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GABRIELE MARINO

MARCO QUARTUCCIO

SIMONA RIZZO

VITTORIO LO PRESTI

ANTONINA ZANGHI'

TÜBİTAK

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Ectopic adrenal tissue in equine gonads: morphofunctional features

Gabriele MARINO*, Marco QUARTUCCIO, Simona RIZZO, Vittorio LO PRESTI, Antonina ZANGHI
Department of Veterinary Public Health, University of Messina, Messina – ITALY

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Abstract: Ectopic adrenal tissue has been reported to occur in equine ovaries and testes, more frequently in stressed, ill, or pregnant animals. In this study, the presence and endocrine activity of ectopic adrenal tissue in equine gonads was investigated. In 278 pairs of ovaries and 100 pairs of testes, the anomaly was observed with a comparable prevalence of 6%–9%; the tissue appeared as nodules, found on the ovarian surface and in the mediastinum. Microscopically, these usually appeared disorganized, with a tendency toward differentiation into the zonae in larger nodules. The endocrine activity of the large ectopic nodules was demonstrated by the extraction of 17-β estradiol (1.1 ng/g), progesterone (12.4 ng/g), testosterone (11.3 ng/g), and cortisol (814.8 ng/g), but in none of the cases was gonadal function apparently influenced.

Key words: Ectopic adrenal tissue, gonad, equine

Introduction

The adrenal gland has a dual embryological origin, the cortex being of mesodermal origin, formed by a thickening of the mesonephros, while the medulla originates from the ectoderm, specifically from the neural crest (1). Embryogenetic defects may lead to an abnormal location of the whole gland or portions of it (ectopia). Ectopic adrenal tissue has been reported with variable frequency in equines, bovines, ovines, carnivores, and primates (2–11).

Ectopic adrenal glands have been widely documented in humans, where they are hormonally inactive and asymptomatic (9,12). Occasionally they may proliferate and undergo adenomatous change, which may be responsible for corticotropin-independent Cushing's syndrome (13–15). Carcinoma of the ectopic adrenal gland has been also reported (16). For these reasons, the incidental finding of ectopic adrenal rests during routine surgical approach in the urogenital tract strongly suggests their excision (17).

In equines, ectopic sites of adrenal gland tissue are generally areas adjacent to the gland, as well as the kidney, retroperitoneal tissue, broad ligament, ovaries, and testes (2,5,6,8,10,11). In a former study, 59% of the examined mares had ectopic adrenocortical nodules localized at the ovary, usually at the junction between the ovarian medulla and the mesovarium (2). The nodules, single or multiple, yellow-orange, were usually a few millimeters in diameter, but could occasionally reach 2.5 cm. Histologically, the typical areas of the adrenal cortex could be recognized (2). The same authors reported a 94% prevalence of ectopic adrenal tissue in pregnant or ill mares (2).

* E-mail: marinog@unime.it

In a thoroughbred mare of 14 years, a 15-g ectopic adrenal mass, connected to the ovary, was found and progesterone (11.4 ng/g), 20-hydroxyprogesterone (9.5 ng/g), and 17-hydroxyprogesterone (55.2 ng/g), but not testosterone, estrogen, or corticosteroids, were extracted from the mass (11). In the equine testis, ectopic adrenal tissue has been detected in young or elderly horses exposed to chronic stress (5), localized at the base of the spermatic cord, between the epididymal head and testis, and in the mediastinum (5,8). Primary and secondary Cushing's syndrome has been reported in the horse (18,19), which may be complicated by a concomitant ectopic adrenal gland if this tissue is able to produce corticosteroids. Furthermore, the hypothetical interference of a hormonoactive ectopic adrenal tissue on the reproductive function has never been investigated. Considering the scarce data available in the literature on ectopic adrenal tissue in the equine species, this study aimed to investigate the morphofunctional features of this congenital disease.

Material and methods

The horses used in this study (278 mares and 100 stallions) consisted of mixed breeds, with a fleshy body condition, that were reared and slaughtered in the Catania area (Italy) during the spring of 2009. The horses ranged in age from 2 to 5 years (median = 2.5 years). Pregnant and ill animals were not available. Acute stress was minimized according to the European directives of slaughtering. Chronic stress was not evident in the antemortem clinical examination. Equine genital tracts were collected and subjected to careful gross evaluation. Samples of the affected gonads were fixed in 10% buffered formalin, dehydrated by passing through a graded series of alcohol to pure xylene, and embedded in paraffin wax. Sections of 5 μm were stained with hematoxylin and eosin and periodic acid–Schiff. The largest nodules were divided, and half were used for histological examination while the other half were subjected to the following technique for extracting lipids, modified by the authors for steroid hormones. The tissue was sliced with scalpels and homogenized (Ultra-Turrax, IKA; 6000 rpm) with 20 mL of methanol for 2 min and then with the addition of 40 mL of chloroform for 3 min. The mixture obtained was filtered and the above treatment was performed again on the residue. The residue of the second treatment was again recovered and treated with 20 mL of methanol and 20 mL of chloroform for 10 min in an ultrasonic bath. After filtration, the solution was added to those from the 2 previous treatments, reduced to about 3 mL using a Rotavapor (Büchi), and then dried under a nitrogen flow. The material was resuspended using a physiological buffer with albumin (RIA buffer, Peninsula Laboratories), centrifuged, and assayed with an enzyme-linked fluorescent assay technique for 17-β estradiol, progesterone, and testosterone, and with chemiluminescence for cortisol.

Data were analyzed using Fisher's exact test; differences between means were analyzed with Student's t-test (2 samples); $P < 0.05$ was considered statistically significant.

Results

In 278 female genital tracts, ectopic adrenal tissue was observed in the ovary in 9% of the cases (n = 25). The nodules were found unilaterally (80%, $P < 0.05$), indifferently at the left or right ovary ($P > 0.05$). They were single or multiple ($P > 0.05$), located generally (90%; $P < 0.05$) on the ovarian surface, in some cases in the mesovarium (6%) and near the ovulatory fossa (4%) (Table 1). They were ovoid or irregular, pale yellow to orange, and with a lobulated appearance (Figure 1). The diameter was between 1 and 15 mm (mean: 7 ± 1 mm). Histologically, the nodules, formed by adrenal cells arranged in lobes or small islands, were located in the subserosa, enclosed by a connective tissue capsule in continuity with the albuginea, sometimes located deeper in the ovarian stroma. In larger nodules, the 3 typical areas of the adrenal cortex were easily recognizable (Figure 2). In the first, the most peripheral adrenal cells formed tubular structures, usually arranged in arcs, with palisade-arranged cells, with elongated eosinophilic cytoplasm and oval nucleus (zona arcuata). In the second, the most represented area, ovoid adrenal cells were arranged in nests, and occasionally in parallel bundles; they appeared round with a highly vacuolated cytoplasm and a small, round nucleus (zona fasciculata). In the third, the adrenal cells were polyhedral, with small, finely reticulated cytoplasm and a pyknotic nucleus (zona reticularis). In smaller nodules the 3 zonae were not arranged in an orderly

Table 1. Ectopic adrenal tissue in the ovary: gross findings. mo = mesovarium; os = ovarian surface; of = ovulatory fossa.

No.	Affected ovary	Number of nodules	Maximum diameter (mm)	Corpus luteum
1	Left (mo)	1	10	Present
2	Right (os)	3	10	Present
3	Both (mo, os)	3	10	Present
4	Left (os)	>5	6	Present
5	Right (os)	1	4	Present
6	Left (os)	1	6	Present
7	Right (os)	1	7	Present
8	Right (os)	>5	12	Present
9	Left (os)	>5	1	Present
10	Both (os)	2	10	Present
11	Left (os)	1	15	Present
12	Left (os)	1	7	Present
13	Right (os)	>5	4	Present
14	Right (of)	1	5	Present
15	Right (os)	2	5	Present
16	Both (os)	>5	8	Present
17	Left (os)	>5	7	Present
18	Left (os)	1	3	Present
19	Left (os)	1	2	Present
20	Right (os)	>5	1	Present
21	Both (os)	>5	5	Present
22	Left (os)	1	4	Present
23	Right (os)	1	7	Present
24	Both (os)	>5	2	Present
25	Left (os)	>5	14	Present

fashion and were not always recognizable. At gross and microscopic examination of the affected ovaries, follicles at various developmental stages and corpora lutea were always ($P < 0.05$) detected in the absence of obvious degenerative lesions. In 100 male genital tracts, ectopic adrenal tissue was found in 6% of the cases, only at the testicular level. The prevalence of the abnormality in the male was not significantly different from that in the female ($P > 0.05$). Ectopic adrenal nodules were generally found in a testis (left or right) or in both testes, with no significant prevalence ($P > 0.05$). They always had a multiple appearance ($P > 0.05$). The diameter of the ectopic tissue ranged from 1 to 3 mm (mean: 2 ± 1 mm), which was significantly less than that found in the ovary ($t = 2.5$; $P < 0.05$). The finding of these nodules was possible by examining the surface of a longitudinal section of the testis, since they were all localized in the mediastinum ($P > 0.05$). They were round and light yellow; the larger ones were only prominent on the surface of the section (Figure 1). Histologically, nests of adrenal cells surrounded by the mediastinal connective tissue, in some cases forming lobules, lined by a connective tissue capsule, were seen. Scattered cells of the arcuata and zonae fasciculata were recognizable (Figure 3). The gross and microscopic examination of the testicular parenchyma showed no degenerative features, spermatogenesis being maintained in all of the cases ($P < 0.05$).

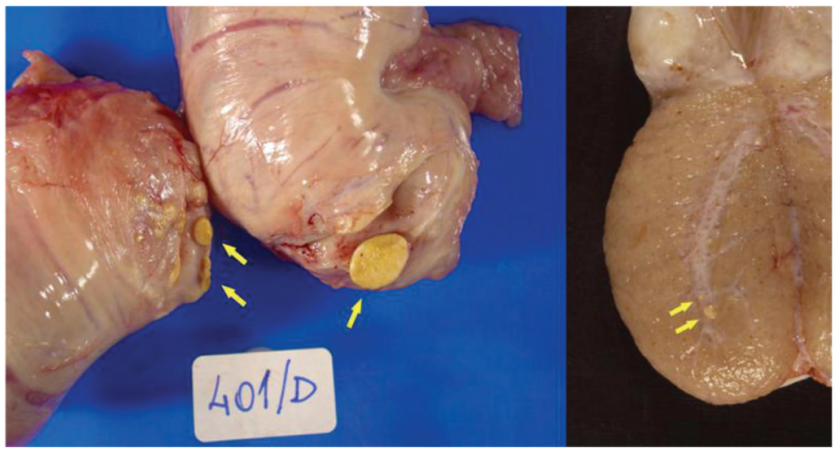

Figure 1. Nodules of ectopic adrenal tissue on the ovarian surface (arrows) and in the testicular mediastinum (arrow).

From the 2 larger nodules (>5 mm) found in 2 mares, 1.1 ng/g of 17-β estradiol, 12.4 ng/g of progesterone, 11.3 ng/g of testosterone, and 814.8 ng/g of cortisol were extracted.

Discussion

This study confirms the frequent occurrence of ectopic adrenal tissue in equine gonads. The observed prevalence was lower than that reported in the literature (2), and this may be explained by the fact that stressed animals were not available in this study. The presence of areas of adrenal tissue in the gonads is justified by the common embryologic origin of the cortical adrenal gland and gonadal ridge (1). Ectopic adrenal medulla was not detected in any case, and this can probably be related to the different embryological origins (1). It is likely that ectopia affects only a gonad (especially in the ovary), while the bilateral occurrence of ectopic adrenal glands is uncommon. The ectopic nodules are single or multiple in the ovary, always multiple in the testis, and generally located on the ovarian surface or in the testicular mediastinum. The different sites may also explain the different sizes of the nodules, comparing the ovary with the testis. The mediastinum testis is a network of fibrous connective tissue that extends from the upper to near the lower extremity of the testis, surrounded by testicular parenchyma. The

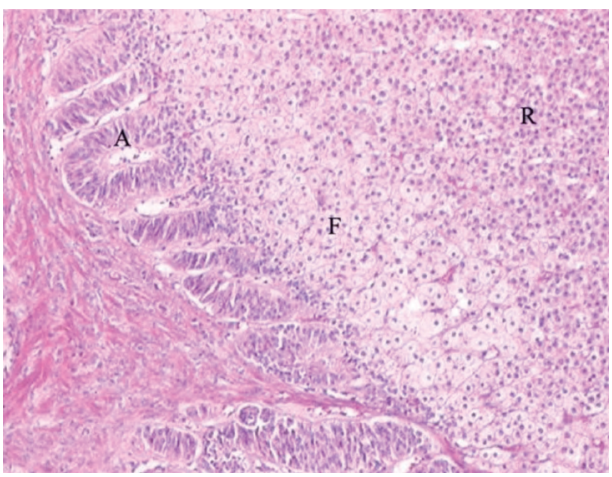

Figure 2. Microscopic layer organization in a large ectopic adrenal nodule. Regular distribution of zonae arcuata (A), fasciculata (F), and reticularis (R). Hematoxylin and eosin, original magnification 100×.

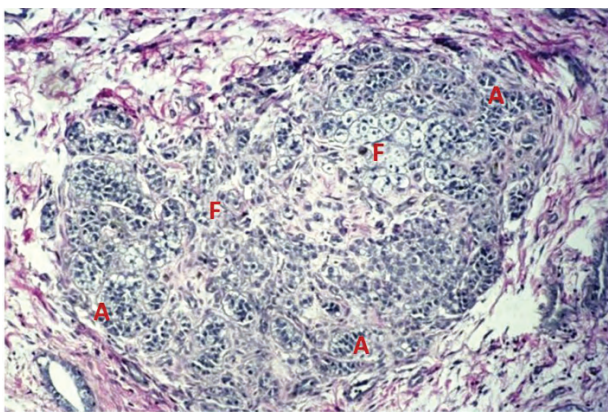

Figure 3. Microscopic appearance of an ectopic adrenal nodule in the mediastinum testis. Irregular organization of zonae arcuata (A) and fasciculate (F). Periodic acid–Schiff, original magnification 200×.

Table 2. Ectopic adrenal tissue in the testis. m = mediastinum.

No.	Affected testis	Number of nodules	Maximum diameter (mm)	Spermatogenesis
1	Both (m)	>5	2	Present
2	Right (m)	>5	2	Present
3	Left (m)	>5	3	Present
4	Right (m)	>5	3	Present
5	Right (m)	>5	3	Present
6	Left (m)	>5	3	Present

increased pressure that is present at the mediastinum may explain the reduced size of the ectopic nodules and the increased fragmentation.

It has been reported that these nodules develop hyperplasia under stress, during illness, or pregnancy (2,5), and this seems to suggest the sensitivity of ectopic adrenal tissue to adrenocorticotropic hormone, also confirmed by the high levels of cortisol found in this study. The extraction of 17-β estradiol, progesterone, and testosterone confirm the production of these hormones, previously established only for progesterone and some derivatives (11). For technical reasons, being that most nodules were only a few millimeters in diameter, it was not possible to extract steroids from each case, but rather from only 2 cases; thus, it cannot be excluded that small ectopic nodules were hormonoactive. It is possible that the hormones produced by the ectopic adrenal nodules spread to the adjacent parenchyma of the gonad, where they can act with a paracrine effect on the delicate gonadal activity. However, in the cases studied, the presence of corpora lutea in the females and regular spermatogenesis in the males suggests, at least at a histological level, a nonsignificant interference with the normal function of the gonads. Despite this, we suggest that large ectopic nodules always have to be kept under observation. This study failed to find proliferative changes in the ectopic tissue, as occasionally reported in humans (13–16). There were no data to support a preventive excision of the ectopic adrenal tissue in the gonads of the horse.

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