity and decreased brain mineralocorticoid receptor binding capacity in aged dogs. Endocrinology 132, 161–168.

Saiardi, A., Bozzi, Y., Ja-Hyun Baik, J., Borrelli, E., 1997. Antiproliferative role of dopamine: loss of D2 receptors causes hormonal dysfunction and pituitary hyperplasia. Neuron 19, 115–126.

Selvais, P., Donckier, J., Buysschaert, M., Maiter, D., 1998. Cushing's disease: a comparison of pituitary corticotroph microadenomas and macroadenomas. European Journal of Endocrinology 138, 153–159.

Speert, D., McClennen, S., Seasholtz, A., 2002. Sexually dimorphic expression of corticotropin-releasing hormone-binding protein in the mouse pituitary. Endocrinology 143, 4730–4741.

Tanaka, S., 2003. Comparative aspects of intracellular proteolytic processing of peptide hormone precursors: studies of proopiomelanocortine processing. Zoological Science 20, 1183–1198.

Van der Vlugt-Meijer, R.H., Voorhout, G., Meij, B.P., 2002. Imaging of the pituitary gland in dogs with PDH. Molecular and Cellular Endocrinology 197, 81–87.

Van der Vlugt-Meijer, R.H., Meij, B.P., Van den Ingh, T.S., Rijnberk, A., Voorhout, G., 2003. Dynamic computed tomography of the pituitary gland in dogs with pituitary17 dependent hyperadrenocorticism. Journal of Veterinary Internal Medicine 17, 773–780.

Vidal, S., Roman, A., Moya, L., 1995. Inmunohistochemical identification and morphometric study of ACTH cells of mink (*Mustela vison*) during growth and different stages of sexual activity in the adult. General and Comparative Endocrinology 100, 18–26.

Voutetakis, A., Argropoulou, M., Sertedaki, A., Livadas, S., Xekouki, P., Maniati-Christidi, M., Bossis, I., Thalassinos, N., Patronas, N., Dacou-Voutetakis, C., 2004. Pituitary magnetic resonance imaging in 15 patients with prop1 gene mutations: pituitary enlargement may originate from the intermediate lobe. Journal of Clinical Endocrinology and Metabolism 89, 2200–2206.

Westphal, C.H., Muller, L., Zhou, A., Bonner-Weir, S., Schambelan, M., Steiner, D.F., Lindberg, I., Leder, P., 1999. The neuroendocrine protein 7B2 is required for peptide hormone processing in vivo and provides a novel mechanism for pituitary Cushing's disease. Cell 96, 689–700.

Zhou, A., Bloomquist, B.T., Mains, R.E., 1993. The prohormone convertases PC1 and PC2 mediate distinct endoproteolytic cleavages in a strict temporal order during pro opiomelanocortin biosynthetic processing. The Journal of Biological Chemistry 268, 1763–1769.

Please cite this article in press as: Gallelli, M.F., et al. A comparative study by age and gender of the pituitary adenoma and ACTH and α-MSH secretion in dogs with pituitary-dependent hyperadrenocorticism. Res. Vet. Sci. (2009), doi:10.1016/j.rvsc.2009.06.011