

Spontaneous fibroadenoma in a twenty-one-week-old female Wistar rat – a case report

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ABSTRACT

We describe the characteristic macro- and microscopic findings of a new case of spontaneous fibroadenoma in a 21-week-old, non-treated female Wistar rat. A mobile and non-ulcerated mass was detected affecting the caudal inguinal mammary gland. At necropsy, the mass was 5.5 cm in size, spherical in shape, and well demarcated with a smooth surface. The cut surface was pink, with a multilobulated pattern. Microscopically, fibroadenoma was composed of dense connective tissue surrounding luminal epithelial tissue, organized in tubular structures. The luminal epithelial cells were typical and, in some areas, presented lipid vacuoles in the cytoplasm and proteinaceous material in the lumen of the tubules. The interlobular and intralobular stroma showed variable amounts of mast cells. Immunohistochemically, epithelial cells expressed both oestrogen and progesterone receptors in the nucleus. Fibroadenoma is a frequent tumour, but it is uncommon at an early age.

Key words: immunohistochemistry; fibroadenoma; rat

Introduction

Fibroadenomas (FAs) are benign tumours of the mammary gland characterized by proliferation of both fibrous and epithelial components. They are the most common benign tumours occurring in young women, laboratory rats and even companion rats, but are uncommon in domestic animals (dogs and cats) (RUSSO, 2015; VERGNEAU-GROSSET et al., 2016; ZAPULLI et al., 2019). As spontaneous mammary tumours, FAs have a sex predilection, and are occasionally observed in

males. In female rats the incidence is high, between 30% to 68% (DINSE et al., 2010; RUSSO, 2015) being variable in almost all strains of female rats. Sprague-Dawley (SD) (72%) and companion rats (*Rattus norvegicus*) (53%) have a higher incidence than Wistar rats (22,3%) or ACI/N (4,8%) (SON et al., 2010; RUSSO, 2015; VERGNEAU-GROSSET et al., 2016; WEBER, 2017). Nevertheless, in males the incidence in Wistar rats is higher (11%) than in other strains such as SD (1-2%) (KROES

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et al., 1981). Mammary tumours in the rat, like most other neoplasms, occur spontaneously with increasing frequency as the animal ages. In general, FAs in Wistar rats occur more often between 31 and 36 months of age, being uncommon in Wistar and SD rats younger than 26 weeks. Some studies have observed that the incidence rate of mammary gland tumours in female rats that underwent ovariectomy at 90 days old was significantly lower than in older, sexually intact females (RUSSO, 2015; VERGNEAU-GROSSET et al. 2016).

Laboratory animals, in particular rodents (rats and mice), are a fundamental part of biomedical research as models in carcinogenicity studies of different cancer types and toxicological studies, so knowledge of both their behaviour and their possible pathologies are studied by veterinary experts to avoid any influence on studies using them. The rat mammary gland has been utilized extensively as a model for mammary carcinogenesis due to its similarity to human mammary glands (RUSSO, 2015). As always, the welfare of these animals, as well as the avoidance of stress and suffering, is a major concern in animal experimentation and is strictly regulated by the European Normative (Directive 2010/63/EU).

One of the main pathologies that appear in rodents is the development of spontaneous tumours, and there is a great deal of information on tumour development in adult rodents based on long-term studies, but this information is limited in young rats (KUZUTANI et al., 2012; MILLÁN et al., 2014). It would be useful to have data on the incidence of spontaneous tumours in animals in lower age groups to compare them in short-term studies.

To report new data about spontaneous tumours and avoid interference in future carcinogenicity studies, the aim of this study was to describe a spontaneous mass that appeared in a Wistar rat 21 weeks of age, that was non-treated but diagnosed as a mammary fibroadenoma.

Case description

A 21-week-old female Wistar rat from the breeding colony of the Experimental Animal

Service (SAEX) of the University of Córdoba (Córdoba, Spain) presented with a large mass in the mammary area. The rat was nulliparous, non-pregnant, and was housed in an individual ventilated cage, with the temperature at 21 ± 2 °C, 40-70% humidity, a 12h-12h light-dark cycle, and a minimum of 15-20 filtered air changes per hour. It had *ad libitum* access to a standard food pellet diet (A04S SAFE®) and drinking water, and was maintained in accordance with the European Union Directive 2010/63/EU guidelines for the protection of animals used for scientific purposes.

The rat had a raised mobile and non-ulcerated mass, 5.5 cm in diameter, on the left part of the inguinal mammary gland area, between the fourth and the sixth pair of mammary glands (Fig. 1) that grew quickly over 21 days after the initial clinical detection. During the clinical examination, no systemic signs appeared, the animal was alert, showing fully normal behaviour. It was eating and drinking normally, and urine and faeces were completely normal. Heart and breathing rates were within physiological parameters, and it showed normal reflexes and movements.



Fig. 1. A female 21-week-old Wistar rat with a non-ulcerated mass on the left part of the mammary gland area, between the fourth and the sixth pair of mammary glands.

Due to the large size of the mass, we decided to euthanize the rat at 24 weeks old, using a CO₂ chamber, as a recommended method in the 2010/63/UE Directive. We carried out a detailed necropsy which did not reveal any other macroscopically visible lesions. At necropsy, a raised mass was observed on the left part of the inguinal mammary

gland area. The skin was not ulcerated, and the tumour was spherical and freely movable in the subcutis (Fig. 1). On cut section, the mass was multilobulated with white and pink areas, and their consistency was soft and rubbery (Fig. 2). No gross lesions were observed in any other organs at necropsy.

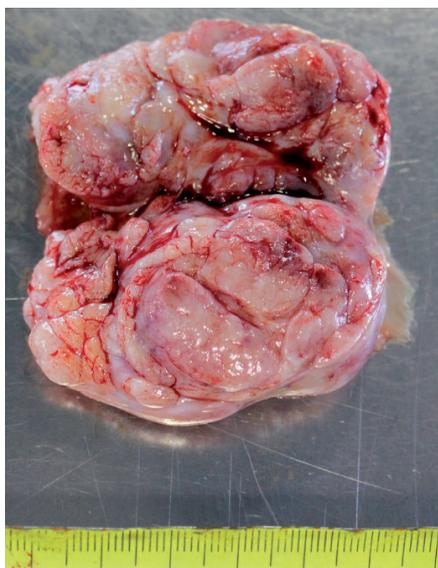


Fig. 2. Cut section showing a well circumscribed and multilobulated mass, 5.5 cm in size, with white and pink areas.

Tissue samples were taken from the mass, together with all the organs, fixed in 10% neutral buffered formalin, and processed routinely using standard histological procedures. All tissue sections, 3 μ m thick, were stained with hematoxylin, eosin and Giemsa. A immunohistochemical study was performed on several sections of the tumour. We used the avidin-biotin-peroxidase complex (ABC Kit Elite; Vector Corporation, Burlingame, CA, USA). The antibodies we used were mouse monoclonal antibody to human progesterone receptor (clone PR10A9, Immunotech) diluted to 1:500, and a polyclonal antibody to human oestrogen receptor (Zymed) diluted to 1:200. All these antibodies showed cross-reactivity with the tissue samples used as an internal positive control (GAYTÁN et al., 2005). As negative controls, the primary antibodies were replaced by mouse

IgG2 (Dako, Burlingame, CA, USA) for anti-PR and immunoglobulin fraction of serum from non-immunised rabbits (Dako, Burlingame, CA, USA) for anti-ER.

Results and discussion

Microscopically, the lesion was well-circumscribed, encapsulated and located in the dermis and subcutis. The mass had a lobular pattern, and every lobule was composed of both well-differentiated epithelial and fibrous connective tissue, in variable proportions. FA was predominantly composed of dense connective tissue that surrounded scarce luminal epithelial tissue, organized in glandular structures. Tubules were lined by cuboidal or columnar neoplastic cells with round and uniform nuclei. In some areas we could see a large proportion of epithelial cells that showed lipid vacuoles in the cytoplasm and proteinaceous material in the lumen, that would suggest secretory activity (Fig. 3). The fibroblastic stromal cells had elongated nuclei, with scant cytoplasm and indistinct cell margins. The inter- and intralobular stroma showed a variable infiltrate of mast cells with cytoplasmic metachromatic granules visible after Giemsa staining. Expression of both ER and PR was observed in the nuclei of the luminal epithelial cells and occasional myoepithelial cells of the acini (Fig. 4a and 4b).

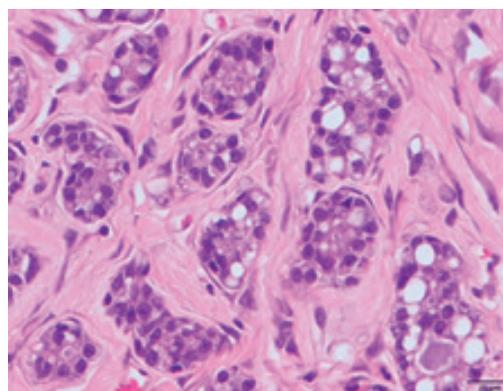


Fig. 3. Area of fibroadenoma with white vacuoles in the cytoplasm of the epithelial cells and proteinaceous material in the lumen. HE.

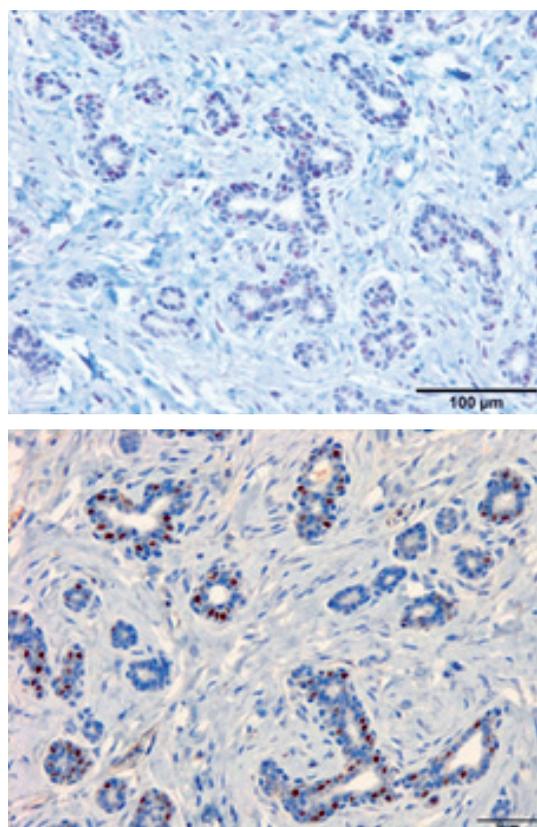


Fig. 4. Immuno-expression of ER (a) and PR (b) in the nucleus of luminal epithelial cells and occasional myoepithelial cells of the acini. ABC.

In this short paper we describe a spontaneous fibroadenoma in 21 week old, non-treated, female Wistar rat (one of the few reports of a young rat). FA is a common benign tumour observed in laboratory and domestic rodents, and in women of childbearing age, but it is but uncommon in domestic animals (VERGNEAU-GROSSET et al., 2016; RUSSO, 2015; TAVASSOLI and DEVILEE, 2003; ZAPULLI et al., 2019). Although FA is a benign tumour in laboratory rats, it is considered to be a fatal tumour because of the large size it can reach. This was a factor in the decision to euthanize the animal for humane reasons and its welfare.

In general, FA in Wistar rats occurs more often between 31 and 36 months of age and is uncommon in Wistar and SD rats younger than 26 weeks. The incidence in female rats may be age related and can be influenced by various factors (IKEZAKI et

al., 2011; RUSSO, 2015; SON et al., 2010). Only one case of FA has been reported in Wistar rats in 13-week studies (WEBER, 2017). VERGNEAU-GROSSET et al. (2016) analysed the prevalence of mammary gland tumours in companion rats, and observed that the age at diagnosis of fibroadenomas was from 8 to 54 months of age.

Our rat presented a nodule that affected the last inguinal mammary glands, between the fourth and sixth pair of left-hand mammary glands. Mammary tumours do not occur equally in all six pairs of mammary glands. It has been observed that virgin rats treated with a carcinogen develop a greater number of tumours in the thoracic mammary glands than in the abdominal-inguinal glands owing to the asynchronous development of mammary glands (RUSSO, 2015). However, in domestic rats spontaneous FA was observed more often in the axillary and ventral regions than in the abdominal-inguinal mammary glands (VERGNEAU-GROSSET et al., 2016). In our case, FA was easily recognized by its size (5.5 cm) and macroscopic appearance, as previously described (MANN et al., 1996; RUSSO, 2015) but some authors, such as IKEZAKI et al. (2011), analysing the kinds of spontaneous lesions in rats, observed no macroscopic lesions in 50% of FAs diagnosed (IKEZAKI et al., 2011).

In general, FAs are composed of both connective tissues and mammary epithelial cells in variable proportions, giving rise to two distinct growth patterns of FA without clinical significance, the intracanalicular and pericanalicular subtypes (low-cellularity and high-cellularity, respectively), in domestic animals, women and laboratory animals (RUSSO, 2015; TAVASSOLI and DEVILEE, 2003; ZAPULLI et al., 2019). The epithelial elements consist of elongated and branching tubules, lined by cuboidal or columnar epithelial cells, with a small round nucleus and a single nucleolus, and a basal layer of myoepithelial cells. The tubules may be widely distended with epithelial cells secreting lipids and proteinaceous material. An extensive stroma surrounds the tubules. Some FAs have loose connective tissue, rich in mucopolysaccharides, and with variable cellularity, while others have dense connective tissue with a variable number of

mast cells (MANN et al., 1996). The fibroblasts have elongated nuclei with scant cytoplasm and indistinct cell margins. Mitosis can be found in both stromal and epithelial components. Some fibroadenomas may contain areas of typical and atypical hyperplasia, with solid and papillary growths within the tubules and large cysts. The small foci of cellular atypia in FAs have been suggested to present an early premalignant change of adenocarcinoma (RUSSO, 2015). In women and other species, FA must be differentiated from other benign biphasic tumours, such as complex adenoma in rats and dogs, fibroadenomatous changes in cats, or phyllodes tumours in women (RUSSO, 2015; TAVASSOLI and DEVILEE, 2003; ZAPULLI et al., 2019).-

FA is presumed to arise from the terminal duct lobular unit (TDLU) area and to be enlarged by hormonal stimuli, such as oestrogen, progesterone, and prolactin (RUSSO, 2015; TAVASSOLI and DEVILEE, 2003). Our FA expressed nuclear expression of ER and PR in the epithelial cells of the acini, which could indicate a hormonal influence. Oestrogen and progesterone hormones (ovarian steroids) exert their functions by binding to their respective receptors (ER and PR). It is well-known that 96% of benign mammary tumours express ER and/or PR in the nucleus of their luminal cells (ZAPULLI et al., 2019).

In general, mast cells are normally observed in connective tissue but, in our FA, with Giemsa staining we identified large amounts of mast cells in some areas of the connective tissue. Mast cells are innate granulated immune cells, characterized by their cargo of inflammatory mediators, and they are normally found in the stroma of breast tumours. There is evidence that mast cells have both pro- and antitumor roles in breast cancer, and they have been correlated with luminal phenotype and the expression of ER and PR in the epithelial cells of mammary tumours (APONTE-LÓPEZ et al., 2018; MAJORINI et al., 2020).

FAs develop spontaneously in women and in other female animals (domestic and laboratory rodents, bitches, queens), influenced by hormone stimuli (RUSSO, 2015; ZAPULLI et al., 2019). In accordance with that, a study performed in

domestic rats observed that some rats with FA had concomitant cystic endometrial hyperplasia, a lesion caused by progesterone stimulation associated with oestrogen priming (VERGNEAU-GROSSET et al., 2016). In mice, spontaneous mammary tumours are also either linked to the infection of female mice with an exogenous mouse mammary tumour virus (MMTV) or a less virulent endogenous provirus (RUSSO, 2015).

Experimentally, FAs can be induced using hormones, especially high doses of oestrogen and chemical carcinogens. The carcinogens most used are DMBA (7, 12- dimethylbenz anthracene) and NMU (*N*-methyl-*N*-nitrosourea) with latency of 8-21 weeks. The sensitivity of the mammary glands to both carcinogens depends on the animal's age and genetic factors of different strains (RUSSO, 2015).

The susceptibility of the rodent mammary gland to the development of neoplasms has made this organ a unique target for testing the carcinogenic potential of specific chemicals. The Wistar rat is a strain of laboratory rodents widely used in toxicity studies, especially in long-term toxicological and carcinogenic studies, because they possess a number of advantageous characteristics, including a higher survival rate, a smaller size, lower incidences of tumours, and a well-defined tumour profile with relatively low incidences of tumours compared to those of other strains such as the SD or Fisher 344 (F344) rats (RUSSO, 2015; SON et al., 2010; WEBER, 2017).

In short- and long-term toxicity studies it is normal to observe unexpected tumours in young rats in the studies, and it would be useful to evaluate whether these tumours are incidental or associated with the toxicology study. Moreover, we should take notice of the fact that tumour development is affected by various factors such as diet, husbandry, environmental conditions, housing style and the source of the animals. Tumour data in the Wistar strain are not easily accessible in general for the younger age group, and it is for that reason, that we describe a case of spontaneous mammary fibroadenoma in a Wistar rat at 21 weeks of age that was not included in any study.

Conflict of Interest

The authors declare that they have no conflict of interest

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SAŽETAK

U ovom se radu opisuju karakteristični makroskopski i mikroskopski nalazi novog slučaja spontanog fibroadenoma u 21 tjedan stare ženke Wistar štakora. Otkrivena je pomična i neulcerirana masa koja je zahvatila kaudalnu ingvinalnu mliječnu žlijezdu. Obdukcijom je ustanovljena masa veličine 5,5 cm, sferična i dobro ograničena glatkom površinom, kod koje je na presjeku reza uočena ružičasta boja i višeslojni uzorak. Mikroskopski se fibroadenom sastojao od gustog vezivnog tkiva koje okružuje luminalno epitelno tkivo organizirano u tubularnim strukturama. Luminalne epitelne stanice bile su tipične i u nekim su područjima imale lipidne vakuole u citoplazmi i proteinski materijal u lumenu tubula. Interlobularna i intralobularna stroma pokazala je varijabilnu količinu mastocita. Imunohistokemijski su epitelne stanice ekspimirale i estrogenske i progesteronske receptore u jezgrama. Fibroadenom je čest tumor, ali je u mlađoj dobi njegova pojavnost rjeđa.

Ključne riječi: imunohistokemija; fibroadenoma; štakor
