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# Study of Some Physiological and Pathological Aspects of Feral Queen's Reproductive System in Iraq

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## Abstract

The present study is carried out to investigate some aspects of reproduction in Iraqi feral cats. Sixty blood samples and female genital specimens from sexually mature queens were trapped during the period from December 2008 to December 2009.During the entire study period, the results showed that incidence rate of follicular phase was 24.56 % and the highest incidence was in Winter 62.5%, while no follicular activity was noted in animals during Autumn . The hormonal assay showed that the mean of the estradiol -17ß in 14 follicular phase animals was 47.5pg/ml, while The dominance of vaginal epithelial cells in 14 estrus queens were nucleated superficial cells 61.5 %. The incidence of luteal phase was 29.82% and the highest incidence was in Spring 56.66%, and the average of corpora lutea per animal was 4.7 % and the incidence rate of pregnant queens was 19.29%, with high incidence in Summer 33.33% with no pregnant queens was occurred in animals with 100% implantation rate . The maximum number of embryos was occurred in animals with 100% implantation rate of pseudopregnancy 11.76%, and characterized by present of active corpora lutea on ovaries , with slight enlargement of uterus and uterine tube .

In all animals with diestrus the mean of plasma progesterone level was 48 ng/ml ,and the cell comprising the vaginal smear include intermediate cells 47% parabasal cells45% and nucleated cells 5% .The anestrus phase was recognized in incidence rate 36.84% and the highest incidence of anestrus phase was noted in Autumn 75%, Cells comprising the vaginal smear in anestrus queens include intermediate cells 87.9%, parabasal cells 8.6%,nucleated superficial cells 2.7% , anucleated superficial cells 0.8% .The pathological study was revealed 3 out of 60 queens with gross reproductive systems abnormalities ,with incidence rate 5% ,these cases include paraovarian cyst, follicular cysts and pyometra.

#### Introduction

Domestic cat (*Felis catus* ) is one of seven species of the genus Felis (a group of small size cats), which is a part of Felids , which include in addition to domestic cat, lion, tiger, cougar and many others (1). Scientifically, domestic cats are used as laboratory animals due to the fact that they have some physiological features more in common with human than the laboratory rabbit or other rodent, hence they have extremely used in behavioral and biomedical researches particularly in neurological signs

#### Animals

The study was carried out in three governorates include ,Babylon, Al-diwaniya and An-najaf from December 2008 to (2).Recently, the domestic cat (*Felis catus*) is a useful model for studying comparative biology of the Felidae family and for developing assisted reproductive techniques for propagating related, endangered species (3). For all above, and due to the rarity of the available references about the feline reproductive system particularly in Iraq, the present study is designed to spotlight on some physiological features and pathological abnormalities of Iraqi feral queens.

# Materials and Methods

December 2009, 60 mature female cat were captured by local made feral cat trap, each captured animal was anaesthetized according to (2), then the anaesthetized animal was

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examined clinically with recording of obtained data in special examination card .

# **Blood Samples**

Sixty blood samples collected from cephalic vein according to (4) the collected blood was poured in labeled plastic plain tube ,and placed in room temperature for 2 hours ,the serum collected by Pasteur's pipette after centrifugation at 3000/rpm for 10 minutes and stored in plastic container at  $-20^{\circ}$ C. The stored sera were send private laboratory for determination of estradiol 17- $\beta$  and progesterone hormones by ELISA apparatus (Bio active DNM-9602/Germany) with specific kit ( Abbot laboratories USA) and according to the instruction of the company .

# Vaginal Smears

The vaginal smears was carried out according to the method that is recommended by (5) ,briefly by introducing a cotton- tipped swab moistened with sterile saline in to the vestibule and caudal vagina with avoiding of clitoral fossa The cotton tipped swab was rolled onto a glass slide allowed to air- dry and stained with Giemsa stain. The correlation between vaginal cytology and stages of the estrus was interpreted according to (6).

## **Female Reproductive Tract Samples**

Sixty samples of the female reproductive tract are collected directly after euthanizing of animal. the female reproductive tract was taken entirely from the ovaries to the vulva ,the non pregnant samples classified in to reproductive tract with or without pathological changes. After detection of physiological evidences of different phases of estrous cycle in non

## 1.Physiological study A.Follicular Phase

The incidence rate of follicular phase was 4.56%(14 out of 57of non pregnant pathological changes free queens). The highest incidence was in Winter 62.5%,followed by Spring and Summer in percentage 44.4% and 33.3% respectively,

pregnant samples, a longitudinal incision was made along whole tract to expose the luminal surface for examination to observe any abnormalities or pathological changes, if reproductive systems showed clear gross pathological lesions, small piece 1cm<sup>3</sup> were fixed in 10% buffered neutral formalin for 3 days for histopathological examination according to (7), while another piece were taken for bacteriological examination, the bacteriological isolation and identification performed according to (8). The reproductive tracts with pregnancy were examined to record the number of fetuses per animal, number of fetuses in each uterine horn and number of corpora lutea were the obtained,the transuterine migration of embryos and implantation rate were recorded according to (9) as following :The number of ovulation was estimated by number of CL in the ovaries .The number of CL was determined by external examination of the ovary with active CL which being round shape with a 3mm diameter and raised 2mm above surface of the ovary, and transuterine migration of embryos was estimated to have occurred prior to implantation when the number of fetuses in a given horn was more than the number of CL present in the ipsilateral ovary. The implantation rate considered as 100% when the number of total CL was equal to the total number of fetuses.

#### **Statistical Analysis**

The results was analyzed statistically by chi square test and student's t test to determine any significant variation between different means(10).

#### Results

while no follicular activity was noted in animals during Autumn, figure 1. According to the months of study, the highest incidence was in the February 100% and the lowest was in the August, while it was absent in September, October ,November and April ,and all follicular activity was observed in animals above 1 years old.

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Figure (1) reveal the incidence of follicular phase in different seasons

The size of recorded follicles ranged 0.5 - 2 mm, and the number was ranged between 1- 3 in the same ovary. Out of 47 observed follicles,27 were present in right ovaries and 20 in the left ovaries. The results showed that the uterus shape was differ

according to size of follicle\s ,the uteri of queens with follicles less than 1 mm were slightly enlarged and congested, with straight uterine horns ,while the uterine horns were Spiral in shape if the follicles larger than 1 mm figure 2.



Figure(2) reveal the spiral appearance of uterus in follicular phase

The hormonal assay showed that the blood level of the estradiol 17-ß in 14 follicular phase animals was 47.5pg/ml, ranged from 25-70pg/ml ,while The percentage and range of vaginal epithelial cells of parabasal cells , intermediate cells, nucleated superficial cells and anucleated superficial cells were .92% (0-3) , 12.21 %(0-25), 61.5 %(6-88) and 27.71%(4-88) respectively.

#### **B.** Luteal phase

The luteal phase was recognized in 17 out of 57 non pregnant pathological change free queens, with incidence rate 29.82 %. The highest incidence was in Spring 56.66%,followed by Summer and Autumn in percentage 41% and 20% respectively, while it was 6.66% in Winter, figure 3. According to the months of study, the highest incidence of luteal phase cases was in the April 80% and the lowest was in the January 20%, while it was absent in February, October ,November and December .The average of corpora lutea per animal was 4.7  $\% \pm 0.12$  (Mean  $\pm$  SE ; range 2-5)with approximately equal distribution between right and left ovaries as  $2.41\% \pm 18$ (Mean  $\pm$ SE; range 0-5) in the right ovary vs.  $2.35\% \pm$ 0.58(Mean  $\pm$  SE ; range 0-9) in the left ovary .The number of corpora lutea per ovary was same in 3 queens (17,81%), greater in right ovary in 7 queens (41.17%) and greater in left ovary in 7 queens (41.17%).

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Figure (3) reveal the incidence of luteal phase in different seasons

The animals with this phase were gathered into 4 groups which describe as following :

#### **Recently ovulated queen:**

only one queen was recorded as recently ovulated, it has 2 corpus hemorrhagicum on the right ovary with 3 corpus hemorrhagicum on the left ovary figure 4 ,the oviduct and uterus were markedly enlarged and congested and The estimated estradiol 17- $\beta$  and progesterone were 16 pg/ml and 2ng/ml respectively.



Figure (4) reveal the corpus hemorphagicum in recently ovulated cat (arrow)

#### **Pregnant queens**

The incidence rate pregnancy was19.29%(11/57), the highest incidence was in Summer 33.33%, followed by Spring and Autumn in incidence rate 28.57% and 25% respectively, while no pregnant cat was captured in Winter figure 5. According to months, the highest incidence of pregnancy was observed in March 50%, followed by

July ,September ,August ,June, April and May in incidence rate 40% ,40%, 33.33% ,25%,20% and 20% respectivel. The number of fetuses per each uterine horn was same in 7/11 queens (63.63%),greater in right uterine horn in 2/11 queens (18.18%) and greater in left uterine horn in 2/11 queens (18.18%).



Figure (5) reveal the pregnancy percentage in different seasons

According to 100% ovulation rate, the implantation rate was 86.06%, and it was

100% in 6/11 queens,80% in 1/11 queens , 75% in 2/11 queens , 66.66% in 1/11 queens

and 50% in 1/11 queens (9.09%). The transuterine migration of embryos was observed in 3/11 queens with incidence rate 27.27% table 1. All transuterine migration of embryos was occurred in animals with 100% implantation rate ,and the maximum number of embryos that migrate per animal was 2 ,in

the first queen , two embryos were migrated from right to left uterine horn figure 6, in the second queen one embryo migrated from right to left uterine horns ,while in the third queen one embryo was migrated from left to right.

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Table (1) reveal the relationship	p between number of cor	pora lutea, number of fetuses
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CL/animal	No	Average	Implantation
	.of	fetus	rate %
	cats	no.(range)	
2	3	2(1-2)	100
3	3	3(1-3)	100
4	2	3(2-4)	75
5	1	4(-)	80
6	1	4(-)	66.66
8	1	4	50



Figure (6) reveal the migration of 2 embryos from right to left uterine horn

#### **Pseudopregnant queens**

The pseudopregnancy was noted in 20ut of 17 queens (11.76 %), the first queen has only 2 active corpora lutea on the right ovary, while the other queen has 2 active corpora lutea on each ovary, the uterus and the uterine tube enlarged slightly. The means of length and diameter of right and left uterine horn were  $45 \times 8$  mm and 47.5  $\times 8$  mm respectively, while the means of length and diameter of uterine body were  $21.25 \times 6.5$  mm.In both pregnant and pseudopregnant animals, the mean of plasma progesterone concentration was 48 ng/ml (range 22-56) ,and the cell comprising the vaginal smear in diestrus cats (mean percentage followed by range) included intermediate cells (47%; 18-78) parabasal cells(45%;12-82), anucleated superficial cells (0%),nucleated cells (5%; 0-5).

# Queens with involuted uterus

The involution was noted in 3out of 17 queens with incidence rate (17.64%) ,all these queens were lactating , and had inactive corpora lutea on their ovaries ,the uteri were straight and markedly dilated. The mean  $\pm$ SE of length and diameter of right and left uterine horn were 45.66× 12mm and 40×11.66 mm respectively , while the means of length and diameter of uterine body were 21× 6.66 mm respectively.

## C. Anestrus phase

The anestrus phase was recognized in 26 out of 57 pathological free queens , with

incidence rate 36.84% .The highest incidence of anestrus phase was noted in Autumn 86.66%, followed by Winter ,Summer and Spring in incidence rate 46.66% ,20% and 7.14% respectively, figure

7. According to the months , the highest incidence of the anestrus phase cases was in October and November 100% and the lowest was in May and July 20% ,while it was absent in February and March.

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Winter Spring Summer Aut

Figure (7) reveal the mean of the anestrus phase according to the seasons

In all observed animals with anestrus phase the ovaries were entirely smooth, and the mean of uterine horns measurements was at the minimum of all recorded measurements. The mean ±SE of length and diameter of right and left uterine horn were 50.5  $\pm 1.54$   $\times$  3.2  $\pm$  0.6mm and 54.65  $\pm 1.87$  $\times 11.66 \pm 1.45$ mm respectively , while the means±SE of length and diameter of uterine body were 16.2±2.73×4.5±0.45 respectively.Cells comprising the vaginal smear in anestrus queens (Mean percentage followed by range )included intermediate cells 87.9%(50-100%), parabasal cells 8.6% (0-50%) ,nucleated superficial cells 2.7%(0-22%), anucleated superficial cells 0.8%(0-2%).

### 2.Pathological Study

During the study period, out of 60 only 3 queens with gross reproductive systems abnormalities were observed ,with incidence rate 5% ,these cases were described as following :

# • Case (1):Parovarian(Periovarian) Cyst

One queen with parovarian cyst was captured in this study, this queen was above one year old and weigh 5 kg ,the clinical examination revealed that she was healthy. a large After dissecting the animal parovarian cyst was located on the left ovary , it was lobulated, thin walled cyst filled with clear fluid (about 6ml) ,this cyst was surround the ovary entirely, figure 8. The ovary and uterine horns was normal grossly .The right ovary was with previous ovarian activity, with 1 small follicle on its surface. The histpathological examination of the ovary with the parovarian cyst reveal normal microscopical structure of the ovary and oviduct.



Figure (8) reveal lobulated parovarian cyst

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#### • Case (2): Follicular Cyst

Only one queen in this study was reported with follicular cyst. The queen was over one year old and weight about 4.5kg ,the clinical examination revealed that the queen was healthy, after dissecting the animal ,a follicular cyst was found on the left ovary ,thin walled cyst ,filled with clear fluid ,5mm in diameter , the ovaries and uterine horns were normal grossly ,Figure 9. The histopathological examination of the ovary with the follicular cyst reveal normal micrscopical structure of the ovary with present of relatively large follicles as well as normal sized follicles, figure 10.



Figure (10) reveal presence of relatively large follicle (left) compared with normal follicle(H &E.X100).

#### • Case (3): Pyometra

The third case which reported in this study was pyometra, the captured queen was above one year old and weight about 4kg, the clinical examination revealed that the queen was healthy, after dissecting the

animal ,the uterus was enlarged with congested appearance ,figure 11. The color of the two uterine horns was abnormal and the endometrium was very congested.



Figure (11) reveal the marked thickness of the endometrium and presence of cheesy pieces(arrow)

The histopathological examination of the uterus with the endometritis reveal sever infiltration of neutrophils in sub epithelium and between the uterine glands ,large number of necrotic neutrophils in the lumen of the uterine glands and uterine lumen and the exfoliation of the epithelium, figure 12. The histopathological appearance of the uterine tube reveal the dilation of the uterine tube and epithelial atrophy, infiltration of the epithelium, fusion of villi formed vacuole filled with pus and mucous, the lumen of the uterine tube filled with mucous

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Figure (12) reveal sever infiltration of neutrophils in sub epithelium and between the uterine glands ,large number of necrotic neutrophils in the lumen of the uterine glands and uterine lumen and the exfoliation of the epithelium (arrow),(H&E.X200)

The bacterial isolation and identification of exudates culturing reveals 2 isolates , one is  $G^{-ve}$  bacteria , the growing

on selective media, feature of colony and biochemical test reveal the characteristics of *E.coli* and *Staphylococcus spp*.

## Discussion

## **Incidence of Sexual Activity**

Although several researchers refer to that in the tropical areas ,the domestic cat may breed throughout the year(5 ,6, 11),there are several factors may influence or determine breeding of domestic cat include length of day, environmental temperature and breed (12),so the fluctuation in breeding may vary from country to country . In temperate zone the high environmental temperature may decrease the estrous incidence(13),while (14) revealed that the effect of heat and humidity on domestic cat breeding depend on individual variation.

The present study was preformed along different 4 seasons on feral cat, which they are roaming outdoor and expose to natural irregular light as well as possibility of high individual variations existence, the fluctuation in incidence of follicular and luteal phases was frankly observed as shown in figure 1 and 2.

## **Incidence of Sexual Inactivity**

The highest incidence period of anestrous in this study are in agreement with (15), that said it occurs in late Autumn months include October, November and December.other authors explained this as result of day light shortage under natural conditions(6).

The ovarian inactivity in Winter may be due to elevation of Prolactin and melatonin in circulating plasma(6,16,17),while the relatively high incidence of anestrous phase in Summer may be due to increase of the environmental temperature as recorded by (18).

#### **Pregnancy and Pseudopregnancy**

The incidence rate of pregnancy which recorded in this study as demonstrated in figure 3, are in agreement with (19) who reported that under the natural conditions the free ranging domestic cats can produce occasionally 3 litters /year, with 1-5 kitten in each litter, and the incidence of pregnancy is Vol./10

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in marked contrast to pregnancy of bitch, which may breed only twice a year (6,20).In this study ,the number of fetuses per each uterine horn, implantation rate, relationship between number of corpora lutea, number of fetuses, transuterine migration of embryos and the maximum number of embryos that migrate per animal are in agreement with (9).Little references are available about transuterine migration of embryos domestic cat ,but it has been recorded in other litter bearing animals ,(21) reported it in 40-50% of pregnant sows, while (22) observed it in 50% of pregnant bitches. All previous our data are near to that of (9) who recorded that the transutrine migration of embryos occurred from the side with ovary containing the greater number of CL to the side of fewer CL and the migration of embryos more than 3 embryo was not observed, while the migration rate recorded by the above authors was more than that recorded in this study, may be due to the difference in the number of examined cats between studies. The queens may undergo as many as 4-5 pseudopregnancies during the course of one poly estrus season (20) .In other hand (23) observed enlargement of abdomen with increase of appetite and queens weight gains in with pseudopregnancy, while (18) revealed that the average of duration of luteal activity in pseudopregnancy was 36.5 days. All these data are close to that obtained in present study where the pseudopregnancy was noted in 2out of 17(11.76 %) queens with at least 2 corpora lutea active with slightly enlargement of uterus and the uterine tube. Vaginal Cytology

The cyclical changes of vaginal exfoliated epithelium in canine have been extensively studied and are widely used clinically (6,24), while only few reports are available on vaginal cytology in feline species.In the present study, the morphological changes of vaginal epithelium during estrus, phase are similar with that obtained by (15) and (25)

documented that the number of superficial cells is fairly constant throughout behavior estrous and ranged from 40-60% of total cells present, and the vaginal cytology of diestrus phase are close to data of (26) which reported 50%, 48% and 2% for intermediate cells, parabasal cells and superficial nucleated cells respectively. Also, the rate of cells comprising the vaginal smear in anestrous queens are incompatible with finding of (24) noted that during anoestrus the cells are rare, mucus is obvious and a bund ,and the majority of cells are basal or parabasal with some intermediate cells, while the present results are in agreement with (15) described that the exfoliated vaginal epithelial cells consist of less than 10% parabasal cells,40% to70% intermediate cells, and 30% to 40% nucleated superficial cells, background debris is obvious.

## Hormonal Changes

The hormonal assay in present study was revealed that the highest mean of serum estradiol was recorded in the follicular phase 47.5 pg/ml, and it was 16 pg/ml during the recently ovulated queen, these finding are in agreement with (27) who recorded а concentration of 20-50 or higher during proestrous and estrus phases, and within the range of (20) who observed that the level of serum estradiol was increase from 25 pg/ml in first day of estrus to the peak 50 pg/ml in  $5^{\text{th}}$  day, then decline to 10 pg/ml in the  $10^{\text{th}}$ day.During the luteal phase the mean of progesterone level was 2 ng/ml in recently obvious ovulated cat (with corpus hemoragicum), increase to reach 48 ng/ml (range 22-56) in animals with diestrus (pregnancy and pseudopregnancy),these results are in disagreement with (15) they progesterone suspected the serum concentrations ranged from 1.5ng/ml to more than 20 ng/ml in the queens with diestrus.All examined blood samples which obtained from the queens in anestrous phase reveal that the level of estrogen and progesterone at the basal level, these are

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compatible with levels mentioned by others (6,15).

## Pathological Study

The relatively low incidence of reproductive problems in present study (5%) might be due to small sample size, or due to fact, that the feral cat has short life comparable with household queens which receive more care and hygienic conditions. There were no available reference refer to recording of parovarian cyst in cat ,except (28) explained that it arises either from remnant of the mesonephric (walffian) tubules or the paramesonephric (Mullarian) tubules. (29) suggested that many tiny parovarian cysts ranged 1-3cm in diameter, are very common incidental finding in slaughtered cattle. (30) recorded two cases of parovarian cysts in Iraqi she camel.The size and description of follicular cyst was

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found in present study was in agreement with (20) and (28) they thought that the normal follicles of the queens is up 2-3mm in diameter and the larger follicles are said to be cystic, and with (31) whose observed cysts as unilateral in 16 cats and bilateral in four from 20 cats. The prevalence of endometritis in cats increases with age in sexually intact female cats and mainly after parturition(32) ,these close to case was reported in this study, which was lactating over 1 years old queen .All observed gross and microscopical changes were compatible with description of (32,33). The isolates of concurrent case (E.coli and Staphylococcus spp.) were in agreement with results of (34,35), while (36) reported E.coli and Staphylococcus spp., as vaginal flora in various stages of queen estrous.

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# دراسة بعض الجوانب الفسلجية والمرضية لجهاز تناسل القطط السائبة في العراق

حلا جواد كاظم ضياء حسين جاسم ثاير علوان عبد كلية الطب البيطري / جامعة القادسية

### الخلاصة

أجريت الدراسة الحالية لمعرفة بعض جوانب التكاثر في القطط السائبة في العراق ، حيث جمعت 60 عينة دم وجهاز تناسلي من إناث قطط تم اصطيادها للفترة من تشرين الثاني 2008 إلى تشرين الثاني 2009. أظهرت النتائج خلال فترة الدراسة إن معدل حدوث الطور الجريبي هو 24.56 % وإن أعلى نسبة لحدوثه في الشتاء 62.5 % ، ولم تسجل إي حالة طور جريبي خلال الخريف وكان معدل تركيز هرمون الاستراديول 17 جبيتا 47.5 بيكوغرام /مل، بينما أظهرت المسحات المهبلية سيادة الخلايا السطحية ذات النواة 61.5 كما أظهرت نتائج الدراسة إن نسبة حدوث الطور اللوتيني هي 29.82% وان أعلى نسبة حدوث في الربيع 56.66% ،وكان معدل الأجسام الصفراء لكل حيوان هو 4.7 ± 0.16 و إن نسبة الحمل المسجلة في العينات المفحوصة هي 19.29 % وكان أعلى حدوث لها في الصيف 3.33% ،و لم تسجل إي حالة حمل في الشتاء وكان معدل الإنبات هو 100% وسجل أقصبي عدد لهجرة الأجنة 2 جنين / حيوان بنسبة حدوث 27.27% وكل حالات الهجرة المسجلة كانت في حيوانات ذات نسبة إنبات 100%. كانت نسبة حدوث الحمل الكاذب المسجلة في هذه الدر أسة 11.76 % وتميزت بوجود اجسام صفراء فعالة مع تضخم بسيط في قناتي البيض والرحم . ان معدل هرمون البرجسترون في الدم المسجل خلال الطور اللوتيني 48 نانوغرام /مل ونسب الخلايا في مسحات المهبل للخلايا الوسطية ،جنب القاعدية وٱلوسطية ذات النواة هي 47% ،45% و 5% على التوالي كان معدل حدوث طور اللاشبق خلال فترة الدراسة 36.84 % ، وسجلت أعلى نسبة حدوث في الخريف 66.66% ، يليها الشتاء والصيف ثم الربيع بمعدل حدوث 46.66%، 20% و 7.14% على التوالي وكانت نسب الخلايا في مسحات المهبل هي 87.9%، 8.6% ، 2.7% في 8.0% للخلايا الوسطية ، جنب القاعدية ، السطّحية ذات النواة والسطحيّة عديمة النواة على التوالي . بينت الدراسة المرضية وجود ثلاث حالات من مجموع 60 حيوان تظهر تغيرات مرضية عيانية في أجهزتها التناسلية بنسبة حدوث 5% وهي تكيس جنيب المبيض ، تكيس المبايض الجريبي وتقيح الرحم