

Pathological and Hormonal Study of clinical repeat breeder cases in local Iraqi cows of Basra province

Ali H. Ali¹, Jihad A. Ahmed², Ihsan A. Habeb³

^{1,3}. Department of pathology and poultry disease, College of Veterinary Medicine, University of Basrah, Basrah, Iraq

² Department of Theriogenology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq,

E-mail : faw33f33@gmail.com

E-mail: Jihad.ahmed@uobasrah.edu.iq

E-mail : ihsan.ali.habeb@gmail.com

ABSTRACT

Cattle breeding are an important economic field in the world, in which there is a great connection between the benefit from the productivity and fertility of cows and the condition of females' genital systems. Genital pathological, especially uterine infected and mineral deficiency, plays an essential role in animal husbandry, causing repeat breeder in cows.

The requirement for additional inseminations, increasing culling rates and lower dairy output is a significant cause of infertility which has become a major cause of economic loss in the cattle business. The research examined at the primary reasons of repeat breeding, such as infectious disease, mineral deficiency, and hormonal problems.

The study was conducted at the laboratories of the pathology and poultry diseases department and Theriogenology Department, College of Veterinary Medicine, Basrah University, during the period extended from December 2020 to the end of July 2021. A total samples 250 dairy cows was used in this study. The ages of cows were various range from 3 to 7 years, and Samples were divided into three groups:

The 1st group of the study was 100 cows (repeat breeder) conducted clinically in fields of dairy cows.

The 2nd group of the study was 100 samples (repeat breeder) done through the gross investigation of the female reproductive tract in the Basrah slaughterhouse.

The 3rd group of the study was 50 samples of the female reproductive tract in the Basrah abattoir and fields (normal estrus cycle) as the control group to evaluate hormonal profile (FSH, LH and Progesterone), haematology parameter complete blood picture (CBC), biochemical parameters (Zinc, magnesium and Copper), Bacteria in the reproductive system and Histopathological study cases of the uterus.

Results showed the total number and percentage of endometrial uterine infections which appears in the present study showed increased value compared with another complication cases Tumor, Mineral deficiency, Para ovarian cyst, Follicular cyst and

Piosalpingitis in cows. The results appear during bacterial isolates from cows uterine discharge increased percentage of *Escherichia coli* (63.46%) compare with *Staphylococcus aureus* (9.61%) and *Streptococcus pyogenes* (26.93%).

The white blood cells and Lymphocytes in the present study were found to be significantly ($P \leq 0.05$) higher in the abattoir group and live female group cows repeat breeding compare with the control group. The haemoglobin and PCV values were significantly ($P \leq 0.05$) lower in the repeat breeding cows (abattoir and live female groups) than in the control group. Furthermore, the red blood cells (RBCs $\times 10^{12}/L$) values were significantly ($P \leq 0.05$) lower in the repeat breeding cows (abattoir group and live female group) than in the control group. The mean serum copper concentration in the repeat breeders groups was more significant ($P < 0.05$) than in the control group. On the other hand, the serum Zn concentrations of the cows with repeat breeding (Abattoir group, Live group) significant ($P < 0.05$) values than in control group. Furthermore, the results also showed magnesium concentrations in the repeat breeder group were significant ($P < 0.05$) values than control group.

From 250 samples of females' genital system of slaughtered and filed cows were examined, found Inflammatory gross changes of endometritis were found in 61 (24 %) and classified according to histological examination as; chronic endometritis 19 /61 (31%), subacute endometritis 22/61 (36 %) and acute endometritis 20/61 (32 %), while metritis cases 43/250 (17.2 %) were of the chronic type of inflammation. All endometritis and metritis cases were characterized by increased thickness of uterine serosa with yellowish coloured pus accumulation.

KEY WORDS: cow, repeat breeder, endometritis, metritis.

Introduction

The most prevalent large domesticated ungulate is cattle, sometimes known as cows. They are the most widely distributed species in the genus (*Bos*) and are categorised as (*Bos Taurus*). They are a notable contemporary member of the subfamily (*Bovinae*), and they are the most widespread species of the genus (*Bos*) (Bollongino R. *et al.*, 2012). According to zoology, cows are classified as the following:

- ❖ Kingdom: (*Animalia*)
 - ❖ Phylum: (*Chordata*)
 - ❖ Class: (*Mammalia*)
 - ❖ Order: (*Artiodactyla*)
 - ❖ Family: (*Bovidae*)
 - ❖ Subfamily: (*Bovinae*)
 - ❖ Genus: (*Bos*)
- Species: *B.taurus* Leroy *et al.*, 2004).

From as few as eighty ancestors, cattle were domesticated in Central Anatolia, Western Iran, and the Levant about 10,500 years ago. There are 1.4 billion cattle worldwide, according to 2011 estimates. Cattle were one of the first farm animals to have their genomes entirely sequenced in 2009. Many view cattle breeding as the

oldest source of material profit and wealth, and hence herd raiding is one of the first types of robbery (Brown *et al.*, 2009).

The animal fortune represents an important source for the national economy. In this fortune, the cattle represent the key part because of their importance to human beings. It is the largest economic component of large, medium, and small-scale farmers in developing countries, in conjunction with small ruminants (Rodriguez-Martinez, 2012).

In some countries, cattle productions are critical because they have broad uses and can be multi-purpose. The main use of cows is to provide milk and meat (Toghiani, 2012), but also the cows may be used as clothing, fertiliser, draft power, and sometimes as status or as a form of currency (Ball and Peter 2008).

The primary purpose of cattle production is reproduction. So any defect in reproduction means economic loss, and the cows should be culled. The goal of reproduction is to get one calve in a year (Duarte *et al.*, 2012). Although cows who cannot be pregnant after three inseminations or more show typical estrus indications every 18 to 21 days have been called “repeat breeders” or “repeat breeder syndrome.” Infertility has become a significant cause of economic losses in the cattle business due to the need for more inseminations, lower milk production, longer calving intervals, and higher culling levels. All these factors result in repeat breeding. (Parkinson, 2001).

One of the most common causes of livestock infertility is repeated breeding. (Amirkalaliet *al.*, 2010). Repeat breeding in dairy cows occurs at a rate of 3 to 10% across the world (Goumenouet *al.*, 2003). Subclinical endometritis is one of the most common reasons for repeated breeding (Haluzik, et al .,1999). Insufficiency in trace minerals and vitamin A, poor heat detection, and endocrine dysfunction are the most common causes of nutritional deficiency. Repeat breeding in cross-bred dairy cows is highly common in Iraq (35 percent), which might result from post-partum (PP) preclinical endometritis, nutritional deficiency, inadequate management, and natural servicing of subclinically contaminated bulls or heat stress. Due to waste of semen, insemination costs, rising intervals to conception, increasing culling and replacement expenses, and loss of genetic gain due to longer generation intervals and decreasing fertility, the repeat breeder syndrome contributes to lower dairy profit (Bartlett *et al.*, 1986).

Materials and Methods

Experimental Samples

A total sample pool of 250 dairy cows was used in this study. The onset of estrus was recorded based on the data provided by owners. The ages of cows were various range between 3 to 7 years. Cows are examined clinically depending upon the case history and rectal palpation to detect the pregnancy and uterine infections. Infected cows with uterine infections are examined vaginally by sterile vaginal speculum as well as by massage of the reproductive tracts through rectal palpation to evaluate the nature of

the discharge based on the color and volume of pus. If the vaginal discharge shows clear translucent mucus character, the score is 0 and when it shows clear mucus pus containing flecks of white pus character, the score is 1 and when it contains $\leq 50\%$ white or yellow pus character, the score is 2, and when it contain $\geq 50\%$ white, yellow or bloody pus character, the score is 3 (Sheldon *et al.*, 2006).

Through the macroscopic study of a total 250 specimens of the female genital tracts, the uteri are classified non pregnant . Uterus was incised to examine grossly to identify the infected and non infected uterus. Tissue samples were taken from the abnormal infected uterus for histopathological study. The samples were fixed in 10 %neutral formalin and processed for paraffin embedding and stained with Haematoxylin and Eosin(Hammon*et al.*,2006).

Experimental Design

Cows were divided into three groups: The 1st group the study was 100 cows conducted clinically fields of cross and native dairy cows. The 2nd group of the study was 100 samples done through the gross investigation of the female reproductive tract in the Basrah slaughter house and The 3rd group of the study was 50 samples of the female reproductive tract in the Basrah abattoir as the control group as following:

Group 1: 100 cows conducted clinically on fields of cross and native dairy cows, to evaluate blood parameters such as. Hormonal profile FSH, LH andProgesterone,Hematology parameter complete blood picture (CBC),Biochemical parameters (Zn, magnesium andCopper)and Uterine swab collection from uterus and valve to evaluate bacteria in reproductive system .

Group 2: 100 samples conducted of the study was done through the gross investigation of the female reproductive tract in the Basrah slaughter house to evaluate pathological study cases of uterus ,Blood parameters such as hormonal profile (FSH, LH andProgesterone). Hematology parameter complete blood picture (CBC). Biochemical parameters (Zn, Magnesium andCopper)uterine swab collection from uterus and valve.

Group 3: 50 samples conducted of the study as a control group was collected done through the gross investigation of the female reproductive tract in the Basrah slaughter house to evaluate :Histopathological study cases of uterus, blood parameters as hormonal profile (FSH, LH andprogesterone), hematology parameter complete blood picture (CBC), Biochemical parameters such as (Zn, Magnesium andCopper)and Evaluate uterine swab collection from uterus and valve.

Clinical Examinations

The general health status, appetite, rectal temperature ,pulse rate, respiration rate, and other clinical signs such as arched back, colic, pain, paresis, and presence of fresh discharge on the vulva, perineum, or tail were recorded. Rectal examination of uterus, oviducts ,ovaries (if palpable), and cervix was performed on each cow. The vulva was wiped with damp clean tow-els, disinfected with iodine - povidine, and washed again with water. A sterile vaginal speculum was lubricated with sterile petroleum jelly and inserted into the vagina to the level of the external os of the cervix. Inspection of the cervix and vagina was performed with illumination from a penlight. The examination lasted for 4 to 5 mint. cows diagnosed with metritis or endometritis were required to

meet the following 2 criteria: 1) cows must have been febrile (i.e., rectal temperature ($>39^{\circ}\text{C}$) and 2) have had a flaccid, non retractable uterus located in the abdomen, a cervical diameter >7.5 cm, and a watery, fetid vulval discharge..

Blood samples collected : Ten ml of blood were collected from jugular vein of the live cows or after slaughter from abattoir. Five ml of blood use test tube with gel and five ml without EDTA to evaluation CBC . The blood was transported within 2 h to the laboratory in an ice box and centrifuged at $1700 \times g$ for 15 min to isolated the serum then collected in tube for laboratory analysis to evaluation hormones and biochemical parameters .

Uterine Swab Collection and Bacteriology:

Sampling for bacteriological examination was performed immediately after clinical examination and diagnosis of endometritis and metritis . The vulval lips were parted and an outer protective sterile plastic straw sheath was advanced into the vagina and fixed in the external opening of the cervix. A guarded inseminating pipette was advanced through the outer tube into the uterus by cervical manipulation similar to the technique for AI.

The sterile swab was pushed out of its protective sterile plastic sheath (to protect from contamination with cervix) and moved about slightly in the body of the uterus. After retraction into its cover, the swab was removed from the vagina. Swabs were transferred into sterile tubes containing thioglycolate broth as a transport media, transported to the laboratory at 4°C , and processed for bacteriological examination. Swabs were cultured aerobically on MacConkey agar, nutrient agar and blood agar. Bacteria were identified after a 24-h incubation at 37°C for aerobic growth and 7-d incubation for anaerobic bacterial growth. Identification was based on the characteristic of colony, hemolysis, gram stain, morphology (Azawietal.,2007).

Results

Clinical Study:

The study's findings emerged from a clinical and vaginal investigation of cows. The cows were classified as repeat breeders based on their inability to conceive after regular service with known fertility bulls and/or insemination with high-quality sperm. There were suspected conditions according to macroscopic inspection during the gross or macroscopic inspection of 250 samples (100 live samples, 100 sample abattoir and 50 normal samples) .According to the kind of discharge evident after uterine, 61 samples (24.4 per cent) of female reproductive systems indicated endometrial uterine infections macroscopically. The results also appeared 43 cases (17.2%) of the total affected reproductive system contains metritis. Moreover, the clinical study showed 6 cases (2.4%) of the total affected reproductive system contains tumour lesions, the result appeared 50 cases(20%) of the total affected reproductive system experience mineral deficiency, as well as it was showed 30 cases (20%) of the total affected reproductive system contains Para ovarian cyst, as more as 10 cases (6.6%) of the total affected reproductive system contains follicular cyst, During the gross or macroscopic inspection of cases 4(1.2%) suffered from uterine tube infections (Piosalpingitis) as below in (Table 1).

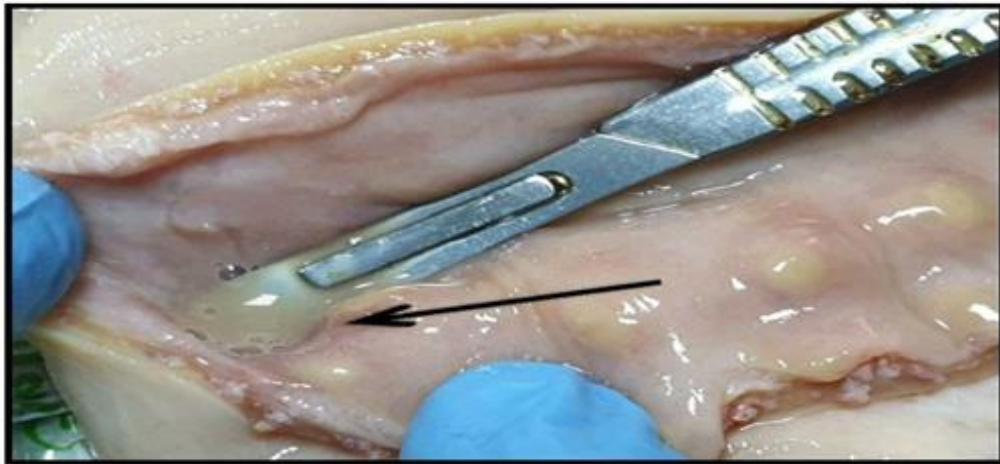
TABLE .1: Investigation of female reproductive tracts in the clinical and macroscopic study of cows

Specimens/cows	Macroscopic study	Total Percentage
Endometrial uterine infections	61	24.4%
Metritis	43	17.2%
Tumor	6	2.4%
Mineral deficiency	50	20%
Para ovarian cyst	30	20%
Follicular cyst	10	6.6%
Piosalpingitis	4	1.2%
Total	204	91.8%

Percentages of reproductive problems were tested by χ^2

After necropsy finding for the female reproductive system (abattoir samples), clear suppuration pus was found locally in the body of the uterus, which extends to the horns of the uterus and oviducts, as well as the anatomical characteristic the pus of suppuration different in colour (white, yellow or bloody pus character) as (Fig1). and the lack of asymmetry in horns of the uterus.

Healthy embryonic growth is supported by the uterine environment. As a result, any abnormality endangers the embryo's life and causes the RBC syndrome. After metritis, uterine infections (both specific and generic) will negatively influence reproductive indices by enlarging the uterine and cervical postpartum involution, altering follicular development, and increasing embryo death and repeat estrus frequencies.



(Fig. 1) Purulent discharge in uterine horns (black arrow).

Bacterial isolation from uterine cows with endometritis and metritis:

The results showed that all cows with (abattoir samples and live samples) endometritis and metritis had a history of obstetrical problems, including dystocia, retained placenta, prolapse of the vaginal and prolapse, and prolapse of the uterine. Clinical examination of cows uterine infection was indicated depression, anorexia, paresis, difficulty in rising constipation with scanty black faeces, staggering gait, frequent and recurrent painful straining. The results also appear to increase rectal temperature, increased pulse heart rate, and increased respiration rate compared to the control group. During the examination of all vaginal cows infected with metritis and endometritis, there is a watery fetid white or yellow discharge from the female reproductive tract. The variety and prevalence of bacterial isolates from the uterus of the infected cows are 104 cases (endometritis 61 cases 24.4% and metritis 43 cases 17.2%). The results appeared during bacterial isolates from cows uterine discharge increased percentage of *Escherichia coli* (63.46%), compare, with *Staphylococcus aureus* (9.61%) and *Streptococcus pyogenes* (26.93%) as below in (Table .2).

TABLE . 2: Prevalence of bacterial isolates from reproductive birth cows Repeat breeder.

Bacterial isolate	uterine infected (metritis and endometritis)	Total Percentage
<i>Escherichia coli</i>	66	63.46%
<i>Staphylococcus aureus</i>	10	9.61%
<i>Streptococcus pyogenes</i>	28	26.93%
Total	104	100%

percentages of reproductive problems were tested by χ^2 analysis.

Effectuated Hematological disorder on repeat breeding in cows :

In the current study, the abattoir group and live female group cows repeat breeding had substantially higher white blood cells (WBCs $\times 10^9/L$) and lymphocyte (Lymph $\times 10^9/L$) levels than the control group. The repeat breeding cows (abattoir group and live female group) had substantially lower haemoglobin and PCV levels ($P \leq 0.05$) than the control group. Furthermore, the red blood cells (RBCs $\times 10^{12}/L$) values were significantly ($P \leq 0.05$) lower in the repeat breeding cows (abattoir group and live female group) than in the control group. Moreover, the plate late count PLT value was significantly ($P \leq 0.05$) higher in the repeat breeding cows (abattoir group and live female group) than in the control group. Compared to the control groups, the repeat breeding cows (abattoir group and live female group) had markedly ($P \leq 0.05$) higher mean monocytes counts on average (Table .3).

TABLE .3: Blood Haematological parameters (mean \pm SE) of repeat breeder in cows and Normal cyclical :

Blood parameter	control group (normal cyclical)	Abattoir group (Repeat breeder cow)	Live group (Repeat breeder cow)
WBCs $\times 10^9/L$	6.2 \pm 0.05 A	13 \pm 0.23 C	14.05 \pm 0.17 B
Lymph $\times 10^9/L$	2.5 \pm 0.23 A	4.1 \pm 0.71 C	5.4 \pm 0.22 B
RBCs $\times 10^{12}/L$	5.37 \pm 0.22 A	3.82 \pm 0.13 B	3.15 \pm 0.21 B
HB g/dL	13 \pm 0.11 A	9.4 \pm 0.32 B	10.4 \pm 0.16 B
PCV %	41 \pm 0.12 A	32 \pm 0.37 C	36 \pm 0.19 B
PLT $10^9/L$	252 \pm 0.42 A	423 \pm 0.14 B	357 \pm 0.26 C
Monocyte (%)	1.4 \pm 0.71 A	5.1 \pm 0.22 B	4.6 \pm 0.45 C

Values are means \pm SE; means with different superscripts in the same raw are significantly different at the level $P \leq 0.05$.

Effectuated Elements deficiency on repeat breeding in cows :

The results of the average serum copper, zinc, and magnesium concentrations in cows with repeat breeding in the control group and (Abattoir group, Live group) have been mentioned in Table .4. The repeat breeders groups had substantially lower mean serum copper concentrations ($P < 0.05$) than the control group. Additionally, The serum Zinc concentrations of the repeat breeding cows (Live group and Abattoir

group) were observed to be substantially lower ($P < 0.05$) than those of the control group. Furthermore, according to the results, magnesium concentrations in the repeat breeder group were substantially lower ($P < 0.05$) than in the control group.

TABLE .4:serumbiochemical values in normal cyclical and repeat breeding cows (mean±SE).

Minerals parameter	control group (Normal cyclical)	Abattoir group (Repeat breeding)	Live group (Repeat breeding)
Copper (µmol/L)	31.5±1.53 A	16.1±0.88 B	14.7±0.58 B
Zinc (µmol/L)	21.4±0.44 A	13.05±0.51 B	12.60±0.30 B
Magnesium(mg/Dl)	2.11 ± 0.13 A	1.28 ± 0.21 A	1.56 ± 0.18 A

The difference between the values marked with various letters in the same line is statistically significant ($P < 0.05$).

Effectuated hormonal deficiency on repeat breeding in cows :

The experiment's results are summarised in the table .5. When comparing repeat breeder cows to normal cyclic cows, serum FSH was significant difference lower ($P < 0.05$) values camper with normal cycle cows . While the present study showed serum LH, was non – significantly between groups. On the other hand, the results show that blood serum Progesterone levels in repeat breeder cows was significant difference ($P < 0.05$) values camper with normal cycle cows .

TABLE.5:serumhormone values in normal cyclical and repeat breeding cows (mean±SE).

serum parameter hormone	control group (normal cycle cow)	Abattoir group (Repeat breeder cow)	Live group (Repeat breeder cow)
FSH (mIU/ml)	1.87±0.23 A	1.23±0.21 B	1.22±0.43 B
LH (mIU/ml)	0.45±0.12 A	0.37±0.04 A	0.44±0.51 A

Progesterone (ng/ml)	5.31±0.62	3.26±0.52	3.15±0.21
	A	B	B

The difference between the values marked with various letters in the same line is statistically significant ($P<0.05$).

Histopathological Results:

Out of 250 female cows genitalia collected from a local slaughterhouse and filed at Basrah Province showed Inflammatory gross changes of endometritis were found in 61 (24 %) and classified according to histological examination as; chronic endometritis 19 /61 (31%), subacute endometritis 22/61 (36 %) and acute endometritis 20/61 (32 %), while metritis cases 43/250 (17.2 %) were of the chronic type of inflammation. All endometritis and metritis cases were characterized by increased thickness of uterine serosa with yellowish coloured pus accumulation.

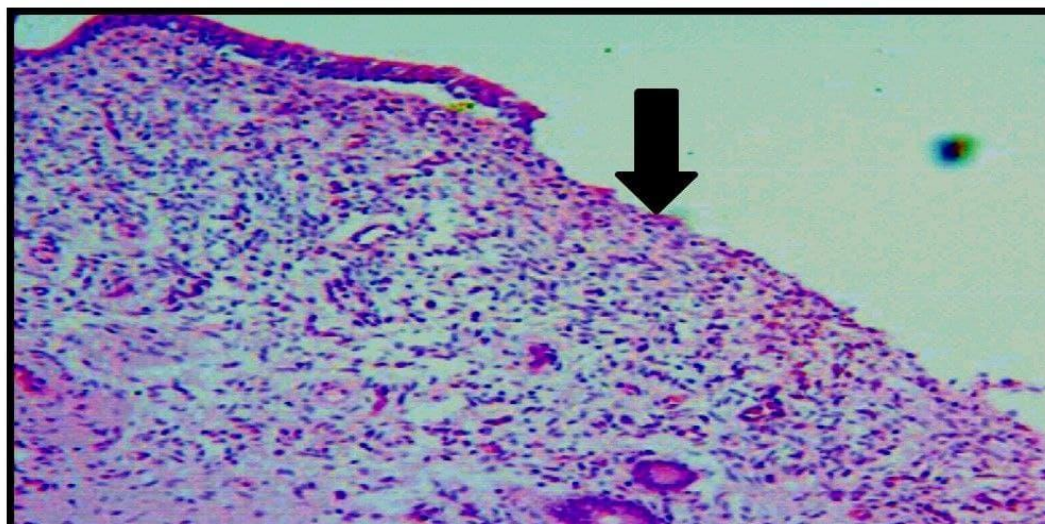


Figure (2): Acute endometritis shows infiltration of polymorpho nuclear inflammatory cells in the uterine mucosal layer there was focal denudation of endometrial epithelium (black arrow) H&E stain 100x .

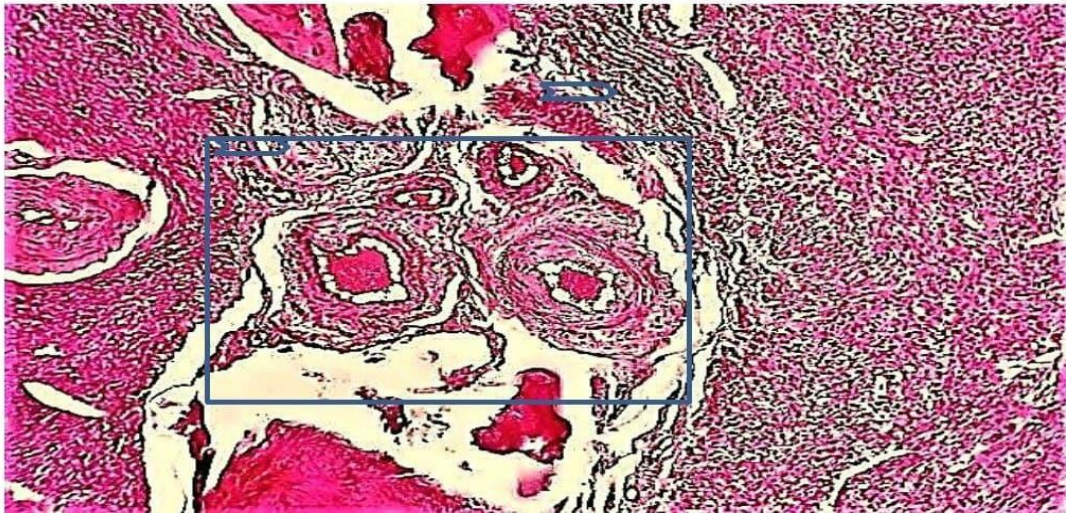


Figure (3) Histopathological section of the uterus showed that hyperplasia and necrosis of interglandular lamina propria (head arrows) and hyperplasia of artery walls (circle). (H and E 100X).

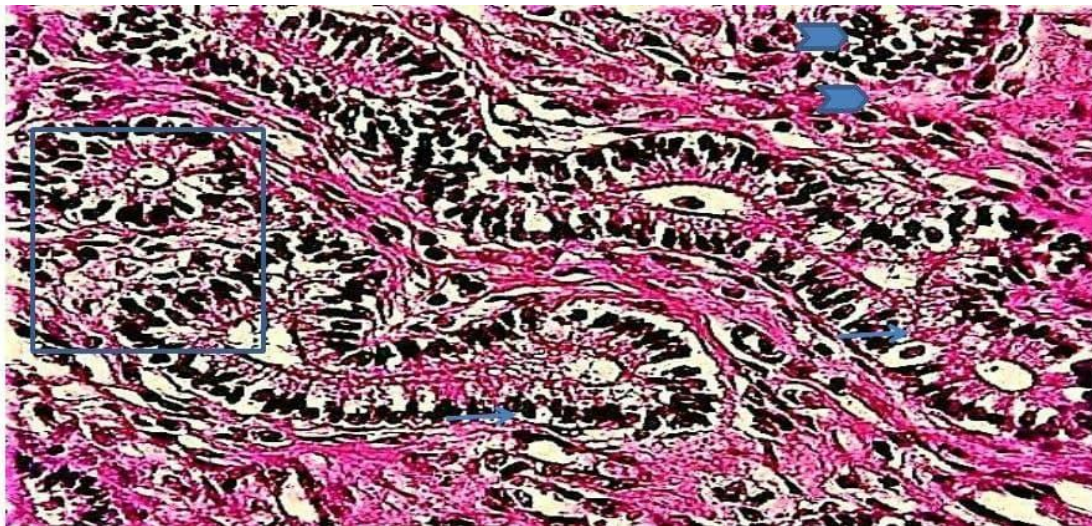


Figure (4) uterus section showed that many cells appeared a large clear (empty) supranuclear vacuole (thin arrows), hyperplasia in some glands (thick arrows) and hypertrophy of uterine glands cells (circle) (H and E 400X).

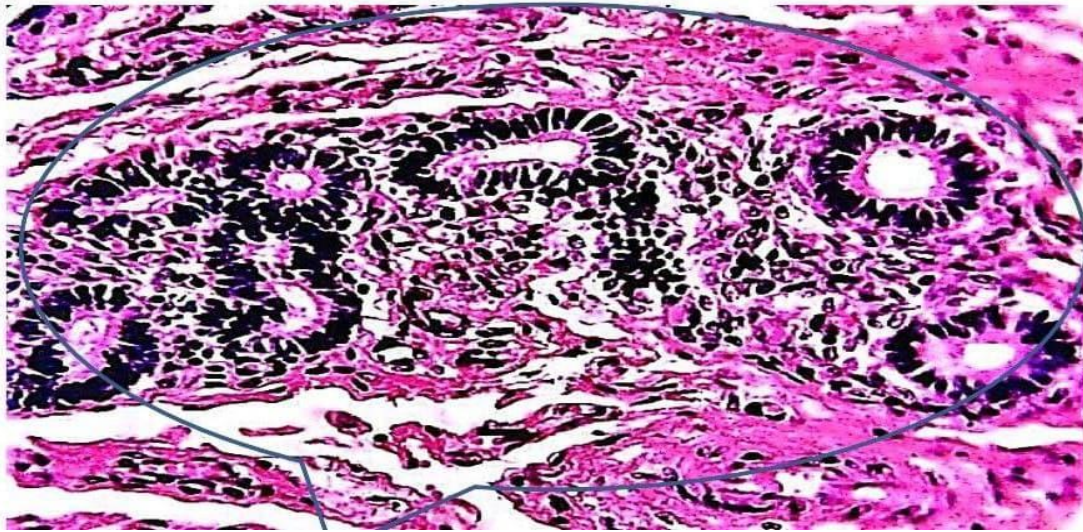


Figure (5) Uterus section showed that clear state of the adenomyosis (uterine subacute endometritis) (circle). (H and E 400X).

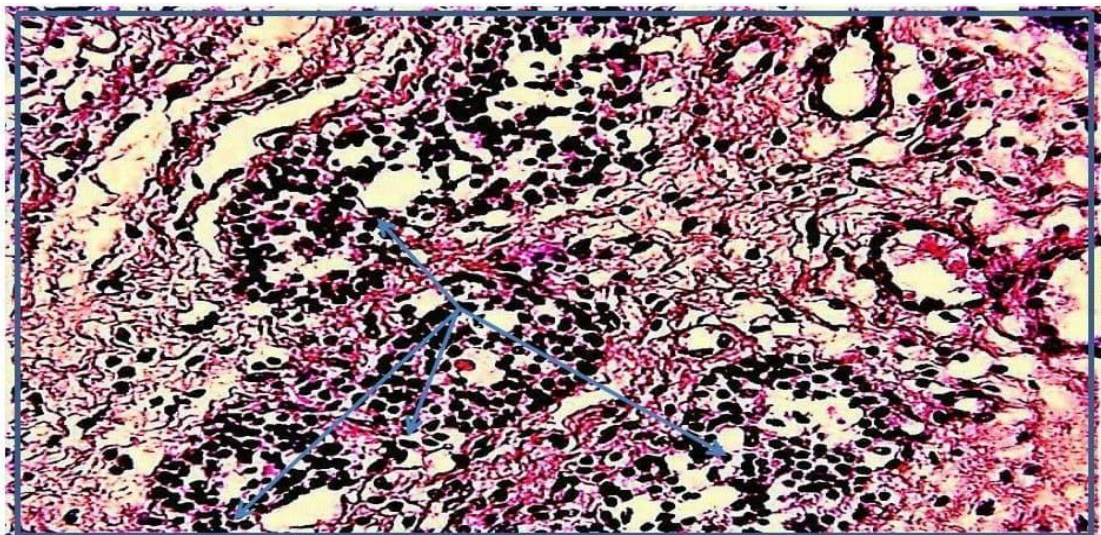


Figure (6) Histopathological section of uterus section showed of infiltration mononuclear inflammatory cells in the uterine parenchyma (arrows), with degeneration and necrosis of endometrial tissues (circle). (H and E 400X).

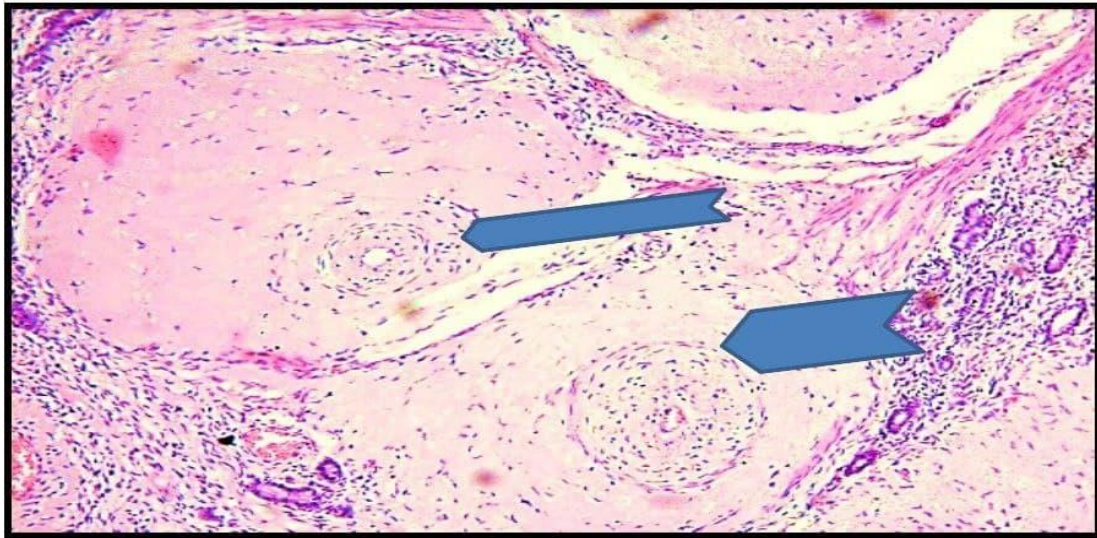


Figure (7). Chronic endometritis: Medial hypertrophy of blood vessels with stenosis of lumina and infiltration of inflammatory cells in lamina propria(H and E 400X).

Discussion

Uterine infection and repeat oestrous cycles

The present study showed that the repeat breeder syndrome in cows is mainly due to subclinical endometritis and metritis after estrus, parturition, retained fetal membranes, dystocia, obstetrical manipulation, uterine prolapse, and vaginal prolapse. The major cause of subclinical endometritis or metritis is the contamination of the cows' genital systems owing to a bacterial infection that has spread downward(Ahmed,2013). The results agreed with Ahmed and Elshiekh (2013), who mentioned contamination of the genital tract during intervention and manoeuvring at the time of parturition.After parturition, the uterus of more than 90% of cows gets infected with germs (Griffin et al., 1974). Opsomer *et al.* (2000) showed that bacteria products cause uterine inflammation, decrease pituitary gland secretion and reduce FSH and LH release. Consequently, ovarian follicular growth and function are perturbed, and the ovulation is delayed in the cows, leading to fertilisation failure. Huszenicza *et al.* (2005) also showed If fertilisation happens, the infection will perturb the embryo survival and implantation.Edwell, *et al.* (2004) reported that subclinical endometritis and metritis lead to failure of conception because they alter the pH of the genital tract.This finding agrees with Grohan and Saoloemi (1995), who mentioned that all the repeat breeder cows in the current study (slaughterhouse and live animals) were infected with the uterus. Cows with subclinical endometritis exhibit quiet heat, failed inseminations and repeated breeding, according to several researchers such as Sarkar (2006).Santana *et al.* (1998b) appear uterine environmental enhancing the normal development embryonic. Repeated breeding cows syndrome is also a result of any disease that impairs the embryo's. Many researchers have found a relationship among cows with repeat estrous cycle and endometrial abnormalities. Lewis (1997) mention reproductive failure appears after metritis. It was found by

Ferreira et al. (2011) that between 3.5% and 5.7% of cows with metritis experienced repeat estrous cycle cycles. Uterine infections (both specific and nonspecific) are thought to have a negative impact on reproductive indices by enlarging the uterus and affecting follicular development during post-partum involution. Santana et al. (1998) appear repeat estrus rates increased after early embryo mortality. Melendez et al. (2004) pointed out that cows recovering from metritis or endometritis can become chronic, causing high economic losses due to prolonged open days and prolonged intercalving intervals, resulting in involuntary culling (Karimiet al., 2004).

The results concurred with Konigsson et al., 2002, who indicated that foetal membranes retention is the real cause of metritis in cows following parturition. It was found by Ferreira et al. (2011) that uterine infection was 25 times more common in cows with retained fetal membranes than in ordinary cow. Retained placenta reduced uterine immunity by impaired uterine function (Kimura et al., 2002). Whereas the present study's bacteriological findings are in line with previous studies on cattle, there are differences in the prevalence of certain bacteria isolated from cows' uterus compared to cattle (Dohmen et al., 2000). Cows with endometritis and metritis were predominantly bacterially insulated from Staph. aureus, F. necrophorum, A. pyogenes and E. coli. At the same time, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staph.aureus*, *Bacillus icheniformis*, and *E. coli* were isolated from cows with normal puerperium.

Elements deficiency and repeat estrous cycles

Several studies have investigated nutrient elements deficiency in cows. Copper, zinc, and Iron deficiencies have been observed and studied in fifty cows selected from Basrah province 2020. For development and reproduction, elements such as Calcium, phosphorus, Zinc, magnesium, and copper are needed and participate in the digestive, physiologic, and biosynthetic processes in the body in many ways (Hurley et al., 1989). According to Boland (2003), the primary purpose of these elements is to give structural support and are made up of bodily organs and tissues. Moreover, they function as electrolytes, bodily fluid components, and enzyme and hormone catalysts. Additionally, they perform many critical activities to preserve animal development and reproduction, and health condition. Spain et al. (2007) found that the decrease of the elements (Manganese, Copper, Selenium, Cobalt, Iron, Iodine, and Zinc) had a negative impact on ruminant fertility. Kumar (2014) has observed that the decline in single or combination trace elements and irregular imbalances may lead to reproductive failure. Also, he mentioned that the deficiency of minerals such as iron, calcium, phosphorus, zinc, and copper had been reported to be a predisposing factor for the helping of occurrence repeat breeder in cows (Sheeta et al., 2014). The present study agreed with Constable et al. (2017), who mentioned that copper acts as a component of metalloenzymes, including in several physiological reactions such as carbohydrate and lipid metabolism, antioxidant activities, respiration, and the formation of the collagen. Tomlinson et al. (2004) pointed copper is also a component of polyphenyl oxidase which catalyses the conversion of tyrosine to melanin and incorporation of sulfide group into keratin in the hair (Underwood and Suttle, 1999). Maxwell (1972) noted that the most prevalent signs are prenatal mortality, especially early embryonic loss in cattle, as hypocuprosis (copper

deficiency) is related to female reproductive disorders. Most of the relevant studies, such as McChowell and Haile. (2014) recorded a low copper content in the ewe diet prevented implantation or induced embryonic loss and fetal death. Anke *et al.* (2012), who recorded one of the earliest signs of copper deficiency, showed nymphomania and a decline in fertility in cows. Copper has excellent roles in body metabolism and immune system function as cofactors for some metalloenzymes, making them vital for cattle (Tomaszewska *et al.*, 2016). A primary copper deficiency in cows caused decreased conception rates and depressed immunity, ill-thrift, decreased milk production, anemia, and rough coat and discolored its color (Philip *et al.*, 2011; Constable *et al.*, 2017). On the other hand, zinc has been recognised as indispensable for average growth and health in animals for several decades. also, mention its lack causes various malformations and has deleterious effects on sexual functions (Tomaszewska *et al.*, 2016). Wilkinson *et al.* (2008) noted that zinc is an essential metabolically active trace element in more than 300 distinct animal health and productivity enzyme systems as prostheses. The result agreed with Vitti (2018), who pointed out that zinc played a crucial role in the reproductive activities of cows, such as enhanced pregnancy rates, robust oestrus cycles, reduced metritis, and promotion of normal uterine involution post-partum. Constable *et al.*, 2017 who recorded Zn deficiency in cows caused decreased conception rates and depressed immunity. Korovina and Morozow (1999) showed fertility of sheep had been improved when both rams and ewes received zinc supplementation. eight also showed the various factors responsible for the poor fertility of ewes on a low zinc ratio was early embryonic mortality, the fertilised ovum of ewes fed low zinc diets does not implant in the uterine mucosa. On the other hand, Anke *et al.* (2017) pointed Feeding goats' semipurified diets low in zinc caused a low conception rate and reduced the number of kids per goat. Furthermore, according to Dyrendahl (2007), the element is important for mending and maintaining the uterine lining after calving, allowing for a faster recovery to healthy reproductive performance and oestrus. Reduced fertility, irregular oestrus, and abortion have all been attributed to insufficient Zinc concentrations. Moreover, Zinc enzyme regulates the arachidonic acid cascade as a result of its involvement in the prostaglandins formation (Chanmugam *et al.*, 2010). Prostaglandins secretion into the uterine cavity and away from the uterine vasculature is enhanced in pregnant pigs, indicating that prostaglandins are necessary for pregnancy maintenance. Conversely, suppression of PGF₂ synthesis, on the other hand, has an influence on the establishment of pregnancy in swine, because Zinc's effects on PGF₂ production may promote conceptus development (Kraeling *et al.*, 2011). In this study, Compared to normal cyclic cows, repeat breeder cows had substantially lower serum magnesium concentrations (P<0.05). Magnesium insufficiency in repeat breeder cows had previously been observed (Das *et al.*, 2009). Despite the fact that magnesium has no consistent role in reproduction, it is engaged in a variety of biochemical processes catalysed by ATP-linked enzymes. In addition, magnesium influences phosphorus and calcium absorption (Sharma *et al.*, 2004). As a result, magnesium deficiency can have a secondary effect on reproduction.

The effect of the hormone on repeat breeder syndrome in cows:

Hormones are chemicals that govern and regulate particular cells or organs generated in the body. Dairy cattle reproduction is regulated by many hormones generated by

multiple endocrine glands, according to Short and Adams (1988). The glandular cells produce these hormones, which then enter the bloodstream and are carried throughout the body to perform their tasks; As a result, a variety of variables impact the reproductive cycle. As a result, a variety of variables have an impact on the reproductive cycle. According to Kaswan and Bedwal (1995), zinc may cause hypothalamic GnRH secretion to be decreased, resulting in a reduction in LH and FSH. In repeat breeding animals, the low-level estradiol and progesterone corroborate the current findings in which FSH and LH play their part in keeping a low ovarian progesterone level. Akhtar et al. (2010) and Ahmed et al. (2010) also noted that blood progesterone level was substantially low in repeat breeder buffaloes ($p < 0.05$) in comparison to normal animals during the mid-luteal stage of the oestrous cycle. In contrast, Kumar *et al.* 2014 found the blood progesterone sub-estrus cows in anoestrous and repeated breeders, even in normal cycle cows, to be considerably greater. Progesterone is an important hormone for the study of reproductive activity. It is generally used to monitor ovarian function in farms animals since it represents the various stages of corpora lutea following ovulation. Prostaglandin F₂-a mobilizes intracellular calcium in the luteal cells, produces oxygen radicals, removes ascorbic acid, and suppresses steroidogenesis, finally leading to cell mortality. This results in a regression of the corpus luteum and a reduction in progesterone production, which might be the cause why the repeat breeding animal is substantially below its value. Abnormalities in ovulation, reasons of fertilization failure, and endocrine dysfunction, according to Haimanot and Tewodros (2016), are the main risk factors for repeated breeding in the dairy cow. In contrast, Bage (2002) indicated that the adrenal progesterone is suprabasal in induced stress cows. Stress might therefore be regarded as a possible cause for repeated syndrome breeding. Furthermore, Opsomer et al. (2000) reportedly causes uterine inflammation by bacteria and their products, reduces pituitary FSH and suppresses the release of LH. Consequently, ovarian follicular development and function are disrupted in dairy cows, and ovulation is delayed, resulting in fertilization failure. If fertilization does occur, the infection will disrupt embryo survival and implantation. Sarkar (2006) reported that repeat breeding could also be due to trace elements deficiencies, particularly Zinc and Magnesium deficiency. Zn and Mg are essential for initiating follicular wave dynamic, ovarian activities, the release of best quality mature oocytes and improvement of the rate of service per conception in dairy cows. At the same time, Copper deficiency has been linked to decreased fertility in cattle and sheep, according to Akhtar et al. (2009).

Histopathological evaluation of repeat breeding :

The evaluation of clinical and postmortem examination findings with the histopathological and microbiological findings is important in determining the aetiology of RB and in developing more appropriate treatment protocols (Azawiet *al.* 2008; Ferreira *et al.* 2008; Kaya 2008; Polat 2008; Ergünet *al.* 2009; Cannazik and Polat 2015). Endometritis is the inflammatory reaction that occurs against various factors (parturition, mating, infection, etc.) in the endometrium of the uterus. Histopathologically, it is characterised by degeneration, desquamation, and inflammatory cell infiltration in the lamina epithelial, different degrees of inflammatory cell infiltration, vascular, glandular, and stromal changes in the lamina propria. (Alaçam 2007, Kaya 2008). However, it is reported that there may be a small

number of neutrophils in the endometrium in the pro oestrus, oestrus, and metoestrus stages in healthy cows, and this situation should be considered in the evaluations to be made in the endometrium (Kaya 2008, Espejel and Medrano 2017). In this study, endometritis was detected in 39% of repeat breeder cows. Other studies reported rates ranging from 12% to 100% (Pothmann *et al.* 2015). Endometritis has a vast range in other studies thought to be related to the number of selected animals, the difference in the management of the enterprises, and time. Haemorrhage was determined in the lumen of one uterus sample in the macroscopic examination. It was thought in the histopathologic examination that neutrophil infiltration, mononuclear cell infiltration, and increased connective tissue indicated chronic endometritis, and the determined haemorrhage might be associated with metoestrus. Degeneration and desquamation at different grades were detected in lamina epithelialis in 25 out of the uterus samples evaluated in the study. Similar findings were determined in cases of endometritis in other studies (Dogan *et al.* 2002; Azawiet *al* 2008; Doğruer and Güler, 2010). Furthermore, degeneration and desquamation were detected in lamina epithelialis in two of uterus samples of the control group, but no inflammatory cells were found in the study. In these two examples, the changes observed in lamina epithelialis are thought to be caused by errors in the preparation stages. The study observed increased connective tissues at different grades around the glands in the lamina propria of 12 uterus samples. Dogan *et al.* (2002) determined periglandular fibrosis of 13.33% in their study. It was reported that severe periglandular fibrosis might cause embryonic death by inhibiting the glandular functions required by the embryo (Polat 2008). In the study, the histopathological findings such as haemorrhage, oedema etc., in the endometrium of 33 (52.34%) uterine samples that are not detected endometritis were the changes based on the cycle phase. Dogan *et al.* (2002) stated in their study that they found similar findings in both fertile and RB cows, and these were the normal histological changes that did not affect fertility. In addition, it was also reported that the haemorrhage and stromal oedema in the endometrium might result from the estrogenic effects in the follicular phase (Espejel and Medrano, 2017).

References :

- Ahmed, F. O., & Elsheikh, A. S. (2013). *Intrauterine infusion of lugol's iodine improves the reproductive traits of postpartum infected dairy cows.*
- Amirkalali, B., Sharifi, F., Fakhrzadeh, H., Mirarefein, M., Ghaderpanahi, M., Badamchizadeh, Z., & Larijani, B. (2010). Low serum leptin serves as a biomarker of malnutrition in elderly patients. *Nutrition Research*, 30(5), 314–319.
- Akhtar, M. S., Farooq, A. A., & Mushtaq, M. (2010). Biochemical and hormonal profile in anoestrus Nili-Ravi buffaloes. *Indian Veterinary Journal*, 87(6), 603–604.
- Anke, M., Hennig, A., Grun, M., Partschefeld, M., Groppe, B., & Ludke, H. (2012). Arsen, a new essential trace element. *Arch Tierernahr.*
- artlett, P. C., Kirk, J. H., & Mather, E. C. (1986). Repeated insemination in Michigan

- Holstein-Friesian cattle: Incidence, descriptive epidemiology and estimated economic impact. *Theriogenology*, 26(3), 309–322.
- Azawi, O. I., Omran, S. N., & Hadad, J. J. (2008). A study of endometritis causing repeat breeding of cycling Iraqi buffalo cows. *Reproduction in Domestic Animals*, 43(6), 735–743.
- Ball, P. J. H., & Peters, A. R. (2008). *Reproduction in cattle*. John Wiley & Sons.
- Båge, R. (2002). *On repeat breeding in dairy heifers* (Vol. 139, Issue 139).
- Boland, M. P., & Lonergan, P. (2003). Trace minerals in production and reproduction in dairy cows. *Advances in Dairy Technology*, 15, 319–330.
- Bollongino, R., Burger, J., Powell, A., Mashkour, M., Vigne, J.-D., & Thomas, M. G. (2012). Modern taurine cattle descended from small number of near-eastern founders. *Molecular Biology and Evolution*, 29(9), 2101–2104.
- Brown, D. (2009). Scientists unravel genome of the cow. Sign of human intervention seen. *Washington Post*.
- Chanmugam, P., Wheeler, C., & Hwang, D. H. (2010). The effect of zinc deficiency on prostaglandin synthesis in rat testes. *The Journal of Nutrition*, 114(11), 2066–2072.
- CANNAZİK, O., & POLAT, B. (2015). İneklerde postpartum dönemde endometritisin sınıflandırılması ve tanımlanmasında kullanılan muayene yöntemleri. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi*, 10(3).
- Das, J. M., Dutta, P., Deka, K. C., Biswas, R. K., Sarmah, B. C., & Dhali, A. (2009). *Comparative study on serum macro and micro mineral profiles during oestrus in repeat breeding crossbred cattle with impaired and normal ovulation*.
- Dogan, İ., Sonmez, G., & Sagirkaya, H. (2002). *Histopathological investigation of endometrium in repeat breeder cows*.
- DOĞRUER, G., & GÜLER, M. (2010). İneklerde endometritisin tanısında klinik muayene, endometriyal sitoloji, biyopsi ve mikrobiyolojik muayene bulgularının karşılaştırılması. *Kocatepe Veterinary Journal*, 3(1), 19–24.
- Duarte, A. B. G., Araújo, V. R., Chaves, R. N., Silva, G. M., Magalhaes-Padilha, D. M., Satrapa, R. A., Donato, M. A. M., Peixoto, C. A., Campello, C. C., & Matos, M. H. T. (2012). Bovine dominant follicular fluid promotes the in vitro development of goat preantral follicles. *Reproduction, Fertility and Development*, 24(3), 490–500.
- Dohmen, M. J. W., Joop, K., Sturk, A., Bols, P. E. J., & Lohuis, J. (2000). Relationship between intra-uterine bacterial contamination, endotoxin levels and the development of endometritis in postpartum cows with dystocia or retained placenta. *Theriogenology*, 54(7), 1019–1032.
- Espejel, M. C., & Medrano, A. (2017). Histological Cyclic Endometrial Changes in

Dairy Cows: An Overview. Review. *Journal of Dairy and Veterinary Sciences*, 2(8), 2017.

Ferreira, R. M., Ayres, H., Chiaratti, M. R., Ferraz, M. L., Araújo, A. B., Rodrigues, C. A., Watanabe, Y. F., Vireque, A. A., Joaquim, D. C., & Smith, L. C. (2011). The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. *Journal of Dairy Science*, 94(5), 2383–2392.

Ferreira, R. M., Ayres, H., Chiaratti, M. R., Ferraz, M. L., Araújo, A. B., Rodrigues, C. A., Watanabe, Y. F., Vireque, A. A., Joaquim, D. C., & Smith, L. C. (2011). The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. *Journal of Dairy Science*, 94(5), 2383–2392.

Gröhn, Y., Erb, H. N., McCulloch, C. E., & Saloniemi, H. S. (1990). Epidemiology of reproductive disorders in dairy cattle: associations among host characteristics, disease and production. *Preventive Veterinary Medicine*, 8(1), 25–39.

Goumenou, A. G., Matalliotakis, I. M., Koumantakis, G. E., & Panidis, D. K. (2003). The role of leptin in fertility. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 106(2), 118–124.

Haile, A., Tsegaye, Y., & Tesfaye, N. (2014). Assessment of major reproductive disorders of dairy cattle in urban and per urban area of Hosanna, Southern Ethiopia. *Environment*, 8, 22.

Haluzik, M., Fiedler, J., Nedvidkova, J., & Češka, R. (1999). Hyperlipidemia: Relationship to Serum Lipids and. *Physiol. Res*, 48, 363–368.

Hurley, W. L., & Doane, R. M. (1989). Recent developments in the roles of vitamins and minerals in reproduction. *Journal of Dairy Science*, 72(3), 784–804.

Huszenicza, G. Y., Jánosi, S. Z., Kulcsar, M., Korodi, P., Reiczigel, J., Katai, L., Peters, A. R., & De Rensis, F. (2005). Effects of clinical mastitis on ovarian function in post-partum dairy cows. *Reproduction in Domestic Animals*, 40(3), 199–204.

KAYA, D. Y., & SALMANOĞLU, M. R. (2008). *İneklerde kronik endometritis olgularında Lotagen®, EucaComp® ve PGF2□□ uygulamalarının fertilité parametreleri üzerindeki etkilerinin araştırılması*. Ankara Üniversitesi Sağlık Bilimleri Enstitüsü Doğum ve Jinekoloji Anabilim Dalı.

Kaswan, S., & Bedwal, R. S. (1995). Light and electron microscopic changes in the ovary of zinc deficient BALB/c mice. *Indian Journal of Experimental Biology*, 33(7), 469–479.

Karimi, H. I., Manspeker, H. A. I., Anvari, R., Moradina, A. F., Neamattillahi, A. I., & Niksam, K. (2004). The study of important factors on reproduction of Azerbaijan buffaloes (*Bubalus bubalis*). 23rd World Buiatrics Cong. *Quebec & Eacute, Canada*, 613.

- Ketodihydro-PGF2 α , Progesterone and Uterine Involution in Primiparous Cows with Induced Retained Placenta and Post-partal Endometritis Treated with Oxytetracycline and Flunixin. *Reproduction in Domestic Animals*, 37(1), 43–51
- .Kumar, A. S. (2014). Blood biochemical profile in repeat breeding crossbred dairy cows. *International Journal of Veterinary Science*, 3(4), 172–173.
- Kraeling, R. R., Rampacek, G. B., & Fiorello, N. A. (2011). Inhibition of pregnancy with indomethacin in mature guts and prepuberal guts induced to ovulate. *Biology of Reproduction*, 32(1), 105–110.
- Korovina, E. K., & Morozov, A. Z. (1999). Influence of zinc supplement of the fertility of sheep under zinc deficiency conditions. *Soviet Agriculture Sciences*.
- Königsson, K., Gustafsson, H., & Kindahl, H. (2002). Leroy, J., Vanholder, T., Delanghe, J. R., Opsomer, G., Van Soom, A., Bols, P. E. J., & de Kruif, A. (2004). Metabolite and ionic composition of follicular fluid from different-sized follicles and their relationship to serum concentrations in dairy cows. *Animal Reproduction Science*, 80(3–4), 201–211.
- Kumar, A. S. (2014). Blood biochemical profile in repeat breeding crossbred dairy cows. *International Journal of Veterinary Science*, 3(4), 172–173.
- Kimura, K., Goff, J. P., Kehrlı Jr, M. E., & Reinhardt, T. A. (2002). Decreased neutrophil function as a cause of retained placenta in dairy cattle. *Journal of Dairy Science*, 85(3), 544–550.
- Lewis, G. S. (1997). Symposium: health problems of the postpartum cow, uterine health and disorders. *J Dairy Sci*, 80, 984–994.
- Maxwell, J. A. L. (2015). Selenium and ewe infertility in western Australia. *Australian Veterinary Journal*, 48(11), 598–600.
- Melendez, P., McHale, J., Bartolome, J., Archbald, L. F., & Donovan, G. A. (2004). Uterine involution and fertility of Holstein cows subsequent to early postpartum PGF2 α treatment for acute puerperal metritis. *Journal of Dairy Science*, 87(10), 3238–3246.
- Opsomer, G., Gröhn, Y. T., Hertl, J., Coryn, M., Deluyker, H., & de Kruif, A. (2000). Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology*, 53(4), 841–857.
- Opsomer, G., Gröhn, Y. T., Hertl, J., Coryn, M., Deluyker, H., & de Kruif, A. (2000). Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology*, 53(4), 841–857.
- Pothmann, H., Prunner, I., Wagener, K., Jaureguiberry, M., de la Sota, R. L., Erber, R., Aurich, C., Ehling-Schulz, M., & Drillich, M. (2015). The prevalence of subclinical endometritis and intrauterine infections in repeat breeder cows. *Theriogenology*, 83(8), 1249–1253.
- Rodriguez-Martinez, H. (2012). Assisted reproductive techniques for cattle breeding

in developing countries: a critical appraisal of their value and limitations. *Reproduction in Domestic Animals*, 47, 21–26.

- Sarkar, A. K. (2006). Therapeutic Management of Anoestrus Cows with Diluted Logul's Iodine and Massage on Reproductive Organs-uncontrolled Case Study. *Journal of Animal and Veterinary Science*, 1(1), 30–32.
- Sharma, M. C., Joshi, C., Saxena, N., & Das, G. (2004). Role of minerals in reproductive performance of livestock. *Livest. Intl*, 8, 5–10.
- Short, R. E., & Adams, D. C. (1988). Nutritional and hormonal interrelationships in beef cattle reproduction. *Canadian Journal of Animal Science*, 68(1), 29–39.
- Sheetal, S. K., Choudhary, S. K., & Sengupta, D. (2014). Mineral deficiency predisposes occurrence of retention of placenta in crossbred. *Veterinary World*, 7(12).
- SPAIN, J. N., LUCY, M. C., & HARDIN, D. K. (2007). Effects of nutrition on reproduction in dairy cattle. In *Current Therapy in Large Animal Theriogenology* (pp. 442–450). Elsevier.
- Tomlinson, D. J., Mülling, C. H., & Fakler, T. M. (2004). Invited review: formation of keratins in the bovine claw: roles of hormones, minerals, and vitamins in functional claw integrity. *Journal of Dairy Science*, 87(4), 797–809.
- Underwood, E. J. (1999). *The mineral nutrition of livestock*. Cabi.
- Tomaszewska, E., Dobrowolski, P., & Kwiecień, M. (2016). Intestinal alterations, basal hematology, and biochemical parameters in adolescent rats fed different sources of dietary copper. *Biological Trace Element Research*, 171(1), 185–191.
- Wilkinson, R. G., Mackenzie, A. M., Pattinson, S. E., & Wilde, D. (2008). Effect of organic or inorganic selenium and zinc supplementation of ewes on ewe and lamb performance and mineral status. *Trace Elements*, 299.