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Ovarian Pathology in Rhesus Macaques: A 12-year Retrospective

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Abstract

Background—Ovarian pathology is an important cause of decreased fertility and reproductive capability and may impact multiple systems, particularly in aging rhesus macaques.

Methods—Retrospective histopathologic and immunohistochemical analysis of 458 female rhesus macaque necropsies over 12 years at the New England Primate Research Center in Southborough, MA.

Results—Degenerative and inflammatory changes in the ovaries included mineralization, infiltration by lymphocytes, macrophages and multinucleated giant cells, endometriosis, and arteriopathy. Cystic changes included follicular cysts, cystic rete, and mesonephric duct cysts with cystic rete the most common. Neoplasms included granulosa cell tumors, cystadenoma, cystadenocarcinoma, and teratoma.

Conclusions—Ovarian lesions of the rhesus macaque are similar to those of cynomolgus macaques and humans. These lesions are frequently incidental findings but may impact metabolic and neurocognitive studies.

Introduction

Rhesus macaques are increasingly used in aging research, and a thorough understanding of ovarian pathology is important as alterations in normal physiologic function could have implications for research outcomes. Ovarian tissue is dynamic with changes precipitated by hormone levels, age, and health status of the animal. During the reproductive cycle, ovarian tissue transforms to allow for ovum development (follicles), ovum release (corpus hemorrhagicum), pregnancy support (corpus lutea), and regression (corpus albicans). As age and health status change, various degenerative, inflammatory, and neoplastic ovarian conditions can develop that negatively impact fertility, metabolic, and neurocognitive ability.

Rhesus and cynomolgus macaque species are important animal models for human disease and are used extensively in biomedical research. Although excellent reviews of ovarian pathology of cynomolgus macaques have been recently published there is little comprehensive literature regarding the rhesus macaque [3]. In the cynomolgus macaque, commonly reported lesions include cortical mineralization, polyovular follicles, various

ovarian cysts, ovarian surface epithelial hyperplasia, and ectopic ovarian tissue [3]. Ovarian neoplasms in this species include granulosa cell tumors, teratomas, and ovarian carcinomas [3]. In the rhesus macaque, dysgerminoma, teratoma, granulosa cell tumor, ovarian epithelial tumors, choriocarcinoma, and a placental site trophoblastic tumor have been reported, with teratomas reported most commonly [2,4,6,8,9,12,14–20].

Ovarian neoplasms represent 6% of all cancers in women [5]. Ovarian carcinomas (tumors derived from surface epithelium including serous, mucinous, endometrioid, and clear cell carcinomas, Brenner tumors, and cystadenofibromas) represent 65–70% of all ovarian neoplasms [5]. Germ cell tumors (teratoma, dysgerminoma, endodermal sinus tumor, and choriocarcinoma) represent greater than 15–20%, and sex cord-stromal tumors (fibroma, granulosa-theca cell tumor, and Sertoli-Leydig cell tumor) represent 5–10% of tumors in women [5]. An additional 5% of ovarian tumors are metastatic neoplasms to the ovary. Of the total ovarian tumors in women, approximately 70% of the carcinomas occur in women between 40 and 65 years of age [5].

Ovarian cysts in women can be solitary or multiple and commonly arise from remnants of the mesonephric duct or tubule, rete ovarii, or follicles. Other than follicular cysts, most cysts are non-functional and are common incidental findings [5]. Some cysts can become quite large and can be mistaken for neoplasms on clinical examination. There is currently no literature describing the incidence of ovarian cysts in rhesus macaques; however in the closely related cynomolgus macaque cystic rete, follicular cysts, and paraovarian cysts have been described [3]. Due to the scarcity of data available for the rhesus macaque, we performed a thorough 12-year retrospective study to characterize ovarian pathology in this species.

Materials and Methods

A database of necropsy and biopsy records at the New England Primate Research Center (NEPRC) was analyzed from 1997–2009 for ovarian pathology in Indian origin rhesus macaques (*Macaca mulatta*). Necropsy records from 1977 female rhesus macaques were reviewed, of which 458 had reproductive tissues including ovaries available for retrospective histologic analysis. Cases were reviewed by two board certified veterinary pathologists (ADM and SVW). All animals except for 2 were euthanized per experimental protocols. All animal procedures were followed in accordance with *The Guide for the Care and Use of Animals* and the standards of the Harvard Medical School Standing Committee on Animals and The Association for the Assessment and Accreditation of Laboratory Animal Care. All animals were necropsied within 24 hours of death and representative sections of all major organs were collected, fixed in 10% neutral buffered formalin (NBF), embedded in paraffin, sectioned at 5µm, and stained with hematoxylin and eosin (HE). Additional sections were prepared for immunohistochemistry.

Routine avidin-biotin-horseradish peroxidase immunohistochemistry was used to analyze the expression patterns of vimentin (M7020 clone VIM 3B4; 5' proteinase K pretreatment; 1:200; Dako, Carpinteria, CA), cytokeratin (M3515 clone AE1/AE3; 20' microwave pretreatment; 1:100; Dako), inhibin alpha (MS-1863-SO; 20' microwave pretreatment; 1:50; NeoMarkers, Fremont, CA) and smooth muscle actin (M0851 clone 1A4; 20' microwave pretreatment; 1:560; Dako) on paraffin sections. Sections were developed with the chromagen diaminobenzidine (DAB, Dako), and the slides were counterstained with Mayer's hematoxylin, dehydrated with xylene, mounted, and coverslipped. Negative controls consisted of adjacent sections stained with isotype- and concentration-matched irrelevant antibodies.

Results

Ovarian tissues were examined from 458 female Indian origin rhesus macaques ranging from infant to 31 years of age with a mean of 7.9 years and median of 6.3 years. The majority of the examined specimens (337/458, 73.5%) were identified as normal cycling ovaries. Of the 458 animals studied, 410 of these were infected with simian immunodeficiency virus (SIV).

Of the 337 specimens that were identified as normal cycling ovaries the following findings were noted: corpora lutea (47/337; 13.9%), corpora albicans (5/337; 1.5%), Graafian follicles (7/337; 2.1%), and regressing follicles (23/337; 6.8%) (Fig 1A). The remainder had no notable features.

The remaining 121/458 (26.4%) of cases exhibited a spectrum of pathologic changes. Multifocal cortical mineralization was the most common pathologic feature observed (42/458, 9.2%) (Fig.1B). Endometriosis involving the ovary occurred in 25/458 female rhesus (5.5%) with a mean age of 15.6 (Fig. 1D). 3/25 animals with endometriosis were infected with SIV. Six of twenty-five (6/25, 24%) of the animals with endometriosis had undergone at least 1 cesarean section. A small number of cases (31/458) had infiltration of inflammatory cells including lymphocytes (12/458, 2.6%), macrophages (12/458, 2.6%), and multinucleated giant cells (7/458, 1.5%) (Fig.1C). All of the cases with multinucleated giant cells (7/7) were SIV infected. One of the cases with macrophage infiltration had intranuclear cytomegalovirus inclusions within the macrophages. One case (1/458) had arterial intimal and medial hypertrophy consistent with SIV arteriopathy affecting intra-ovarian arteries.

Cysts or neoplasms were found in 22/458 (4.8%) of animals ranging from 1.1 to 31 years of age with the mean age of 12.8 years and median of 8.5 years. Cysts involving the ovary included follicular cysts (1/458; 0.2%), cystic rete (7/458; 1.5%), and mesonephric duct cysts (8/458; 1.7%). Follicular cysts were lined by vimentin and cytokeratin positive granulosa cells and were embedded in a smooth muscle actin (SMA) positive stroma (table 1). Cystic rete were multilocular, lined by a single layer of epithelial cells that was cytokeratin positive. These were supported by a stroma that was vimentin positive and SMA negative (Table 1) (Fig. 2 A, B, C). Lastly, mesonephric duct cysts were lined by a single layer of ciliated cuboidal epithelium that was cytokeratin and variably vimentin positive and surrounded by a thin rim of SMA positive stroma (table 1) (Fig. 3 A, B, C). Neoplasia was recorded in 6/458 animals (1.5%) with a mean age of 14.4 years. The most common neoplasm in female rhesus macaques was the granulosa cell tumor, observed in 4/458 animals examined (0.9%) (Fig. 4A). The granulosa cell tumors were variably-sized ranging from 1.5 cm to 3.0 cm, unencapsulated, expansile neoplasms that were composed of sheets of round to polygonal cells that often surrounded small, proteinaceous brightly eosinophilic concretions (Call-Exner bodies) (Fig. 4B). The neoplastic granulosa cells were variably immunopositive for cytokeratin, vimentin, inhibin, and smooth muscle actin (Fig. 4C). Two animals had developed epithelial tumors (2/458, 0.4%) consistent with a cystadenoma and a cystadenocarcinoma. These tumors were composed of multilocular masses that effaced much of the ovarian parenchyma. Small projections of neoplastic epithelial cells often protruded into the cystic spaces. Interestingly, the animal with the cystadenocarcinoma also had a granulosa cell tumor in the contralateral ovary. Lastly, one animal (1/458, 0.2%), 8 years of age had a unilateral teratoma. This neoplasm consisted of variably mature tissue types derived from mesoderm, endoderm, and ectoderm. No concurrent inflammation, hemorrhage, or degenerative changes were associated with the neoplasms.

Discussion

The goal of this 12-year retrospective analysis was to describe ovarian lesions observed in rhesus macaques. In the present study conducted at the NEPRC spanning a 12-year period, 121/458 (26.4%) of female Indian origin rhesus macaques exhibited ovarian pathology. In a normal aging human, expected changes based on age, reproductive cycle status, and health include normal stages of follicle and corpus luteum development. Although the majority of animals included in this study were infected with SIV, only rare lesions (giant cell oophoritis and arteriopathy) were directly associated with SIV infection. The rest of the lesions were considered to arise unassociated with the concurrent SIV infection. The most common pathologic feature recorded in the current study was mineralization in the ovarian cortex. This mineralization was random and not associated with a specific cell type. Baboons and cynomolgus macaques also have an increased incidence of mineralization, which likely is a type of dystrophic mineralization resulting from regressed follicles [3,13]. Inflammatory infiltrates such as lymphocytes, macrophages, and multinucleated giant cells within the human ovary have been associated with viral infection such as mumps virus and human immunodeficiency virus [5]. Oophoritis is an uncommon finding in rhesus macaques. All cases with multinucleated giant cell-associated oophoritis in the present study were concurrently infected with SIV.

In the present study 5.5% of animals examined had endometriosis involving the ovary. Various macaque species are commonly afflicted by endometriosis, which can have serious consequences on fertility and subsequent breeding. Endometriosis has been linked to uterine surgeries and genetics and is one of the most common reproductive disorders in rhesus macaques [11]. Uterine surgeries are thought to lead to implantation of uterine tissue in the coelomic cavity that can give rise to endometrial tissue. Other factors involved in the genesis of endometriosis include retrograde menstruation and the ability to mount an immune response. The incidence of endometriosis in captive rhesus macaque facilities is colony dependent and in one study was reported as high as 25% [7,10]. Endometriosis currently affects at least 5.5 million women in North America [11]. Severe cases often extend to involve the ovary and alter normal cycling and folliculogenesis.

Cysts were a common lesion encountered (16/458) with a relatively equal incidence between cystic rete ovarii and mesonephric duct cysts. Ovarian cysts are common in women and include follicular cysts, cystic rete, and mesonephric duct cysts [5]. Follicular cysts are lined by multiple layers of granulosa cells, which are cytokeratin and vimentin positive with the surrounding stroma smooth muscle actin positive. Cystic rete are dilated, often multilocular, masses that arise in the center of the ovary and are typically cytokeratin positive embedded in stroma that is vimentin positive and smooth muscle actin negative. The rete ovarii consists of three anatomical parts. These are the intraovarian rete, a connecting rete, and an extraovarian rete. In the current case series all cystic rete ovarii arose in from the intraovarian rete. No smooth muscle surrounds the rete tubules which distinguishes them from mesonephric tubules which are surrounded by a thin layer of smooth muscle. These cysts often compress the ovarian cortex and can retard folliculogenesis. Mesonephric duct cysts are lined by a single layer of ciliated cuboidal epithelium that is cytokeratin and variably vimentin positive surrounded by a thin layer of smooth muscle that is strongly positive for smooth muscle actin. In cynomolgus macaques, cysts adjacent to, but not within the ovary are reported to be the most frequent and are derived from the mesonephric duct [3]. In the present study, mesonephric duct cysts were the second most common cyst noted after cystic rete ovarii although both occurred at similar frequencies. None of the cystic lesions in the current study were severe enough to negatively impact normal ovarian function and can be considered incidental findings; however hormone producing cysts are

well described in the literature and, if secretory, could significantly alter scientific studies [5].

Neoplasia was uncommonly encountered (6/458 animals, 1.5%) and included epithelial tumors, granulosa cell tumors, and a teratoma. The incidence of neoplasia in the current study is slightly lower than might be expected; however, due to early termination in experimental studies, rhesus macaques are frequently submitted to the necropsy service at a young age and this likely decreases tumor incidence. Those animals in which tumors did arise were older than 10 years of age, except for the animal with the teratoma. All of the findings reported herein were largely incidental in young adult animals with a mean age of 7.9 years and represent a potential confounder for metabolic and cognitive studies that involve female rhesus macaques. The most common neoplasm was the granulosa cell tumor (0.9%), which is also relatively common in domestic animals. Only two animals (mean age 17.5 years) had epithelial tumors. In all cases, these tumors were moderate to large in size and likely would have negatively impacted normal ovarian function. Although no clinical tests were performed, it is possible that the granulosa cell tumors in the present cases were functional producing estrogens. Lastly there was a single case of a teratoma in an eight year old rhesus as an incidental finding.

Ovarian tumors are a common finding in aging women and most commonly arise from either the epithelial or follicular structures within the ovary. Ovarian neoplasms account for 6% of all cancers in the human female and are the sixth most common form of cancer in women in the United States [5]. Due to the difficulty of early detection, these neoplasms are responsible for almost half of the deaths from cancer of the female genital tract. About 80% of ovarian neoplasms are benign, and these occur mostly in young women between the ages of 20 and 45 years [5]. The malignant tumors are more common in older women between the ages of 40 and 65 years [5]. Although a broad retrospective of ovarian neoplasms in the rhesus macaque has not been performed, ovarian surface epithelial tumors, granulosa cell tumors, teratomas, and dysgerminomas have all been reported in addition to a single case each of choriocarcinoma and placental site trophoblastic tumor [2,4,6,8,9,12,14–20]. Of those reported in the literature, ovarian teratomas appear to be the most common ovarian tumor in rhesus macaques with granulosa cell tumors reported to be the second most common. This is interesting for animal model research as ovarian neoplasms derived from the surface epithelium are the most common ovarian tumor in women which does not appear to be the case in the rhesus macaque [5].

In conclusion, the rhesus macaque has similar ovarian pathology to cynomolgus macaques and humans. Cystic rete were the most common ovarian cysts followed by mesonephric duct cysts and follicular cysts. Tumors were generally uncommon with granulosa cell tumors the most represented followed by surface epithelial tumors. Additional data regarding spontaneous ovarian pathology should be valuable to diagnostic pathologists and research pathologists that commonly encounter rhesus macaques. These data are especially important to consider given the increased use of rhesus macaques in metabolic, dietary, and neurocognitive studies and the negative impact that hormone alterations can have on test animals.

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1. .

2. .

3. .

4. .

Table 1

Summary of Cystic and Neoplastic Ovarian Lesions in Rhesus Macaques

Animal Number	Age (days)	Cytokeratin	Vimentin	Inhibin	SMA	Immunohistochemical Diagnosis
Rhesus No. 1	3042	+	+	ND	+	Follicular cyst
Rhesus No. 2	8037	+	-	-	+	Cystic rete
Rhesus No. 3	418	+	+	ND	-	Cystic rete
Rhesus No. 4	4884	+	+	ND	+	Cystic rete
Rhesus No. 5	9434	+	+	ND	-	Cystic rete
Rhesus No. 6	8137	+	+	ND	+	Cystic rete
Rhesus No. 7	4847	+	+	ND	+	Cystic rete
Rhesus No. 8	7839	+	+	ND	-	Cystic rete
Rhesus No. 9	6627	+	+	ND	-	Mesonephric duct cyst
Rhesus No. 10	5394	+	+	-	+	Mesonephric duct cyst
Rhesus No. 11	1111	+	+	ND	+	Mesonephric duct cyst
Rhesus No. 12	4716	+	+	ND	+	Mesonephric duct cyst
Rhesus No. 13	2429	+	+	ND	-	Mesonephric duct cyst
Rhesus No. 14	2639	ND	ND	ND	ND	Mesonephric duct cyst
Rhesus No. 15	2010	ND	ND	ND	ND	Mesonephric duct cyst
Rhesus No. 16	1992	ND	ND	ND	ND	Mesonephric duct cyst
Rhesus No. 17	11326	+	-	ND	+	Cystadenocarcinoma
Rhesus No. 17	11326	+	-	+	+	Granulosa cell tumor
Rhesus No. 18	1481	ND	ND	ND	ND	Cystadenoma
Rhesus No. 19	5456	+	+	+	-	Granulosa cell tumor
Rhesus No. 20	7164	ND	ND	ND	ND	Granulosa cell tumor
Rhesus No. 21	3092	-	+	-	-	Granulosa cell tumor
Rhesus No. 22	2945	ND	ND	ND	ND	Teratoma

SMA = smooth muscle actin; ND = not determined