# Hormonal, Hematological, and Oxidant / Antioxidant Disturbances in Aborted Cows

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#### **Abstract**

**Background**: Cattle abortion, increasingly occurred in Iraq in last decades due to unknown causes, associates with various postpartum disturbances and/or complications which might affect markedly of the reproductive performance.

*Aims*: To estimate the possible changes in hormonal, hematological and antioxidant/oxidant profiles in aborted cows due to undiagnosed causes.

*Materials and methods*: A total of 80 adult cows; 40 naturally calving cows as a healthy control in addition to 40 recently aborted cows, were selected from different areas in Al-Qadisiyah province during March-November (2023), and subjected for draining the jugular venous blood that kept into with and without anticoagulant tubes.

Results: In aborted group, the findings of hematology were showed a significant decrease in values of total erythrocytes and hemoglobin, and significant elevation in values of MCV when compared to those of healthy group. However, insignificant variations (P>0.05) between values of both study groups were showed in hematocrit, MCH, MCHC, total leaukocytes, lymphocytes, monocytes, and granulocytes. For hormones, the findings of aborted cows revealed a significant reduction in values of prolactin and a significant elevation in values of insulin. In cows of aborted group, the findings of antioxidants (catalase, glutathione peroxidase and superoxide dismutase) were significantly higher; while, malondialdehyde was significantly lower than seen in cows of healthy groups. Although the findings of glucose and magnesium were differed insignificantly, the concentration of calcium was reduced significantly in cows of healthy group when compared to those of aborted group.

**Conclusion**: To date, abortion in cattle due to unknown causes and their complications remain an actual problem without control. This might represent the first study in Iraq compare between the markers of aborted cows and naturally calving cows; therefore, additional studies appear very important to know all bodily changes after abortion and the methods for decreasing or preventing their negative effects.

Keywords: RBCs, Prolactin, Insulin, Glucose, Calcium, Iraq

#### Introduction

Cattle are domesticated bovine farm animals, which are raised for their meat, milk, or hides or for draft purposes. Although all modern domestic cattle are believed to belong to the species *Bos Taurus* (European) or *Bos indicus* (Indian) and even to be crosses of these two breeds, definition of a breed is difficult and inexplicit (Udeh, 2021; Berry, 2022). In Iraq, four main cattle breeds are recognized; Karradi, Sharabi, Rustaqi and Jenoubi in addition to other

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commercially breeds that introduced during the last five decades including Holstein-Friesian, Jersey, Hereford and Ayrshire (Alshawi et al., 2019). All these breeds were lived in different agro-ecologies and adapted to their environmental challenges such as heat, humidity and infectious diseases. However, reproductive efficacy remains one of the most factors that make cattle industry economically and clinically sound (Galina and Geffroy, 2023). The incidence of reproductive disorders such as infertility and abortion in cattle due to physical, chemical, biological and environmental agents are increasing over years causing substantial economic impact on producers who want the production of viable and healthy offspring (Yoo, 2010; Manyi-Loh et al., 2016).

Abortion may be defined as the loss of a fetus earlier at the day 42 or later due to multiple potential causes that varied significantly between herds in depending on the husbandry system and calving pattern (Diskin et al., 2016; Khan et al., 2016). Although many noninfectious causes are implicated in abortion (Yadav et al., 2021), infectious causes remain the most abundant etiology of abortion due to bacterial (brucellosis, leptospirosis, and listeriosis), viral (BVD and IBR), parasitic (trichomoniasis and neosporosis), and fungal agents (Reichel et al., 2018; Gharban, 2023). Pathophysiology of abortion involves the termination of pregnancy through the hormonal changes that originate from the fetal pituitary and releasing of adrenocorticotrophic hormone (ACTH) and increasing the concentration of fetal blood cortisol that leading to decreasing the concentration of progesterone and increasing the levels of estrogen and oxytocin (Murray, 1990; Ganaie et al., 2018). Abortion may occur also through the failure of several adaptive strategies which normally maintain pregnancy and failure of the cow's immunological system (Corbeil and BonDurant, 2001). Other noninfectious complications of pregnancy which are either fetal or placental origin might associate with abortion in addition to various complications that can be observed after abortion such as hemorrhage, uterine prolapse, uterine perforation, vaginal prolapse, and retained placenta (Purohit and Gaur, 2011; Wolf-Jäckel et al., 2020).

In Iraq, a number of studies have been conducted to identify relationship of retained placenta and fetal membranes to enzymatic and hormonal activities, and the association of some pathological agents in reproductive disorders (Naief et al., 2015; Khudhair et al., 2021); therefore, this might represent the first Iraqi study aims to estimate the possible changes in hormonal, hematological and antioxidant / oxidant profiles in aborted cows due to undiagnosed causes.

#### Materials and methods

#### Ethical approval

This study licensed by the Scientific Committee of the Department of Surgery and Obstetrics (College of Veterinary Medicine, University of Al-Qadisiyah)

## Samples

A total of 80 adult cows; 40 naturally calving cows as a healthy control in addition to 40 recently aborted cows, were selected from different areas in Al-Qadisiyah province during March-November (2023). A 10 ml of jugular venous blood was drained from each study animal under aseptic conditions using a disposable syringe, and divided as 2.5 ml in an EDTA anticoagulant plastic tube and 7.5 ml in a glass-gel tube. The EDTA blood tubes were used for hematology and measurement of glucose; while, the free-anticoagulant blood

samples were centrifuged (5000 rpm for 5 minutes) and the obtained sera were kept into labeled Eppendorf tubes and kept frozen until are used for measurement of hormonal and antioxidant / oxidant markers.

# Hematology

The complete blood account including total erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocytes, lymphocytes, monocytes and granulocytes were measured using the Automated Mythic 18-Vet Analyser (Orphee SA, Switzerland).

## Biochemical analysis

Following the manufacturer instructions of the quantitative ELISA Kits (SunLong Biotech, China), progesterone (CAT NO: SL0042Bo) and Prolactin (CAT NO: SL0166Bo) hormones in addition to insulin (CAT NO: SL0018Bo), catalase (CAT NO: SL0233Bo), glutathione peroxidase (CAT NO: SL0024), superoxide dismutase (CAT NO: SL0014Bo), and malondialdehyde (CAT NO: SL0098Bo). Concentration of glucose in study animals was measured using the Cobas 6000 (Roche, Germany); whereas, levels of calcium and magnesium were measured spectrophotometerically by specific colorimetric assay kits (Linear / Spain).

# Statistical analysis

The *t*-test in the GraphPad Prism Software was applied to detect significant differences between the values of the study healthy and aborted groups at a level of P<0.05 (Gharban et al., 2023).

#### **Results**

#### Hematology

In aborted group, the findings of total erythrocytes and hemoglobin were decreased significantly (P<0.05); while, the finding of MCV was elevated significantly (P<0.0155) when compared to those of healthy group. However, insignificant variations (P>0.05) between values of both study groups were showed in hematocrit, MCH, MCHC, total leaukocytes, lymphocytes, monocytes, and granulocytes (Table 1).

Table (1): Hematological findings for cows of study healthy and aborted groups

Marker	Unit	Healthy	Aborted	p-value
Total erythrocyte	$\times 10^6 / \mu l$	$7.55 \pm 0.28$	$5.06 \pm 0.26$	0.009 S
Hemoglobin	g/dl	$10.03 \pm 0.23$	$7.92 \pm 0.33$	0.0273 S
Hematocrit	%	$28.6 \pm 0.49$	$25.18 \pm 0.55$	0.068 NS
MCV	fl	$39.46 \pm 1.52$	53.15 ± 1.5	0.0155 S
МСН	pg	$13.66 \pm 0.39$	$16.07 \pm 0.29$	0.0558 NS
МСНС	g/dl	$34.98 \pm 0.44$	$30.88 \pm 0.69$	0.0692 NS
Total leukocytes	$\times 10^3 / \mu l$	$9.03 \pm 0.65$	$9.36 \pm 0.41$	0.0982 NS
Lymphocytes	%	$53.95 \pm 2.43$	$52.7 \pm 1.35$	0.0934 NS
Monocytes	%	$5.73 \pm 0.35$	$5.43 \pm 0.16$	0.0892 NS

Granulocytes	%	$43.9 \pm 2.52$	$45.08 \pm 1.42$	0.0924 NS			
S: significance (P<0.05), NS: Non-significance (P>0.05)							

# **Hormones**

In comparison with cows of healthy group, the findings of prolactin in aborted cows were decreased significantly (P<0.009) whereas insulin values were elevated significantly (P<0.008), (Figures 1, 2).

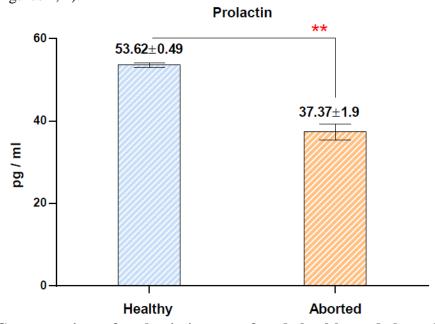


Figure (1): Concentrations of prolactin in cows of study healthy and aborted groups

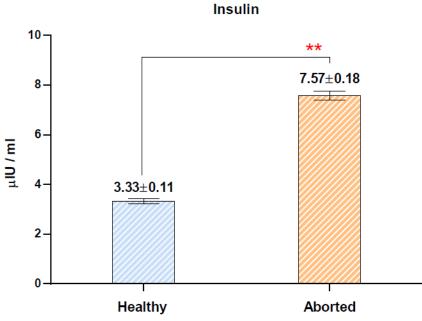


Figure (2): Concentrations of insulin in cows of study healthy and aborted groups

# Antioxidants / oxidant

In cows of aborted group, the findings of antioxidants (catalase, glutathione peroxidase and superoxide dismutase) were significantly higher (P<0.0171, P<0.0451 and P<0.009,

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respectively); while, malondialdehyde was significantly (P<0.009) lower than seen in cows of healthy groups (Figures 3-6).

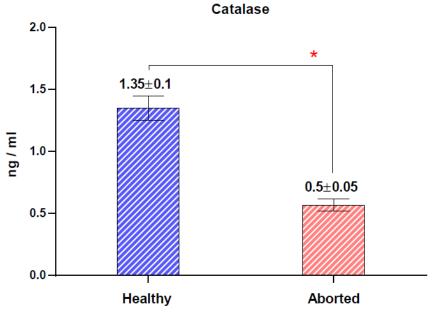


Figure (3): Concentrations of catalase in cows of study healthy and aborted groups

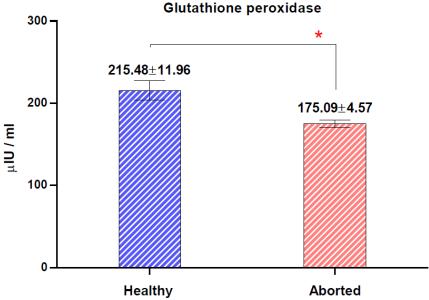


Figure (4): Concentrations of glutathione peroxidase in cows of study healthy and aborted groups

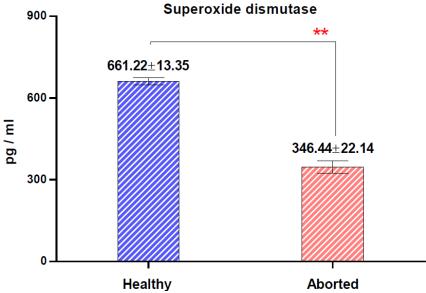


Figure (5): Concentrations of superoxide dismutase in cows of study healthy and aborted groups

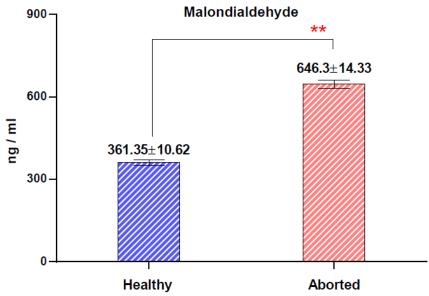


Figure (6): Concentrations of malondialdehyde in cows of study healthy and aborted groups

# Glucose and minerals

Although the findings of glucose and magnesium were differed insignificantly (P<0.0939 and P<0.0554, respectively), the concentration of calcium was reduced significantly (P<0.0398) in cows of healthy group when compared to those of aborted group (Figures 7-9).

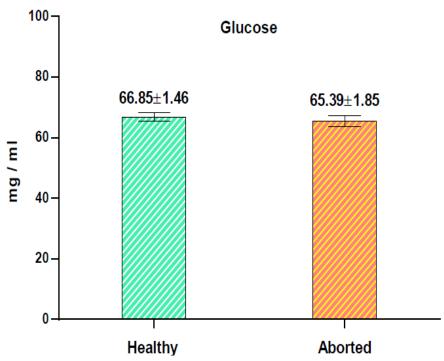


Figure (7): Concentrations of glucose in cows of study healthy and aborted groups

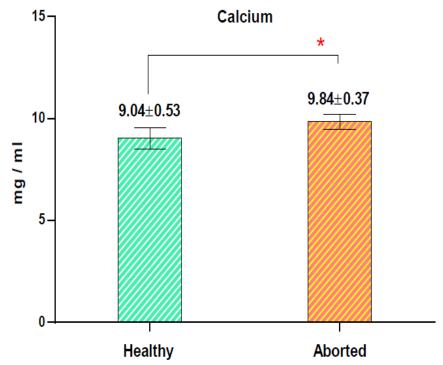


Figure (8): Concentrations of calcium in cows of study healthy and aborted groups

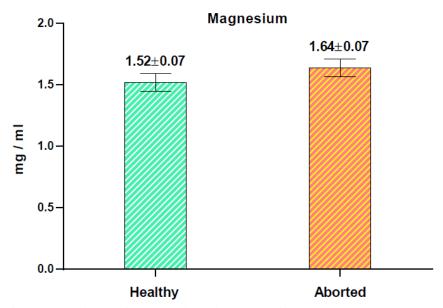


Figure (9): Concentrations of magnesium in cows of study healthy and aborted groups

#### **Discussion**

A strong relationship between reproductive disorders as abortion and reduced fertility was shown in bovine animals that are more prone to reproductive problems resulting in marked economic losses (Tolosa et al., 2021). In the current study, the findings that revealed a significant alteration in total erythrocytes, hemoglobin and MCV could be occurred abortion were similar with reported by other previously and recently studies (Atallah et al., 1997; Hajiabadi et al., 2022). This study suggested that the etiological agent(s) implicated in incidence of abortion might the cause of anemia in aborted animals as reported by *Mycoplasma wenyonii* (Hofmann-Lehmann et al., 2004), chronic intoxication with nitrate (Sezer et al., 2011), epizootic bovine abortion (Coker et al., 2012), leptospirosis (Ismail, 2019), babesiosis and anaplasmosis (Henker et al., 2020), and brucellosis (Hashem et al., 2020). Additionally, the presence of complication during gestation or abortion which causing blood hemolysis or hemorrhage might be another cause of anemia in aborted cows. Abramowicz et al. (2019) mentioned that the prolonged bleeding, low capacity of reticulocyte production and iron deficiency could increase the MCV value and this case is usually known as the normocytic normochromic anemia.

The decreasing of prolactin hormone and increasing of insulin were showed in this study. Chew et al. (1977) detected the decreased levels of prolactin in cattle with retained fetal membrane suggesting the role of high progesterone and low estrogen in this action. García-Ispierto et al. (2009) referred to the interaction between plasma prolactin, plasma cortisol and milk production throughout gestation, and found that the prolactin value was significantly lowered in aborting animals on day 210 of pregnancy suggesting the protective effect of prolactin against the parasite diseases causing abortion such as *Neospora caninum* probably due to its pro-inflammatory action. Although, the glycemic control and spontaneous abortion in diabetic women has a great attention, there is a low available data about the role of insulin in animal pregnancy in particular cattle. However, some studies studied the association of insulin to energy balance, body condition score, ovarian activity and estrous behavior in particular in dairy cows as pregnancy loss in these animals had an upward trend bringing

difficulties for breeders and high annual costs (Saifullizam et al., 2010; Ntallaris et al., 2017; Ghaffari et al., 2023). In an experimental study, authors found that cows with insulin resistance were showed a lowered body condition score, serum urea nitrogen and glucose concentration before calving; while after calving, reduced milk production and high plasma insulin concentration were identified (Kawashima et al., 2016). Weber et al. (2016) reported that dairy cows undergo significant metabolic and endocrine changes during the transition from pregnancy to lactation and impaired insulin action influences nutrient partitioning towards the fetus and the mammary gland. We suggested that different types of stresses could be initiated during gestation and abortion / parturition which certainly play a marked role in increasing the concentration of insulin.

In agreement with our findings, Amin et al. (2023) reported that the concentrations of antioxidants were decreased significantly while oxidant values were increased markedly in abortive cows. Several studies researchers have been studied the activities of antioxidant in maintain of gestation in cattle and other domestic animals (Abuelo et al., 2015; Fávero et al., 2018; Baimishev et al., 2019; Xiao et al., 2021). Antioxidants/oxidants are involved in several reproductive functions such as the regulation of follicular fluid environment, folliculogenesis, steriodogenesis, corpus luteum function and luteolysis (Talukder et al., 2017). Abuelo et al. (2018) recorded cattle can succumb to illness at any given time, but the majority of diseases take place around two clusters; time around calving and the first few weeks after calving due to dysfunctional immune response and increasing the risk of oxidative stress. Sayiner et al. (2021) demonstrated the relationship between alterations in antioxidant enzyme activities and the metabolic profile parameters during different reproductive stages suggesting that evaluation the activity of these parameters in pre- and postpartum periods might contribute to better management of these periods and the prevention of complications.

Our findings showed that the concentrations of glucose and magnesium were differed insignificantly; however, there was a significant reduction in calcium in normally parturition cows when compared to aborted cows. This might attributed to increase demands of calcium for normally growing of fetus in addition to losses of calcium in response to the onset of lactation. Various studies have den documented the role of calcium supplementation during the early postpartum period in reducing the loss of body condition and improving lactation performance and reproduction (Valldecabres et al., 2023; Bharti and Singh, 2024; Ghaderi et al., 2024). Also, the effects of calcium deficiency in increasing the rates of calving and abortion were confirmed by many reports (Cargile and Tracy, 2021; Essawi et al., 2021; Molefe and Mwanza, 2023).

## Conclusion

To date, abortion in cattle due to unknown causes and their complications remain an actual problem without control. This might represent the first study in Iraq which focused on the concentrations of different hematological, hormonal and biochemical markers in aborted cows suggesting their direct or indirect roles in reproductive disorders. Therefore, additional studies appear very important to know all hormonal changes that might be initiated during gestation and after abortion with providing the active schemes for decreasing or preventing their negative effects. Association between prolactin and/or insulin with other reproductive

hormones must be studied in animals suffering reproductive disorders, in particular cows with spontaneous abortion.

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# **Authors' contribution**

BHAA: Collection, processing and examination of blood samples. MSK: Serological examination of hormonal, antioxidants / oxidant with glucose and mineral markers. Authors approved the final copy of manuscript.

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#### **Conflict of interest**

No.

# **Data availability**

All data were involved within the manuscript.

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