Effect of level of Interleukin 1 beta (Il-1B) and Luteinizing hormone (LH) impacts on the size of the ovarian follicles of local cows

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Abstract
This study was conducted in the laboratories of the Department of Animal Production Techniques at the Technical College in Mussaib (TCM) from September 2021 to January 2022 to study the relationship between Interleukin 1 beta (il-1B) and Luteinizing hormone (LH) with ovarian follicle size on local cows. 150 reproductive systems were collected from non-pregnant local cows, which slaughtered in the abattoirs of Babylon Province and were transported to the laboratory within two hours. Ovaries were removed (300 ovaries) and their dimensions were measured. The follicles were measured and classified into three categories. Follicular fluid was drawn from the visible follicles (2-8) on the surface of each ovary and placed in an Eppendorf tube to measure the concentration level of Interleukin 1 beta (il-1B) and Luteinizing hormone (LH) in the follicular fluid. The results showed a significant high superiority ($P<0.01$) of an increase in the level of interleukin-1beta over the size of large, medium and small follicles. A significant high effect ($P<0.01$) of increasing the level of the ovulation hormone on follicle size was observed for all small, medium and large groups. We conclude from this study that the concentrations of cytokines (interleukin-1beta) and hormones (Luteinizing hormone) are influenced by the size of the ovarian follicle.

Keywords: Follicle, Follicular fluid, Interleukin1-beta, Luteinizing hormone

Introduction
The growth and stimulation of ovarian follicles requires a series of coordinated events that induce morphological and functional changes within the Follicle leading to cell differentiation and oocyte development [1]. The process of follicle formation begins when the germ cell or egg is liberated from the egg nest, and its importance lies in the fact that it provides the appropriate environment for the growth and nutrition of the egg [2]. Follicular fluid is a complex fluid, part of which is derived from the blood via the capillary vasculature of thecal cells [3,4] indicated that the follicular fluid is a viscous fluid with a pH of 7.4, which is similar to blood plasma and also has many elements similar to blood serum with slight differences in the proportions of its

components from serum. Interleukin 1-beta is an important pro-inflammatory cytokine that can stimulate T cell proliferation and increase antibody production [5].

The synthesis of interleukin 1-beta occurs mainly in macrophages (macrophages) and monocytes (monocytes) when stimulated, but to a lesser extent it can be synthesized in other cell types such as lymphoid cells, neutrophils, fibroblasts, endothelial cells [6]. Interleukin 1-beta acts to control follicle growth by facilitating granulocyte proliferation and preventing premature differentiation This factor also Affects apoptosis of cells in ovarian granulocytes [7] it appears to be involved in a number of ovulation-related events such as the synthesis and production of Prostaglandins [8]. Explained [9]an rease in the concentration of interleukin1-beta above the normal limit negatively affects the delicate balance between sex hormones and the development of dominant ovarian follicles , which directly affects fertility [10] an interleukin-1-beta affects the development of follicles by modulating the formation of lipophilic hormones in bovine granular ovarian cells depending on the stage of follicle development.studies also showed that Interleukin-1-beta levels were associated with the level of gonadotropic hormone and pregnancy rates [11].Explain. [12] the expression of interleukin1-beta is different in ovarian follicles according to their stage of development and promotes the development of primary follicles , as the results indicate the important role in regulating the formation of ovarian follicles in cows, follicle maturation and ovarian function The ovulation hormone is an asymmetric glycoprotein, each mono-unit is one alpha-glycoprotein molecule and a beta subunit that makes the protein fully functional, secreted from the anterior lobe of the pituitary gland [13]. each secretory pulse of the hormon Luteinizing e is caused by a pulsatile secretion of reproductive stimulation hormone from the hypothalamus through the pyloric network, since it is a gonadotropic hormone due to its role in controlling ovarian function in females and testicles in males, since a sharp rise (pre- Luteinizing hormone increase) in the concentration of the Luteinizing hormone leads to ovulation and the development of the corpus luteum.

Materials and Methods

Reproductive system collection

This study was conducted in the laboratories of the Department of Animal Production Techniques at the Technical College in Mussaib (TCM) from September 2021 to January 2022. The reproductive systems were collected and excised from 150 non-pregnant local cows that were slaughtered in the abattoirs of Babylon Province and were in a healthy condition before slaughter. The genital canals were examined visually and found to be normal and free of congenital anomalies (Figure 1), and were placed in a plastic bag containing normal physiological saline (0.9% NaCl) and the bags were placed in a cooler box and transported to the laboratory within 2 hours.

Figure (1): The female reproductive system of cows

Classifying follicles and measuring their dimensions

The follicles of each ovary were measured by a Vernir and classified according to these measurements into three groups: small with a diameter of less than (3 mm), medium with a diameter of (3-8 mm), and large with a diameter greater than (8 mm). These follicles were counted and recorded for further analysis (Figure 2) according to [14].

Figure (2): Measuring the size of the follicle

Aspiration of follicular fluid

Follicular fluid samples were aspirated from visible follicles on the surface of the ovary with a diameter of 2-8 mm using a 20G needle attached to a plastic syringe of size 2 and 5 milliliters containing a solution of phosphate diuretic with heparin and placed in an Eppendorf tube and placed at a temperature of -20 °C until analysis (Figure 3) according to [15].

Figure (3): Follicular fluid aspiration

Biochemical analyses

Interleukin 1 beta (il-1B) and Luteinizing hormone (LH)

Laboratory analyses were carried out by the indirect method using a ready-made laboratory kit from Sunlong that adopts the enzyme linked-immunosorbent assay (ELISA) method, according to the manufacturer's instructions, using competitive ELISA technology, for the purpose of measuring the levels of Il-1b and LH. All of the components of the detection kit were taken out and placed at room temperature, then the standards for cytokines and hormones were prepared according to the instructions in the booklet for each kit.

Statistical analysis

Statistical Analysis Software [16] was used to analyse the data obtained according to the Complete Random Design (C.R.D.) and according to the mathematical model below:

$Yij = \mu + Ti + eij$

Results and Discussion

Effect of il-1b level impact with follicle size.

The results (Table 1) show that there is a significant effect ($P<0.01$) in the levels of interleukin-1beta in the ovarian follicular fluid on the size of the large and small follicle, as the highest level of interleukin-1beta was recorded 6.77 pg/mL, for the category of small follicles, the lowest level was recorded 5.601 pg/mL, as well as the highest levels was recorded 10.65 pg/mL, and the lowest levels was 9.41 pg/mL, in the presence of significant differences ($p<0.05$) for the levels of interleukin-1beta in the follicular fluid in the volume of the average follicle, the highest level was recorded at 8.671 pg/mL, for interleukin-1beta and the lowest level was 7.57 pg/ml.

Since increased concentration of interleukin-1beta contributes to the development of ovarian follicles and fertility, and that the types of interleukin were differentiated in ovarian follicles depending on the stage of its development and interleukin-1beta enhances the development of primitive follicles the results indicated its important role in arranging the formation of ovarian follicles in cows, as it plays a positive role in ovarian function and follicle maturation, and the results of our study are consistent in the superiority of the levels of interleukin-1beta with the study of [17,18].

Although the immune environment of the reproductive system is often associated with the response to diseases of the uterus after childbirth , the immune system plays a role in healthy cows for normal reproductive functions and the development and maintenance of pregnancy .The results of the study agree with who stated that interleukins affect the quality of eggs and, consequently, the embryo produced by these eggs. Cytokines act as conductors between immune cells located in the reproductive system to regulate the local immune environment and stimulate the appropriate foetal response before conception and throughout pregnancy in cattle. [19] the reproductive system experiences a decrease in the mRNA abundance of pro-inflammatory cytokines, such as interleukin 1-beta and interleukin 6 and an increase in anti-inflammatory cytokines, such as interleukin 10, during the postpartum period [20]. It differs with [21], which found that cows with a high concentration of interleukin-1beta in their blood are less fertile than cows with a low concentration of interleukin-1beta in their blood, the reason for the difference is due to the period during which blood samples were taken for analysis, which was in the first week after birth, and the researcher indicated that the gene expression of interleukin-1beta differs significantly between infertile animals [19].

Table(1): Effect of Interleukin 1 beta (il-1b) level on the follicle size

Moral level	Overall mean ± standard error Interleukin-1 beta level pg/mL			Follicle size
**	0.012 ±6.774 a.m	0.005±6.518 a.m	0.01± 5.601 b	small (less than 3 mm)
*	0.022 ± 7.57 b.m	0.012 ± 7.662 b.m	0.010 ±8.671 a.m	Medium (3-8 mm) (
**	0.027 ± 10.65 a.m	0.033 ± 10.51 a.m	0.0123 ±9.41 b	big mm8 more than) (
Means bearing different letters mean that there are significant differences *(P ≤ 0.05)** (P ≤ 0.01)				

Effect of Luteinizing Hormone level impact with follicle size.

The results (Table 2) that there is a significant high effect(P<0.01) in the level of the ovulation hormone in the ovarian follicular fluid of domestic cows , for the size of the large and small follicle with the level of the ovulation hormone in the follicle size and we note that the level of the ovulation hormone is directly proportional to the size of the follicle and for all small, medium and large groups, for large follicles, the largest volume was 8.843 ng/ml, while the lowest level was recorded in the size of large follicles 8.493 ng/mL, as significant differences were found in the size of the follicle for the category of medium-sized follicles depending on the level of the ovulation hormone (p<0.05) and the relationship was direct between the size of the follicle and the level of the hormone, as the largest size of the medium follicle recorded its level of 8.758 ng/ml, while the smallest size of the medium follicle and its level was 8.662 ng/mL, for the category of small-sized follicles it was no different from the categories of large and medium follicles, as the difference was also significant and the direct relationship between the size of the follicle the level of the ovulation hormone and the largest size of the small follicle was 8.547 ng/ml, while its level in the sizes of small follicles was about 8.337 ng/mL and 7.942 ng/mL, respectively.

The stages of ovulation have a role and importance in the concentration of steroid hormones in the follicular fluid, as the onset of ovulation leads to an increase in the concentration of the hormone ovulation and a decrease in the level of estrogen, that is, their effect is opposite to the process of maturation of the egg and the completion of ovulation [20] .The significant increase in the rate of the level of the ovulation hormone with the growth and development of the size of the developing follicle is probably due to the beginning of follicle growth and the formation of the Lumen in the developing follicle [21].Theca interstitial cells in the developing follicle begin to differentiate and build receptors for the hormone of ovulation,and as a result, these cells have the ability to produce androgens (Androgens), the most important of which is the testicular adipocyte hormone (Testosterone) with the help of the hormone of ovulation [22] .A decrease in the level of the ovulation hormone leads to a decrease in the percentage of Good Eggs and an increase in concentration leads to the development of ovarian follicles during the estrus cycle [23].The results of our study also agreed with [24].showed that the dominant follicle continues to grow as a result of stimulation of the ovulation hormone, which leads to ovulation, which is the most important event in the reproductive process, so the low concentration of the ovulation hormone is one of the most important reproductive causes that lead to the abortion process. The results of our study agree with. [25]. who found that increasing the level of the ovulation hormone necessary in the development of ovarian follicles to the pre-ovulatory stage disrupts the estrus cycle in sexually mature animals, and the weakening of the ovulation hormone leads to a delay in the process of sexual puberty, which precedes sexual maturation by a period and the delay of the first insemination, which determines the age at first birth [26].

Table (2): Effect of Luteinizing Hormone (LH) level with follicle size.

Moral level	Overall mean ± standard error			Follicle size
	ovulating hormone level nanograms /milliliter			
**	0.17 ± 8.547 a.m	0.15 ±8.337 a.m	0.13 ± 7.942 b.m	small (mm3 less than)
*	0.11 ± 8.758 a.m	0.11 ± 8.678 b.m	0.10±8.662b	Medium (mm 8-3)
**	0.13 ± 8.843 a.m	0.11 ± 8.758 a.m	0.10 ± 8.493b	big greater than 8) (mm
** (P Means bearing different letters mean that there are significant differences ≤ 0.01) * (P ≤ 0.05)				

It can be concluded from this study that the concentration of interleukin-1beta was the highest among large follicles greater than (8 mm) and the concentrations of the ovulation hormone recorded more values in the size of medium (3-8 mm) and large follicles greater than (8 mm) of the size of small follicles less than(3 mm), the presence of a strong positive correlation between the concentrations of (Luteinizing hormone interleukin-1beta). An increase in the size of the ovarian follicle is accompanied by an increase in the level of interleukin-1beta, as well as an increase in the Luteinizing hormone.

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