**SHORT PAPER**

**Short Title: Ovulation Fossa Cysts in Miranda Jennets**

# Bilateral Ovulation Fossa Inclusion Cysts in Miranda Jennets

**M. Quaresma[*], R. Payan-Carreira[†], M. dos Anjos Pires[†] and J. F. Edwards[‡]**

[*]*Veterinary Clinics and* [†]*CECAV, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal and* [‡]*College of Veterinary Medicine and Biomedical Sciences, Department of Veterinary Pathobiology, Texas A&M University, Texas, USA*

Correspondence to: R. Payan-Carreira (e-mail: rtpayan@gmail.com).

**Summary**

Multiple cysts ranging from 2–111 mm were noted bilaterally in the ovulation fossa of 11 mature Miranda jennets. These ovulation-fossa inclusion cysts (OFICs) were lined by a simple low to columnar epithelium that included many ciliated cells. Although most cases were incidental findings, two of the jennets were presented with reduced fertility. Extensive cyst formation could have been responsible for the reproductive problems because they replaced most of the ovarian parenchyma. Due to their close proximity to the ovulation fossa, the OFICs may have mechanically interfered with passage of eggs into the oviduct. OFICs are histologically common in equids, but are reported uncommonly as gross lesions in either mares or jennets. Ovarian inclusion cysts are associated with neoplasia in **women**; however, these OFICs showed no evidence of epithelial hyperplasia or cellular atypia and no evidence of independent growth; therefore, they were considered to be non-neoplastic. The bilateral occurrence and high incidence of OFICs in Miranda jennets, a breed with limited genetic variability, suggests that the lesion has a genetic causation.

*Keywords:* ovulation-fossa inclusion cysts; ovary; donkey; jennet

Ovarian inclusion cysts are epithelium-lined structures that form in response to trauma or wounds. Epithelium entrapped below the surface during the healing process forms cysts separate from the overlying epithelium. The development of ovarian inclusion cysts has been associated with repetitive cycles of ovulation with healing as the female ages (Clement, 1994; Kennedy *et al.*, 1998; Heller *et al.*, 2005; Tan *et al.*, 2005). The term ‘germinal inclusion cyst’ has also been used for ovarian inclusion cysts that in

most species are lined by a simple epithelium similar to that of the ovarian surface. Because of the unique structure of the equine ovary, all ovulations occur in a small area of the cortex known as the ovulation fossa. Thus, equine ovulation fossa inclusion cysts (OFICs) are concentrated in the fossa and not distributed over the entire ovary (O'Shea, 1968; MacLachlan and Kennedy, 2002; Schlafer and Miller, 2007). Microscopically, OFICs are common and with ageing they increase in size and number (Prickett, 1966).

Occasionally, OFICs grow and accumulate in a process that can destroy an ovary (MacLachlan, 1987). Non-functional ovaries associated with OFICs are usually reported as being unilateral. While OFICs are well recognized, they are usually microscopic and clinical reports involving OFICs in equids are sparse (Hinrichs *et al.*, 1989). Microscopically, equine OFICs are different from ovarian inclusion cysts in other species in that they are lined by a simple or pseudostratified, columnar epithelium that includes ciliated and non-ciliated cells (O'Shea, 1968). This mixed lining is presumed to occur because of the proximity of the ovulation wound to the tubal infundibulum.

The Miranda donkey is a breed of *Equus asinus* subspecies *europeus* that has been derived from breeding stock in the north eastern region of Portugal and is the only recognized Portuguese donkey breed (Quaresma *et al.*, 2005). Little is known about pathological conditions of this breed that is threatened by extinction. During recent work attempting to rescue the breed, a high incidence of bilateral OFICs was observed in animals during necropsy examinations. Some cases were associated with infertility.

From October 2007 to June 2009, the reproductive tracts of 11 Miranda jennets (17–33 years of age) were obtained at necropsy examination. Ten of these animals were humanely destroyed because of emaciation and one was destroyed due to a broken leg. The animals were otherwise normal and none of them had been used for breeding

in the previous 4 years. Two of the emaciated animals (numbers 1 and 2) had been inseminated on two successive cycles, but had failed to conceive. Six months prior to death, these animals had been subjected to a reproductive evaluation that revealed bilaterally enlarged ovaries that were rounded in shape (the ovary of the jennet is typically bean shaped). Transrectal ultrasound showed follicles and corpora lutea on the ovaries as well as clusters of small, anechoic, fluid-filled cysts within the ovulation fossa. A second evaluation 2 weeks later showed that the cysts in the ovulation fossa were unchanged. The serum concentrations of oestradiol, testosterone and progesterone of these animals were within normal range for the species (Henry *et al.*, 1987).

The reproductive tracts were removed immediately after the animals were humanely destroyed and were fixed in 10% neutral buffered formalin. Subsequently, they were examined, photographed and samples of the ovulation fossa were submitted for microscopical examination. Eight ovaries from four Miranda jennets free of disease and having no macroscopic OFICs were used as control tissue.

Gross evaluation of all 11 reproductive tracts showed an increase in ovarian size due to the presence of multiple, round, thin-walled cysts (2–111 mm) protruding from the ovulation fossa **(Table 1)**. These cysts induced the modified shape of the ovaries described above (Figs. 1, 2). In general, the pairs of ovaries were of different sizes due to either a higher number or a larger size of the cysts. Some affected ovaries were only slightly enlarged when compared to control ovaries, but control ovaries did not have the thin-walled cysts disfiguring the ovulation fossa (Figs. 3, 4). The cyst walls were uniform, thin and smooth, and the prominent surface ovarian blood vessels were smaller and less prominent where they ran over the cysts. Unlike developing follicles, vessels were not noted in the cyst wall. In all cases, the fimbria was adherent, but displaced peripherally by the cysts. The cysts were generally filled by colourless or bright yellow

serous fluid. The ovaries with multiple cysts or larger cysts had reduction of the ovarian parenchyma (Figs. 3, 4). Occasionally, smaller cysts appeared to grow from the wall of larger cysts.

Microscopically, follicles and corpora lutea in different stages were detected in the ovaries from control jennets and most affected jennets. The presence of granulosa cell layers permitted identification of developing and atretic follicles (Fig. 5). In control animals, the external surface of the ovulation fossa was lined by a simple cuboidal epithelium that became pseudostratified within the fossa and near the edge of the infundibulum. The majority of the cells were ciliated. Lining cells had a basilar nucleus and pale eosinophilic cytoplasm. Cysts within the ovulation fossa were lined by a single layer of attenuated, cuboidal or columnar epithelial cells similar to the surface lining of the control ovulation fossa (Figs. 5, 6). Attenuated epithelial cells lined the larger cysts and in cysts that were not compressed a similar number of ciliated epithelial cells were irregularly dispersed between non-ciliated cells. Occasionally, the epithelial cells had apical blebbing (Fig. 6). Mitoses were rare. Based on the location of the cysts and the nature of the lining cells, the structures were diagnosed as OFICs.

Differential diagnoses for enlarged ovaries with one or multiple cysts in equids include mature Graafian follicles, ovarian haematomas, transitional follicles, OFICs and granulosa cell tumours. Bilateral cystic enlargement has not been reported in equids. Study of the 11 jennets in this report showed that the cystic ovarian changes were associated with bilateral OFICs, with the number and the size of cysts often being different between the ovaries of the same jennet. Less-affected ovaries had normal developing follicles and corpora lutea observable grossly and microscopically; however, when ovaries were severely affected, most gonadal parenchyma was lost. Because the cysts occur in the ovulation fossa and persist and accumulate secretion, it is reasonable

to speculate that this bilateral process would result in infertility by preventing the ovum from reaching the oviduct or by causing compression and degeneration of adjacent ovarian parenchyma. Further, the formation of large cysts may predispose to more and larger cyst formation because as OFICs grow and follicles are compressed, the edges of the follicles are moved further apart following ovulation and the normal healing of the follicle edges would be compromised. Two types of epithelium line the surface of the ovulation fossa. A ciliated cuboidal to columnar epithelium similar to that of the infundibulum lines most of the fossa; however, the ciliated epithelium of the fossa interfaces abruptly with a simple cuboidal or squamous epithelium that is continuous with the lining of the remainder of the ovarian surface. Therefore, the lining of these OFICs reflects the proliferation of entrapment of the fossa lining.

The equine ovary is unique among animal species in that the cortical tissue is concentrated around the ovulation fossa and all ovulations occur into the fossa. The medullary tissue of the mare is distributed more peripherally; therefore, cystic epoophoron and rete cysts are located away from the ovulation fossa**. For that reason** these types of cyst were eliminated as diagnoses for the cysts observed in these jennets.

Three types of cyst are commonly found in the equine ovulation fossa (O'Shea, 1968). Fossal cysts are blind-ended, non-follicular cysts within the ovary that are lined by a simple or pseudostratified columnar or cuboidal epithelium, a proportion of which are ciliated. Serial sections of these cysts have shown that they may be tubular and branched and can communicate with other fossal cysts, but that they do not communicate with the surface of the fossa. Sometimes, they are visible grossly and multiple. Larger cysts tend to have an attenuated lining, and the fibres of the supporting connective tissue parallel the basement membrane. Fossal cysts are also called germinal inclusion cysts, but this term is considered a misnomer by some who underscore the fact

that there is no germinal epithelium in the pubertal mare ovulation fossa (O'Shea, 1968; Walt *et al.*, 1979; McEntee, 1990).

Fimbrial cysts and cysts of developing Graafian follicles are also seen in or close to the ovulation fossa (O'Shea, 1968). Although fimbrial cysts have a similar lining to that of fossal cysts, they develop superficially and subjacent to the fimbria, while fossal cysts develop within the ovarian parenchyma in the fossa (Hughes *et al.*, 1980). The hydatid of Morgagni is a congenital fimbrial cyst of paramesonephric origin commonly found in the cranial end of the infundibulum in mares (McEntee, 1990) and it does not resemble OFIC. The cysts of developing follicles are recognizable due to their characteristic granulosa cell lining (MacLachlan, 1987). Again, the location and histology of these cysts are such that they can be differentiated from OFICs.

Because OFICs were found only in old animals and younger jennets of the same breed were unaffected, it seems reasonable that bilateral OFICs are an acquired lesion. Nevertheless, this report involves a population of a donkey breed with limited genetic variability (Quaresma *et al.*, 2005). Bilateral OFICs in Miranda donkeys could represent development of congenital cysts that appear clinically only in aged donkeys. The development of Miranda donkey ovaries should be studied over time to establish when the OFICs develop and when they might begin to affect fertility. Such a study may be an opportunity to study the pathogenesis of OFIC formation. Although most authors believe that entrapment of portions of the fossa and fimbriae following ovulation causes OFICs, the possibility that the lesion represents ingrowths of the fossa epithelium into the ovarian stroma remains a hypothesis that has not been disproven (Bosu and Smith, 1993; MacLachlan and Kennedy, 2002; Schlafer and Miller, 2007).

In **women**, some authors consider inclusion cysts to be precursor lesions for cystadenomas or cystadenocarcinomas (Fenoglio, 1980; Russell, 1994) and early lesions

of ovarian epithelial neoplasia are reported in inclusion cysts, especially in ovaries contralateral to ovaries affected by epithelial neoplasia. However, the relationship of inclusion cysts to ovarian neoplasia remains controversial and some authors believe that inclusion cysts are not associated with ovarian neoplasia (Westholf *et al.*, 1993). In equids, cystadenomas are uncommon and the major veterinary reference textbooks do not describe equine ovarian cystadenomas (Kennedy *et al.*, 1998; MacLachlan and Kennedy, 2002; Schlafer and Miller, 2007). The cystadenomas that are reported in horses resemble OFICs (Hughes *et al.*, 1980; Held *et al.*, 1982; Taylor et al., 1983; Hinrichs *et al.*, 1989). All cases involved unilateral, large, cystic or polycystic ovaries. Two mares had mild elevation of testosterone, but the contralateral ovary was normal, and the mares continued to cycle (Hughes *et al.*, 1980; Hinrichs *et al.*, 1989). The cysts in mares were lined by a simple cuboidal epithelium that included ciliated and non-ciliated cells, but no features of neoplasia were described. One reported cystadenoma in a jennet consisted of a 20 × 17cm cyst (containing 2. 5l of fluid) with a wall that was 3 cm thick. This structure replaced the ovary in that case. The thick fibrous wall had a large area of haemorrhage and necrosis suggesting that the lesion may have been on the verge of rupture at the time of surgery. Microscopically, the wall of this ovary had necrosis, mineralization and leucocyte infiltration. Where the lining persisted, the cysts were lined by simple cuboidal epithelium that included ciliated and non-ciliated cells with small epithelial projections (Taylor *et al.*, 1983). The case in this jennet is difficult to assess because the ovary was necrotic, and it may represent a number of conditions of an enlarged ovary undergoing pressure ischaemia. As with some human cystadenomas, the diagnosis of cystadenoma in the mare may be arbitrary. When viewing potential cases of cystadenomas in equids without strong evidence of paraneoplastic disease,

clonality, anaplasia or atypia, severe involvement with OFICs should be considered as a differential diagnosis.

OFICs are microscopically common in equids, but cases of gross OFICs that deform ovaries and may cause infertility have only been published in reviews in proceedings or textbooks as an uncommon, unilateral condition. Bilateral OFICs have not been previously described. The clinical importance of OFICs has not been established by published clinical studies. The biology of the Miranda donkey is being characterized during efforts to rescue the breed. Veterinarians must be aware of and study the potential adverse effect of OFICs on fertility of this breed. The high incidence of bilateral OFICs in the Miranda donkey suggests the breed has a genetic predisposition to this condition.

## Conflict of Interest

The authors declare that they have no competing interests.

## References

Bosu W, Smith C (1993) Ovarian abnormalities. In: *Equine Reproduction*, AO McKinnon, JL Voss, Eds., Lea & Febiger, Philadelphia, pp. 397–403.

Clement P (1994) Non-neoplastic lesions of the ovary. In: *Blaustein's Pathology of the Female Genital Tract*, RJ Kurman, Ed., Springer-Verlag, New York, pp. 597-645.

Fenoglio C (1980) Ultrastructural features of the common epithelial tumors of the ovary. *Ultrastructural Pathology*, **1**, 419-444.

Held J, Buergelt C, Colahan P (1982) Serous cystadenoma in a mare. *Journal of the American Veterinary Medical Association*, **181**, 496-498.

Heller D, Murphy P, Westhoff C (2005) Are germinal inclusion cysts markers of ovulation? *Gynecological Oncology*, **96**, 496-499.

Henry M, Figueiredo A, Palhares M, Coryn M (1987) Clinical and endocrine aspects of the oestrous cycle in donkeys (*Equus asinus*). *Journal of* ***Reproduction and Fertility***, **Suppl. 35,** 297-303.

Hinrichs K, Frazer G, deGannes R, Richardson D, Kenney R (1989) Serous cystadenoma in a normally cyclic mare with high plasma testosterone values. *Journal of the American Veterinary Medical Association*, **194**, 381-382.

Hughes JP, Kennedy PC, Stabenfeldt GH (1980) Pathology of the ovary and ovarian disorders in the mare. In: *Proceedings of the 9$^{th}$ International Congress on Animal Reproduction*, Madrid, pp. 203–322.

Kennedy P, Cullen J, Edwards J, Goldschmidt M, Larsen S *et al.* (1998) Tumours of the ovary. In: *Histological Classification of the Tumours of the Genital System of Domestic Animals*, Armed Forces Institute of Pathology, Washington DC, pp. 24-28.

MacLachlan N (1987) Ovarian disorders in domestic animals. *Environmental Health Perspectives*, **73**, 27–33.

MacLachlan N, Kennedy P (2002) Tumors of the genital system. In: *Tumours in Domestic Animals*, D Meuten, Ed., Iowa State Press, Ames, pp. 547-573.

McEntee K (1990) Cysts in and around the ovary. In: *Reproductive Pathology of Domestic Mammals*, Academic Press **Inc., San Diego,** pp. 52-68.

O'Shea J (1968) A histological study of non-follicular cysts in the ovulation fossa region of the equine ovary. *Journal of Morphology*, **124**, 313-320.

Prickett ME (1966) Pathology of the equine ovary. In: *Proceedings of the12th Annual American Association of Equine Practitioners Convention*, Los Angeles, pp. 145–153.

Quaresma M, Nóvoa M, Monteiro A, Almeida JM, Portas M (2005) A raça Asinina de Miranda [The donkey breed Asinina de Miranda]. *Revista Portuguesa de Ciências Veterinárias*, **100**, 227-231.

Russell P (1994) Surface epithelial-stromal tumours of the ovary. In: *Blaustein's Pathology of the Female Genital Tract*, RE Kurman, Ed., Springer-Verlag, New York, pp. 705-782.

Schlafer D, Miller R (2007) Pathology of the ovary (non-developmental lesions). In: *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, Vol. 3, 5th Edit., MG Maxie, Ed., Elsevier Limited, St Louis, pp. 444-450.

Tan O, Hurst P, Fleming J (2005) Location of inclusion cysts in mouse ovaries in relation to age, pregnancy, and total ovulation number: implications for ovarian cancer? *Journal of Pathology*, **205**, 483-490.

Taylor T, Bowen J, Montgomery D (1983) Cystadenoma of the ovary in a Jennet. *Compendium on Continuing Education for the Practicing Veterinarian*, **5**, S677-679.

Walt M, Stabenfeldt G, Hughes J, Neely D, Bradbury R (1979) Development of the equine ovary and ovulation fossa. *Journal of Reproduction and Fertility*, **Suppl. 27,** 471-477.

Westholf C, Murphy P, Heller D, Halim A (1993) Is ovarian cancer associated with an increased frequency of germinal inclusion cysts? *American Journal of Epidemiology*, **138**, 90-93.

*Received, November 15th, 2010*

*Accepted,*

**Figure Legends**

Fig. 1. Uterus and ovaries from jennet number 7 showing bilateral OFICs. Ovaries are enlarged, rounded and unequal in diameter.

Fig. 2. Unsectioned fixed ovary from jennet number 7. OFICs fill the ovulation fossa to enlarge the ovary and give it a rounded shape.

Fig. 3. Sectioned fixed ovary from jennet number 10. The ovulation fossa (white arrow) is filled by multiple inclusion cysts. Normal ovarian parenchyma (dark arrow) remains opposite the fossa.

Fig. 4. Sectioned fixed ovary from jennet number 9. Variably sized inclusion cysts replace the ovarian parenchyma and fill the ovulation fossa.

Fig. 5. Jennet ovary. A developing Graafian follicle with a layer of granulosa cells (thick arrow) is distinguishable from the lining of an OFIC (thin arrow). HE. Bar, 100μm.

Fig. 6. Jennet ovary. An OFIC is lined by short columnar cells many of which are ciliated. Note an occasional cell with apical blebbing. HE. Bar, 20μm.

**Table 1**

**Summary of reproductive data and gross evaluation of the reproductive tract of 11 Miranda jennets**

| *Animal number* | *Age* | *Last recorded oestrus* | *Left Ovary (cm)·* | *Right ovary (cm)* | *Apparent ovarian activity*[†] | *Uterine disease* |
|---|---|---|---|---|---|---|
| 1 | ≥ 20 | 2006 | 7.5 × 7 × 5<br>Multiple cysts 3–30 mm | 5 × 2.5 × 3.5<br>Multiple cysts 4–35 mm | Yes | Not detected grossly |
| 2 | ≥ 24 | -- | 8 × 5.5 × 4<br>Multiple cysts 2–30 mm | 6 × 3.5 × 5<br>Multiple cysts 3–35 mm | Yes | Multiple endometrial cysts |
| 3 | 20 | -- | 4.5 × 3 × 4<br>Multiple cysts 2–25 mm | 6 × 5.5 × 4<br>Multiple cysts 5–30 mm | Yes | Not detected grossly |
| 4 | 18 | -- | 9 × 5 × 5<br>Multiple cysts 8–55 mm | 4.5 × 3 × 4<br>Multiple cysts 5–12 mm | Yes;<br>parenchyma of the left ovary compressed by cysts | Not detected grossly |
| 5 | 33 | -- | 5 × 2.5 × 3<br>Four cysts 3–17mm | 4.4 × 4× 2.5<br>Three cysts 2–15 mm | Yes | Not detected grossly |
| 6 | 20 | -- | 5 × 4 × 3<br>Five cysts 4–10mm | 5.4 × 2.5 × 4.5<br>Four cysts 2–15 mm | Yes | Not detected grossly |
| 7 | 18 | -- | 18 × 12 × 10<br>Multiple cysts 4–111mm | 7 × 5 × 4<br>Multiple cysts 4–25 mm | No;<br>parenchyma compressed by cysts | Not detected grossly |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | 23 | -- | 7 × 3.5 × 4<br>Three cysts 3–6mm | 5.5 × 3.5 × 3.5<br>Three cysts 2–5 mm | Yes | Not detected grossly |
| 9 | 21 | -- | 11 × 8 × 8.5<br>Multiple cysts 4–98mm | 8.5 × 8 × 5<br>Multiple cysts 4–40 mm | Yes;<br>parenchyma of the left ovary compressed by cysts | Multiple endometrial cysts |
| 10 | 17 | -- | 4.5 × 4 × 4<br>Multiple cysts 4–10mm | 5.5 × 5 × 4<br>Multiple cysts 3–15 mm | Yes | Not detected grossly |
| 11 | ≥ 20 | -- | 4.5 × 3.5 × 3<br>Multiple cysts 4–10mm | 7 × 4.5 × 4<br>One large cyst (45 mm) and 3 smaller cysts (10mm) | Yes | Not detected grossly |

*Ovary measurements correspond to length × width × thickness.

†Presence of follicles or a corpus luteum were assumed as suggestive of ovarian activity