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Histological and immunohistochemical characterization of the Mongolian gerbil's mammary gland during gestation, lactation and involution

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ABSTRACT

The morphological description of normal tissues is fundamental for making comparisons and in order to identify injuries and lesions. The aim of this work was to describe the morphological characteristics of the female Mongolian gerbil's (*Meriones unguiculatus*) normal mammary gland, the average expression of hormone receptors, and the average proliferation rates in the epithelial cells during the periods of lactation, pregnancy and involution. Dams were euthanized on the 14th and 21st gestational days, 7 and 14 days after parturition, and 3 and 5 days after weaning. The dams' mammary tissues were processed and were submitted to haematoxylin and eosin staining, Periodic Acid Schiff (PAS) staining, and Gomori's Reticulin staining. Additionally, immunohistochemistry was performed for the characterization of myoepithelial cells with α-actin, the proliferation rates with proliferating cell nuclear antigen (PCNA), the estrogen hormonal receptors (ESR1 and ESR2), and progesterone receptor (PR) quantifications. It was observed that the abundant adipose tissues were replaced by glandular epithelia and there was an increase in the epithelial cell's height (from 5.97 to 32.4 μm in 14th and 21st gestational days and from 20.64 to 25.4 μm in 7th and 14th lactational days, respectively) and the acini diameters (from 24.88 to 69.92 μm in 14th and 21st gestational days and from 139.69 to 118.59 μm in 7th and 14th lactational days, respectively) with the progression of gestation and lactation. The PAS staining intensity varied throughout the glands and between the stages that were evaluated. The extracellular matrix showed different phenotypes too, with more of a presence of the Type I collagen during the early gestation and involution and with more reticular fibers (Type III collagen) during the late gestation period and lactation. The myoepithelial layers showed alterations in their distribution with thick patterns as verified by the α-actin labeling. The PCNA showed higher rates of the marked cells in 14th and 21st gestational days (40.25 and 60.28%) and in 7th and 14th lactational days (64.08 and 65.08%). The hormone receptor quantifications showed a high variation in the rates: the average PR staining decreased from 14th to 21st gestational days (from 42.3 to 8.54%), from 7th to 14th lactational days (from 59.83 to 23.18%) and from 3rd to 5th days after weaning (from 39.98 to 12.72). There were higher averages of ESR1 staining in gestational days 14 and 21(from 58.06 to 30.02%). ESR2 staining decreased during gestation (25.7 and 12.94% in 14th and 21st gestational days)and involution (from 50.97 to 30.18% in 3rd and 5th days after weaning). The Mongolian gerbils showed similar morphological characteristics when they were compared to mice and rats. However, the higher proliferation rates with a smaller involution period compared to other murine characterized this species as being adequate for mammary pathologies studies.

1. Introduction

The mammary gland, which distinguishes mammals from all other animals, produces and secretes milk in order to nourish offspring. It is classified as apocrine exocrine tubuloalveolar (Inman et al., 2015; Oftedal, 2002). Although there are structural differences between species, several researches use the mouse and rat

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mammary gland as a model to infer about developmental mechanisms (Macias and Hinck, 2012; Howlin et al., 2006). In general, the primary mammary gland structure originates from the ectoderm (epithelium) and the mesoderm (stroma). The interaction between these tissues promotes the formation of the mammary placodes which develop towards the anterior portion of the body (Balinsky, 1950). Generally after birth, the gland remains quiescent until the beginning of the cyclic reproductive life, in response to hormonal cues. Thus, the mammary gland provides a unique model for biologists to study development and organ specificity (Inman et al., 2015).

In rats, the parenchymal compartment of the mammary gland is composed of different epithelial structures with distinct morphological and functional activities, comprising the luminal epithelium of ducts, ductules, terminal end buds, alveolar buds, alveoli and the underlying myoepithelial layer (Líška et al., 2016; Masso-Welch et al., 2000). As in most glandular tissues, the adult mammary gland comprises multiple cell types, including epithelial, adipose, fibroblasts, immune, lymphatic and vascular cells that work together to sculpt and maintain a functional organ. These different cell types have been demonstrated to be of importance at specific stages of mammary gland development (Inman et al., 2015). The increase of estradiol secretion during puberty promotes the storage of dense connective tissues and adipocytes among the lobules (Sinowatz, 2012). Between the luminal epithelium and basement membrane the contractible myoepithelium is present, promoting the milk excretion under oxytocin stimulation during pregnancy (Masso-Welch et al., 2000).

The gland undergoes deep modifications with its structure during the reproductive cycles due to the hypothalamic-hypophysis-gonadal axis activity. In nonpregnant female, the development of the mammary gland is rigorously controlled by the ovary. In general, estrogens are responsible for mammary ducts growth and progesterone is necessary for lobuloalveolar development in the mouse (Russo and Russo, 1996). Nevertheless, the functional and structural development occurs in the dam exclusively during gestation and lactation, followed by involution (Sinowatz, 2012). During these different stages, the cells of the mammary gland proliferate, differentiate and go through apoptosis in response to stimuli, giving rise to significant remodeling of the glandular tissue architecture (Inman et al., 2015).

In the dam, early in gestation, the combined influences of ovarian estrogen, progesterone, and inhibin, with the production of rat chorionic gonadotropin contribute to stimulate the gland to undergo active cell proliferation (Ying, 1988). In the gestational and the lactational periods, the morphological alterations in the dam include an alveolar expansion of the secretory epithelium regions towards the fat pad, culminating in a considerable remodeling of adipose tissue (Collins and Schnitt, 2012). The end of gestation is the lobuloalveolar phase of the mammary gland's development (Richert et al., 2000). In C57BL/6 mice during lactation, the pre-secretory cells go through an intense activity, releasing their components, together with apical region pieces of the cytoplasm to the lumen. In this strain, after the end of lactation, the absence of a mechanical suckling stimulation promotes the mammary involution: apoptosis, epithelial cell detachment, and an alteration of the acini's shape are characteristics of the first stage of mammary involution (Inman et al., 2015; Monks et al., 2008). In rats, together with the secretory alveolar collapse, there is removal of active cells and secretions by macrophages (Helminen et al., 1968). In human mammary involution the extracellular matrix remodeling that is associated with a new wave of apoptosis then occurs, promoting the substitution of the epithelium by the adipose tissues (Macias and Hinck, 2012). This process of expansion and regression can occur across multiple gestations during the reproductive phase, demonstrating that the mammary epithelial cells have considerable regenerative abilities (Arendt and Kuperwasser, 2015).

In female mice offspring, on day 14 of gestation, the epithelium of the mammary gland responds to estrogen, thanks to the presence of estrogen receptors (ESR) (Sampayo et al., 2013). In the adult mammary gland, however, only a small population of epithelial cells expresses ESRs and progesterone receptors (PRs). It has been stated in several species and, although not all of their functions are clear, it is known that ESR1 is the most important receptor during the mammary ductal morphogenesis. PRs are only necessary during alveologenesis and throughout the gestation period (Macias and Hinck, 2012). Despite ESR1 is related to cellular proliferation, it is known that it exerts a paracrine effect, since the ESR1 positive cells have shown that they are not the same as those that are positive for bromodeoxyuridine in Balb/c mice (Zeps et al., 1998). A paracrine mechanism is also shown by the PR-positive cells in mammary gland of ROSA26 and RAG1 mice (Brisken et al., 1998). Moreover, estrogen receptor gene ESR2 knockout mice have shown no deficiencies in the mammary structure or with lactogenesis (Mehta et al., 2014; Krege et al., 1998). This suggests a greater presence of ESR1 and PR during the development stages (regulating ductal outgrowth and morphogenesis) while ESR2 is associated with the non-proliferative phases of the gland (Macias and Hinck, 2012). The ESR1-knockout mouse is infertile and it presents a rudimentary development of the mammary ductal system, because it lacks the terminal end buds. It seems that the growth of ducts depends on the presence of ESR1 in the stroma, and that the availability of epithelial cells expressing ESR2 is insufficient to evoke a mammary proliferation induced by estrogen (Hamilton et al., 2014; Musumeci et al., 2015). ESRs and PRs are also present in mesenchymal cells surrounding the mammary gland (Bigsby et al., 2004).

In both rats and mice, the mammary glands are aligned ventrolaterally along the mammary or milk lines from de cervical to the inguinal regions. Female mice have five pairs of mammary glands: one cervical, two thoracic, and two abdominal-inguinal pairs. The female rat has six pairs, the thoracic, abdominal, and inguinal glands that vary in their degree of development in the nulliparous rats, with the inguinal being the most differentiated, and the cervical glands being the least differentiated. The anatomic location and distribution of these paired organs is similar between these two species (Russo and Russo, 1996).

Female Mongolian gerbil (*Meriones unguiculatus*), a polyestrous species, have an estrous cycle of 4–6 days (Nishino and Totsukawa, 1996) and reaches puberty at 9–12 weeks. Gestation lasts 24–26 days and weaning happens 3 weeks after birth (Marston and Chang, 1965). They show four pairs of mammary gland: two thoracic and two inguinal (Fig. 1). Each of them show only one galactophore duct (Yu and Anderson, 1975).

A morpho-physiological description of the mammary gland has been made when related to several species (Bellatine et al., 2010; Chandra et al., 2010; Saji et al., 2000; Zeps et al., 1998). However, a descriptive study about the morphological variations of a Mongolian gerbil's mammary gland is not available in the literature. These experimental models have been increasingly used due to their relatively high development rates of spontaneous tumors (Salyards et al., 2013; Campos et al., 2008), what mimics the natural tumor environment and enables the development of research in this particular area. Thus, the aim of this study was to describe the structural characteristics of the female Mongolian gerbil's mammary gland, the average patterns of the hormone receptor expression, and the average proliferation rates in the epithelial cells during the periods of lactation, pregnancy and involution as a contribution to understanding the various aspects that involve the development and functionality of these glands.

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