



Research Article

**Suspected Case of Granulosa Cell Tumour from Ovarian Tissues
of One-Humped Camel in Maiduguri, Nigeria using Abattoir
Samples**

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ABSTRACT

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A total of 150 non-pregnant dromedary camels' ovaries were collected from Maiduguri central abattoir and examined for the presence of any tumour mass. Thorough macroscopic examination was carried out with emphasis on ovarian tissues for the screening of any abnormal swelling or growth around the said tissue. Although histo-pathological examination was not conducted on the ovarian tissues with suspected neoplastic swelling but was fixed in 10% buffered neutral formalin for later further investigations and referencing. Only a single case of granulosa cell tumour was identified on suspicion but not confirmed. It was grossly observed and identified as only a unilateral swelling and or abnormal growth in the affected left ovary of the female camel. A longitudinal section with a sharp scarpel blade of the suspected neoplastic mass appeared cystic. One of the constraints of the study was that the cut tissue of suspected tumour mass was not subjected to microscopic screening because of the constraint of no adequate reagents and lack of availability of expert to read slides for confirmation as a tumour mass. However, the study remained relevant as it demonstrated the usefulness of gross pathological examination in determining the characteristics of granulosa cell tumour as one of the ovarian tumours originating from germ cell especially as camels are known as hardy animals that hardly come down with diseases let alone a tumour growth. It was also the first time a case of granulosa cell tumour was detected even at suspicious level in the study. Despite a known fact that camels are hardy animals that hardly come down with diseases let alone a tumour growth. It is recommended therefore that adequate facilities for pregnancy diagnosis and assessment of hormonal imbalances be made available in most cattle markets, clinics and abattoirs across the Nigeria to help early diagnosis and guard against subsequent future occurrences.

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INTRODUCTION

The name camel refers to *Camelidae* of the old world known as camelini tribes represented today by the three species which includes; the wild Bactrian camel (*Camelus ferus*), the domesticated Bactrian camel (*Camelus bactrianus*) which is also called Asian camel and the domesticated dromedary camel (*Camelus dromedarius*) also called Arabian or Indian camel and whose their wild ancestors went extinct during one (1) millennium B.C. (Renato *et al.*, 2017). The wild bactrian camel (*Camelus ferus*) is a critically endangered species of camel living in parts of Northern China and Southern Mongolia. It is closely related to Bactrian camel (*Camelus bactrianus*). Both types of camels are large, double-humped, even-toed ungulates (AIO, 2012). Until recently, wild bactrian camels were thought to have descended from domesticated bactrian camel. However, genetic studies have established that the wild bactrian camel (*Camelus ferus*) are separate species which diverged from the domesticated bactrian camel about 1.1 million years ago (Silbermayr *et al.*, 2010; Burger *et al.*, 2016; Mohandesan *et al.*, 2017).

The bactrian camel (*Camelus bactrianus*) is a large even-toed ungulate native to the steppes of central Asia. The Bactrian camel has two humps on its back in contrast to the single-humped of dromedary camel. Its population of two million exists mainly in the domesticated form (EDGE, 2010). The name of this specie of camel comes from the ancient historical region of Bacterian. The Arabian camel also called *Camelus dromedarius* is an even-toed large ungulate with one hump on its back. The dromedary is the second largest member of the camelus family after the bactrian camel (Grove *et al.*, 2011). Unlike the dromedary camel with one hump on its back, the bactrian camel (*Camelus bactrianus*) and the wild Bactrian camel (*Camelus ferus*) have two humps on their backs.

Reproductive disorders in she-camel are rapidly becoming a major part of the veterinary care provided to the Camelidae especially when dealing with genetically superior animals. The incidence and pathology of the genital tract affections of she-camel could provide valuable information which can be used in evaluation of animals reproductively. The dromedary camels have ovaries as paired sex glands or gonads in the female and are importantly concerned with germ cell maturation, storage and its release. Basically ovaries are also concerned with steroidogenesis which is very rigorous for normal ovarian processes, such as follicle growth, maturation of oocyte, and ovulation. Contrary to normal functions, altered steroidogenesis as a result of any neoplastic reaction can lead to profound ovarian pathology (Ali *et al.*, 2013).

Group of neoplasms affecting the ovary and having a diverse spectrum of features according to the particular tumour entity are called ovarian tumours. Tumors may develop from any tissue type located within the ovary. Ovarian tumours are subdivided into three main categories; germ cell tumours, epithelial tumours and sex cord stromal tumours (Kennedy *et al.*, 1998). The granulosa cell tumor (GCT) is a sex cord–stromal tumor and is by far the most common tumor of the equine ovary (Bosu *et al.*, 1993; Clark, 1975; Cordes, 1969; Huges *et al.*, 1980; Meagher *et al.*, 1977; Norris *et al.*, 1968). GCTs account for more than 85% of equine reproductive tract tumors (Mc Cue *et al.*, 1998) and approximately 2.5% of all neoplasms in horses (Sundberg *et al.*, 1977). Some sex cord–stromal tumors are composed primarily of granulosa cells and are termed granulosa cell tumors, whereas others contain granulosa and theca cells and are consequently termed granulosa–theca cell tumors (GTCTs) (Jubb *et al.*, 1985). The most common type of ovarian tumours has been reported as teratomas by

Tibary and Anouassi, (2000). In addition, tumors of mesenchymal tissue (i.e., fibromas, hemangiomas) may develop within the ovary, and secondary tumors that originate in another location (i.e., lymphosarcoma, melanoma) may metastasize to the ovary. Dysgerminoma is extremely rare type of tumour but there were reports of these occurrences by El-Khouly *et al.* (1990). Although there are scanty or no information on granulosa cell tumours in camels, Ali *et al* (2013) however reported that granulosa cell tumours were also recognized in camel. Hence, the objective of the present study was to investigate the prevalence, types and gross-pathological details of ovarian granulosa cell tumours in one-humped camels using abattoir samples.

MATERIALS AND METHODS

Study Area

This study was conducted in Maiduguri considered the capital and the largest urban center in Borno state. Maiduguri lies between latitude 10.2⁰ and 13.4⁰ N and longitude 9.8⁰ and 14.4⁰E. The state covers an area of 69,436sq km and is the largest state in the federation of Nigeria in terms of land mass (Musa and Pindar, 2005). It is located in the Northern-Eastern region of the country and occupies the greatest

part of Lake Chad basin. It shares border with republic of Niger to the North Lake Chad (Republic of Chad) to the North-East and Cameroon to the East, In the South and west, it borders the Nigerian states of Adamawa, Gombe and Yobe (NiMET, 2022).

The state has hot and dry climate during the greater part of the year, although the southern part is slightly milder. The period of wet season varies from place to place due to influence of various climatic factors such as the duration of rain, wind and topography, but generally the raining season is from July to September in the north and up to early October in the south with relative humidity of about 45% and evaporation of 203mm/year. The state has two vegetation zones; Sahel savannah in the north and Sudan savannah in the south (NiMET, 2022).

The study area was Maiduguri central abattoir specifically located opposite Maiduguri main cattle market popularly called “Kasuwn Shanu”. The abattoir which lies within the semi-arid zone of northern part of Nigeria, with an estimate of 16-25 camels slaughtered daily. Camels used for this study were trade stock brought in from the neighboring countries of Chad, Cameroon and Niger republics and presented for slaughter at Maiduguri central abattoir.

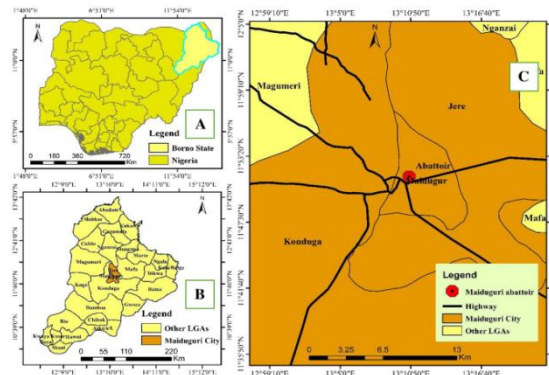


Figure 1: Map of Maiduguri with the location of Maiduguri abattoir as the study area (Source google map)

One-humped camel (*Camelus dromedarius*) is the most predominant type slaughtered at the Maiduguri central abattoir and only few of the

two-humped camels (*Camelus bactrianus*) are slaughtered alone side the former. Both of these camel breeds constitute males and females

brought to the abattoir for slaughter on daily basis. Identification of sexes was based on appearance of external genitalia and the presence or absence of testis and udder as the case may be.

MATERIALS USED FOR THE STUDY

Accessories or materials used during the course of the screening includes: still camera that was used for taking a snap shot of suspected positive samples; measuring tape was placed adjacent to the suspected samples for making a snap shot to give an idea of the specimen size; disposable gloves were used as protective instrument against contaminations; scalpel blades were employed for making incisions during sample screening; post-mortem knife was used also for making incisions; tissue forceps were used for holding tissues of specimen samples during examinations and incisions cardboard paper was used as a background for specimen samples placing during snap shot of the samples.

METHODOLOGY

Criteria for Diagnosis of Suspected Granulosa Cell Tumour

The classic clinical presentation of a she camel with a GCT is a unilaterally enlarged ovary and a small inactive contralateral ovary. The ultrasonographic appearance of the affected ovary, behavioral changes, and endocrine parameters may be of assistance in confirming the diagnosis in live camels. Other techniques, such as laparoscopy, biopsy, or aspiration of an ovarian mass, have occasionally been used for diagnostic purposes (Catone *et al.*, 2004; Fischer, 1986; Pugh *et al.*, 1985; Rambags *et al.*, 2003) but are not routinely used in clinical practice. The definitive diagnosis is made by histopathologic examination.

Palpation and ultrasonography

The detection of a unilaterally enlarged ovary in the presence of a small inactive contralateral ovary during the physiologic breeding season is suggestive of a GCT. GCTs can present in all shapes and sizes. Most affected ovaries range

from 10 to 20 cm in diameter [8]. A small GCT may be difficult to detect and may not have an effect on the opposite ovary if detected early in the course of the disease. Large tumors are much more obvious and are typically associated with suppression of follicular development in the contralateral ovary. One of the largest GCTs recorded was a 59.1kg mass that weighed approximately 350 kg, which was removed from a 9-year-old Quarter Horse mare (Nyack *et al.*, 1983). It has been reported that the ovulation fossa is usually obliterated by the presence of a GCT and that the absence of an ovulation fossa may be a means to differentiate this tumor from other ovarian abnormalities (Baker and Kenney 1980).

Ultrasound examination may be valuable in diagnosing the presence of a GCT, but the appearance of the affected ovary can be quite variable (Hinriches and Hunt 1990; White and Allen 1985). GCTs can appear as a multicystic ('honeycombed') mass, as a solid ovarian mass, or as a single large fluid-filled cyst. The affected ovary often has a thick capsule or tunica albuginea surrounding a multicystic core (Rambags *et al.*, 2003). The opposite ovary usually has little or no follicular activity noted on ultrasound (Hinriches and Hunt 1990).

Procedure

The genitalia were separated immediately after evisceration at the slaughter house. The broad ligaments, the loose connective tissues, and the fat surrounding the vulva and retroperitoneal part of the vagina and other genitalia were removed as far as possible to clear the reproductive organs for a better examination. The flexures of the uterine tubes were straightened out by freeing them from the mesosalpinx. The samples were placed in plastic bags and transported to the laboratory of Department of Veterinary Medicine at the University of Maiduguri. Thorough macroscopic examination was carried on all of the 150 collected ovaries from female one-humped

dromedary camels. Each ovary of the non-pregnant genitalia was carefully observed externally and internally for the presence of different types of gross pathological abnormalities related to tumors such as cysts (follicular, luteal and para ovarian cyst), inflammatory conditions, congestion, and other visible changes and any abnormal swelling was recorded. On gross examination, GCTs will typically appear polycystic with some areas of solid tissue (Nielsen and Kennedy 1990). Focal areas of necrosis and hemorrhage will be common.

Although the study does not involved histopathological assessment in the ovarian tissues due to constrains of unavailability of necessary reagents and the lack of an expert to read slides for histopathological preparations, histologically, the cysts seem to be a disorganized attempt at follicle formation, with multiple layers of granulosa-type cells within the follicular structures and a supporting stroma that may contain theca-type cells (Mac Lachlan, 1987). There is no breed predilection for the formation of GCTs. The data for gross observation was photographed. Specimen suspected to be neoplastic or of having abnormal growth or swelling in the ovarian tissues (section of approximately one cubic centimetre) was fixed in a plastic container that contains 10% buffered formalin for histopathological examination.

Buffered Formalin Fixation of Granulosa Cell Tumour Tissues

Buffered formalin is a good routine fixative. This solution is hypotonic in buffer ions and has

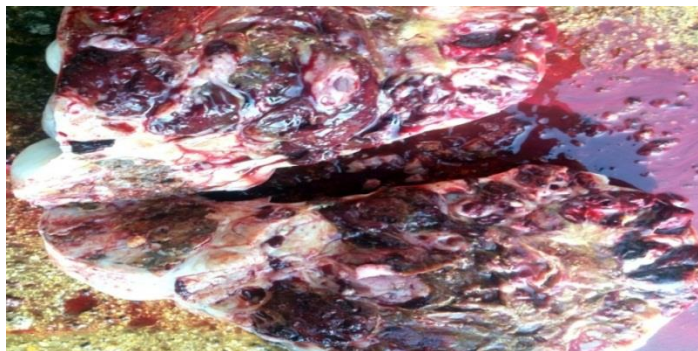
a pH of 6.8. Specimen of granulosa cell tumour was fixed in this solution for longer keeping of the specimen for histopathological examinations. Formalin fixation will form between the aldehydes and the proteins, creating a gel, thus retaining cellular constituents in their in vivo relationship. This will enable proper fixation of the tissues and will be able to withstand the subsequent stages of tissue processing or staining. It has been used previously as a popular general-purpose histological fixative to fix tissues. The chemical composition of the formalin fixative was 10ml of formalin with 0.4gm of acid sodium phosphate (monohydrate) and 0.65gm of anhydrous disodium phosphate mixed in 100 ml of water.

RESULTS

Only a single case of suspected granulosa cell tumour was identified. It was observed grossly as unilateral swelling or a growth of tumour mass in the affected left ovary as depicted in Plate 1. The cut section of the neoplastic mass appeared cystic as shown in Plate 2. One of the constraints of the study was that sample from the tumour mass was not subjected to microscopic screening. This will have a negative perception about the outcome of the study because histopathological investigation as the only confirmatory procedure for granulosa cell tumour was not conducted. That was part of the reasons that the study only reported the presence of the tumour as a suspected case and therefore affects the conclusion and recommendations of the studies.

Table I: Prevalence of Granulosa cell tumour in Ovary of One-Humped Camel from Maiduguri Abattoir

S/No	Months of Sample Collection	Number of Camels Examined	Number of Ovary with Granulosa Cell Tumour	Frequency of Granulosa Cell Tumour
1.	September	18.0	0.00	0.00%
2.	October	24.0	0.00	0.00%
3.	November	36.0	1.00	0.70%
4.	December	52.0	0.00	0.00%
5.	January	20.0	0.00	0.00%
Total		150.0	1.00	0.70%

**Plate I:** Gross appearance of suspected granulosa cell tumor mass from the left ovary of female genitalia of one-humped camel (*Camelus dromedarius*) in Maiduguri central abattoir**Plate II:** A transverse section of suspected granulosa cell tumor mass dissected bilaterally with polycystic appearance on the cut surface of the ovary and haemorrhagic cystic fluid

DISCUSSIONS

An examination of ovaries from female genital tracts of dromedary camels slaughtered in Maiduguri central abattoir during the study period revealed only a single case of suspected granulosa cell tumour. Detection of the suspected case of the tumour is important as there are almost no reportable or rare cases in the prevalence of granulosa cell tumour in this species that were considered as hardy animals

and because tumours generally were reported as one of the common causes of infertility in female animals generally. These observations tallies with the findings of Ali *et al.* (2013) who for the first time reported the prevalence of granulosa cell tumour in female dromedary camels and asserted that it helped in the diagnosis of cases of infertility in this species of animal. These insights were strongly supported by assertions of Anderson *et al.* (2010) who

reported the exhibition of three types of abnormal reproductive behaviours but in mares identified with granulosa cell tumor basing upon the types and amount of hormones produced as related to causes of infertility in the mares. The behaviors could be prolonged anoestrus, persistent or intermittent oestrus (nymphomania) or stallion-like behaviours which all lead to infertility in the long run. Although the findings of the present study recorded a very low prevalence of 0.7% of the suspected granuloma cell tumor in dromedary camel, it was still higher when considered in percentages in comparison with that of Martines *et al.* (2004) who reported the prevalence of 0.3% of granuloma cell tumor in species of cattle after an abattoir examination of considerably high number of 1,113 cows. Number wise, the difference was glaringly higher from the findings of Martines *et al.* (2004) than that obtained in the present study.

Similarly other observations made by array of researchers revealed that granuloma cell tumour had occasional occurrences in horses and cattle but with rarity in malignancy (Parez-Martinez *et al.*, 2004; Crabtree, 2011). However, other scientist had a contrary view regarding the effects of the tumours generally on fertility decline and asserted that the dromedary camel's ovarian tumour constituted a rare cause of reproductive disorders (Tibary and Anouassi 1997; Ali *et al.*, 2009). El-Khouly *et al.* (1990) was however with the view that most of the reported ovarian tumors in camels have been tetratomas. This was a contrary view from the findings of the present studies where only granulosa cell tumour was recorded and therefore emphasized that the most common type of ovarian tumour was granulosa cell tumour.

Dysgerminoma has been reported as a different type of tumour mass but with extremely rare occurrences as observed by El-Khouly *et al.* (1990) in comparison and with reference to

granulosa cell tumor as a tumor that aroused from granulosa cells. These belong to sex cord-gonadal stromal tumors or the non-epithelial tumor group. Adult granulosa cell tumor group of the ovary are often hormonally active (Schumer and Cannistra 2003; Koukourakis *et al.*, 2008) and are often related to Dysgerminoma.

Mares with granulosa cell tumours usually exhibit one of three types of behaviours, depending upon the type and amount of hormones produced by their tumour; these are, prolonged anoestrus, persistent or intermittent oestrus behaviour (nymphomania) or, stallion-like behaviour. Mares exhibiting the latter may also have a crested neck and enlarged clitoris (Crabtree, 2011). These features were however not exhibited in camels probably because of the fact that camels are hardy animals and although tumour type may affect fertility, the exhibition of any of these features has not been reported in camels.

In cattle, clinical signs associated with granulosa cell tumour included anoestrus, nymphomania and virilise, or there may be no abnormal signs (Pérez-Martínez *et al.*, 2004). However, in an affected llama, clinical signs of lethargy and decreased feed intake were observed, likely caused by the metastatic nature of the tumor. However, llama was reported to be able to successfully conceive and maintain a pregnancy in spite of infiltration with the granulosa cell tumour cells (Anderson *et al.*, 2010).

Although the findings of the present studies was skeletal as only a single case of granulosa cell tumour was recorded there was however still some potential complications of the existence of the tumour in the she-camel. And therefore there is the need to bring to the fore the findings of the prevalence of the tumour mass in other species of animals especially where detailed investigations have been conducted to elucidate these potential complications due to the presence of the tumour in camels. And in that respect the

findings in mares reported that medical complications attributable to or associated with the presence of a GCT are uncommon. Although most equine granulosa cells are benign, metastasis has been reported also (Meagher *et al.*, 1977; Gift *et al.*, 1992; Lavoie *et al.*, 1992; Patrick *et al.*, 2003). Patrick and colleagues (2003) noted that only 2 of 112 reported cases of GCTs had metastasized. It was further established that metastasis was generally confined to the abdominal cavity, although extension to thoracic and sublumbar lymph nodes and emboli within the vasculature of the lungs has been noted (Gift *et al.*, 1992).

In a related finding it was reported that large GCTs may cause colic or other gastrointestinal problem (Meagher *et al.*, 1977; Schmidt *et al.*, 1976; Nyack and Johnson 1983). Van der Zaag and coworkers (1996) reported that a postpartum Arabian mare developed colic secondary to a 31.5-kg GCT. Intermittent colic was also noted in a mare with an extremely large GCT (Nyack and Johnson 1983) [35]. Wilson and colleagues (Wilson *et al.*, 1989) described a case in which a GCT caused rupture of the small colon in a 10-year-old Quarter Horse mare. The rupture was secondary to impaction caused by occlusion of the small colon between the enlarged left ovary and its suspensory ligament.

Rupture of juvenile- and adult-form GCTs with subsequent intra-abdominal hemorrhage has been reported in other investigations (Green *et al.*, 1988; Gateway *et al.*, 1990; Alexander *et al.*, 2004). In one case, tumor rupture was diagnosed in a neonatal foal (Green *et al.*, 1988). It was hypothesized that the rupture occurred during foaling. Affected animals are typically presented with abdominal pain, distention, and anemia (Green *et al.*, 1988; Gate wood *et al.*, 1990). Hemoperitoneum was confirmed by transabdominal ultrasonography and abdominocentesis (Alexander *et al.*, 2004). Alexander and coworkers (Alexander *et al.*, 2004)[74] reported on the successful surgical

treatment of two mares with ruptured ovarian GCTs. There are no documented cases of metastasis after rupture of a GCT in mares.

Adhesions of unruptured GCTs to adjacent abdominal structures, including the abdominal wall, bladder, uterus, spleen, omentum, and intestinal tract, have been described (Rambags *et al.*, 2003). Rupture of the tumor or adhesion of the tumor to adjacent structures may follow localized ischemia and necrosis of the tunica albuginea induced by pressure of rapid neoplastic growth within the encapsulated ovary (Rambags *et al.*, 2003).

Torsion of the ovary containing a GTCT has been described in a 3-month-old Arabian filly (Hultgren *et al.*, 1987) and a 12-year-old Morgan mare (Sedrish *et al.*, 1997). The filly had a 3-day history of abdominal distention and colic. Necropsy revealed 360 torsion of the affected ovary, which measured 32 cm 27 cm 27 cm in another research. The mare had a 45-day history of stallion-like behavior, and initial examination revealed an enlarged multicystic left ovary and a small inactive right ovary. Serum levels of testosterone and inhibin were elevated above normal values. A complete blood cell count (CBC) evaluation revealed mild anemia, and abdominocentesis indicated that recent intra-abdominal hemorrhage had occurred. The mare became acutely painful, and surgical exploration of the abdomen confirmed torsion of the enlarged ovary. The ovary was removed, and the mare recovered. Gross and histologic evaluation of the ovary revealed extensive necrosis and hemorrhage.

Hypertrophic osteopathy (HO) associated with the presence of an ovarian GCT with metastasis to the abdominal cavity was noted in a pregnant 8-year-old Quarter Horse mare (Lavoie *et al.*, 1992). Similar syndromes have been reported in a 4-year-old Arab-cross mare with an ovarian dysgerminoma with metastasis to the abdominal lymph nodes (McLennan and Kelly, 1977) and an 8-year-old Arabian mare with metastases

throughout the abdomen (Meuten and Rendano, 1978). Although the pathogenesis of HO is unclear, it has been associated with neoplasia or inflammatory conditions of the thoracic or abdominal cavity (Lavoie *et al.*, 1992).

Hensen and colleagues (Henson *et al.*, 1988) reported the association between a GTCT and histiolymphocytic lymphoma in a 9-year-old Arabian mare. The mare exhibited signs of stallion-like behavior around other mares and had multiple subcutaneous masses on the trunk and limbs. A generalized regression of the subcutaneous tumors occurred within a month after surgical removal of the GTCT. It was hypothesized that steroid hormones produced by the GTCT may have influenced the growth and development of the lymphoma.

Secondary diabetes mellitus was noted in a 10-year-old American Saddle Horse with bilateral GCTs. The horse had markedly elevated serum and urine glucose levels and abnormal results of a glucose tolerance test (McCoy, 1986). The cause of the diabetes condition was not determined, but exposure to gonadal steroids or other hormones produced by the tumor was suggested as a possibility.

CONCLUSION

In conclusion therefore, the present study demonstrated the usefulness of morpho-

pathological examination in determining the characteristics of granulosa cell tumour. Additionally, out of all ovarian tumours originating from germ cell granulosa cell tumour as mass of ovarian growth was the only tumour detected and was therefore a very important finding because of the claim that camels are hardy animals and therefore implied that camels withstand a lot of stresses and diseases. This therefore suggests that GCT is not a common ovarian tumor in camels. A clinical diagnosis can be made based on the presence of a unilaterally enlarged ovary and a small inactive contralateral ovary. It is therefore recommended that further detailed investigations on granulosa cell tumour be carried using especially histopathologic screening and other advanced techniques to assist in confirmation of the suspicion.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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