Effect of Level of Tumor Necrosis Factor-Alpha (TNF-A) And Follicle Stimulating Hormone (FSH) 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 Tumor Necrosis Factor-alpha (TNF-α) and Follicle Stimulating Hormone (FSH) with ovarian follicle size of local cows. 150 reproductive systems were collected from non-pregnant local cows, which were 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 Tumor Necrosis Factor-alpha (TNF-α) and Follicle Stimulating Hormone (FSH) in the follicular fluid. The results showed a significant (P<0.01) increase in the average level of Tumor Necrosis Factor-alpha (TNF-α) in the follicular fluid with an increase in follicular volume and a decrease in large follicles. Also, there was a significant impact (P<0.01) in the average level of Follicle Stimulating Hormone (FSH) in the follicular fluid for the groups of medium and small size of the follicles. It can be concluded that the levels of Tumor Necrosis Factor-alpha (TNF-α) and Follicle Stimulating Hormone (FSH) are impacted by the size of the ovarian follicle.

Keywords: Follicle, Follicular Fluid, Tumor Necrosis Factor-Alpha (TNF-α), Follicle Stimulating Hormone (FSH)

1. 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 (Palma et al, 2012). 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 (Rodgers and Irving-Rodgers, 2010).

Follicular fluid is a complex fluid, part of which is derived from the blood via the capillary vasculature of thecal cells (Zhou et al, 2007). Luck et al. (2001) 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.

Tumor Necrosis Factor-alpha (TNF-α) is a partial cytokine of 17 kDa, produced by activated macrophages produced by granulosa cells in the bovine ovary (Dinarello, 1989). TNF-α is involved in the formation of systemic inflammation, since it is one of the cytokines that make up the acute phase reaction are mainly produced by activated macrophages and can also be produced by many other cell types such as mast cells, lymphocytes, white blood cells, and neurons (Rosenblum and Donato, 1989). Within the ovarian cells and the follicular fluid, the ovaries, granulosa cells, tunica albuginea, bile cells, endothelial cells, and macrophages are also sources of TNF-α. In developing small follicles, TNF-α is inhibited. Ovarian response to gonadotropins, while stimulating the formation of steroids in follicles in the pre-maturity stage (Terranova, 1997). Cytokines have a control over the growth of primordial follicles until fun. pre-ovulatory function as research over the past decade has indicated that TNF-α plays a physiological role in ovarian function in various mammalian species (Benyo and Pate, 1992).

Several studies indicated the role of TNF-α in the process of inhibiting Follicle Stimulating Hormone (FSH) and the synthesis of pregnancy hormone (Progesterone) in ovarian tissues, and thus affecting the growth and maturation of the follicle and the formation of ovarian steroids (Ferreira, 2017).

FSH is from the family of glycoprotein hormones, which are secreted by the anterior lobe of the pituitary gland under the influence of gonadotropic-releasing hormone secreted by the hypothalamus gland, as well as the production of eggs in the first half of the estrous cycle, after its release from the pituitary gland. FSH on the granular cells in the ovaries and plays an essential role in the

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reproductive processes of many vertebrates, and its function is to stimulate the production of the hormone estradiol (estrogen E2), because it stimulates the growth and formation of ovarian follicles and the formation of lipid hormones in the ovaries (Touyz et al, 2000; De Loof et al, 2001; Ulloa-Aguirre et al,1999).
The aim of the experiment is to demonstrate the relationship between some cytokines (interleukin-1beta and TNF-α) and hormones (ovulatory hormone and FSH) with the size of the ovarian follicle of local cows.

2. 2. Material and Methods

2.1 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 were normal and free of congenital anomalies (image 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.

Image (1): The female reproductive system of cows

2.2. 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 (Picture 2) according to Kouamo *et al* (2014).

Image (2): Measuring the size of the follicle

2.3 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 (image3) according to Alves et al (2014).

Image (3) Follicular fluid aspiration

2.4 Biochemical analyses

2.4.1Tumor necrosis factor-alpha (TNF-α) and Follicle stimulating hormone (FSH)

Laboratory analyses were carried out by the indirect method using a ready-made laboratory kit from Sunlong company that adopts the enzyme linked-immunosorbent assay (ELISA) method, according to the manufacturer's instructions, using the competitive type of ELISA technology, for the purpose of measuring the level of **TNF-α** and FSH. All of the components of the detection kit were took 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.

2.5 Statistical analysis

Statistical Analysis Software (SAS 2012) was used to analyse the data obtained according to the Complete Random Design (C.R.D.) and according to the mathematical model below:
$Yij = \mu + Ti + eij$

3. Results and Discussion

3.1 Effect of TNF-α level impact with follicle size

The results of Table 1 showed a highly significant impact ($P < 0.01$) in the level of TNF-α on the ovarian follicular fluid, in the size of small, medium and large follicles, the largest recorded size of large follicles was 10.69 pg.ml-1, while the lowest level of TNF-α was 9.55 pg.ml-1, and in medium follicles, the largest volume was recorded in which the level of TNF-α was 8.98 pg.ml-1, while the lowest level was 7.82 pg.ml-1. and for small follicles, the smallest follicle size was recorded, as the level of TNF-α was 5.25 pg.ml-1, and its level was 6.84 pg.ml-1in the largest size of the small follicle.

Table1. Effect of Tumor Necrosis Factor-alpha (TNF-α) level on the follicle size.				
P value	Overall Mean ± Standard Error			Follicle Size
	Tumor Necrosis Factor-alpha (pg.ml^{-1})			
**	0.003±6.84 a	.004±6.63 a0	0.005±5.25 b	Small (Less than 3 mm)
**	0.006±8.98 a	0.005±8.71 b	0.004±7.82 b	Medium (3-8 mm)
**	0.003±10.69 a	0.005±9.61 b	0.002± 9.55 a	Big (More than 8 mm)
Means with different letters mean that there are significant differences. ** (P≤ 0.01), * (P≤ 0.05)				

The results of Figure 1 show a highly significant (P<0.01) in the rate of the level of TNF-α in the ovarian follicular fluid, as the level of TNF-α in small follicles was 0.004 ± 6.24 pg.ml^{-1}and in medium follicles was 0.005 ± 8.503 pg.ml^{-1}. milliliter, and in large follicles was 0.003 ± 9.95 pg.ml^{-1}.

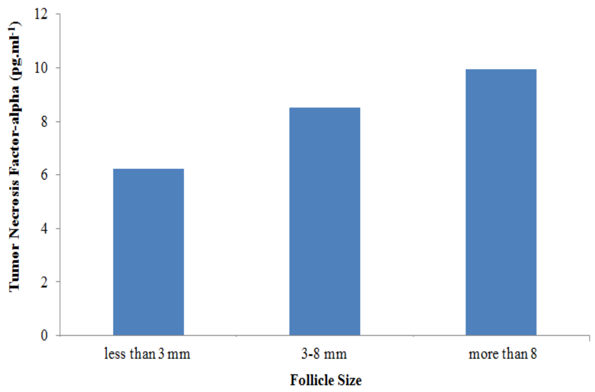

Figure1. The effect of Tumor Necrosis Factor-alpha (TNF-α) level (pg.ml^{-1}) on the follicle size.

Silva *et al* (2020) indicated that TNF-α is involved in the growth, proliferation and death of ovarian follicular cells in mammals, as the expression of these cytokines in different parts of the follicle depends on the stage of follicle development, and cytokines are involved in controlling the growth of primordial follicles until the pre-ovulation stage. Numerous studies have indicated the role of TNF-α in the inhibition of FSH, the activity and induction of aromatide, and the formation of progesterone from ovarian tissue, thus affecting the growth and maturation of the follicle and steroid formation of the ovary (Ferreira, 2017).

The results of our study agree with the findings of Spicer and Alpizar (1994) who found that cytokines regulate ovarian function through their effect on steroid secretion from granulosa cells in the ovarian follicles. The results of our study are consistent with many previous studies. Physiologically, TNF-α is an important component of the normal immune response. It can activate the immune system to regulate; However, inappropriate or excessive production of TNF-α can be harmful and may lead to several diseases such as arthritis (Celis *et al,* 2019).

This study indicated that abnormal TNF-α secretion leads to non-infectious uveitis, inflammatory bowel disease and psoriatic arthritis, which can be classified as a major factor in disease progression (Adegbola *et al,* 2018). The results of this study also agree with Yan *et al* (1993) who found a strong relationship between increased concentration of TNF-α and increased production of estrogen, which stimulates the growth of the ovarian follicle. The results of our study differed from the study of Chaouat *et al* (1995), as they showed that TNF-α affects the pregnancy rate and an increase in its concentration leads to miscarriage. The difference between the results of our study and the other results may be due to the interaction of several immune factors that lead to an increase in the level of TNF-α above the normal limits, which would have a harmful effect on pregnancy and lead to miscarriage.

3.2 Effect of FSH level impact with follicle size

It is noted from the results of Table 2 that there are highly significant effects (P<0.01) of the level of FSH in the ovarian follicular fluid, in medium and small follicles, as the medium follicles of FSH recorded the highest level, reaching 22.651 ng.nl^{-1}, and 22.478 ng.nl^{-1}, As well as in the size of the small follicle, as the highest level was 7.11 ng.nl^{-1}, and the lowest level was 6.011 ng.nl^{-1}, while the differences were not significant between the large follicles according to the level of FSH, as the highest level was 10.17 ng.nl^{-1}, and the lowest level was 9.33 ng.nl^{-1}.

	Table 2. Effect of Follicle Stimulating Hormone (FSH) level with follicle size.			
P value	Overall Mean ± Standard Error			Follicle Size
	Follicle Stimulating Hormone level (ng.ml^{-1})			
**	0.037±7.11 a	0.041±7.01 a	0.047±6.011 b	Small (less than 3 mm)
**	0.017±22.651 a	0.044±22.478a	0.051±8.061 b	Medium (3-8 mm)
N. S	0.76±10.17 a	0.921±9.84 a	1.22±9.33 b	Big (more than 8 mm)
Means with different letters mean that there are significant differences. ** (P≤ 0.01), * (P≤ 0.05)				

The results of Figure 2 showed a highly significant increase (P<0.01) in the mean level of FSH in the ovarian follicular fluid, as its level in medium follicles was 0.037 ± 17.73 ng.nl^{-1}, and its level in small follicles was 0.41 ± 6.710 ng.nl^{-1}, and its level was in ovarian follicular fluid in the large follicles 0.967 ± 9.78 ng.nl^{-1}, respectively.

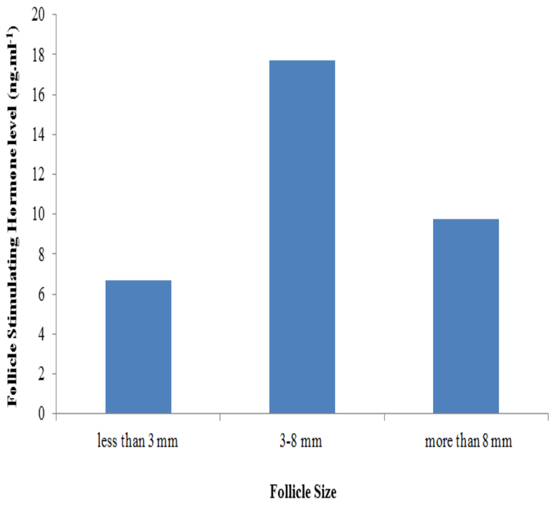

Figure 2. Effect of Follicle Stimulating Hormone (FSH) level (ng.ml^{-1}) level on the follicle size.

The significant increase in FSH with the small size of the follicle and its development in the follicular fluid of the small follicles may be due to the fact that the proliferation of follicle cells with the beginning of their formation is rapid to build layers of different follicle cells that help in the path of feeding the egg and under the influence of FSH, and after building layers of Follicular cells are less dependent on FSH, so its concentration decreases with the growth and development of the follicle (Mishra *et al*, 2003).

The decrease in the level of FSH in the large follicles begins when the formation and stimulation of the estrogen hormone increases when the growth of the follicle is complete. For this reason, its level begins to increase inside the follicle and then its concentration in the blood increases. Through negative feedback, the production of FSH from the pituitary gland is inhibited, as well as with Increased FSH receptors and decreased pre-ovulatory FSH receptors, mean FSH level decreases in large follicles (Ying *et al*, 2011).

The results of our study are consistent with that of Singh *et al* (1999) and Berlinguer *et al* (2007) in their study on sheep. The results of our study agree with study of Rico *et al.* (2009) who showed that differences in the level of sex hormones do not affect the size of the follicles only, but also contribute to significant changes in the length of the estrus cycle and the events of ovulation, which means that the size of the follicles and the degree of maturity of eggs are affected by the level of sex hormones. The results of the study agree with Deshpande and Pathak (2010), Yu *et al* (2005) and Mohanan *et al.* (2018) in their study on goats, as they showed a decrease in FSH with an increase in the size of the follicle, and with Al-Jubouri (2017) in his study on the local goat and Al-Hasnawi(2012) in his study on local sheep.

Recent studies indicated the effect of nutrition on the formation and development of the ovary, as the metabolism contributes to the hormonal regulation that controls the formation and development of ovarian follicles (Scaramuzzi *et al*, 1993; Downing *et al*, 1995). Researchers have found that top feeding or starving the animal reduces the rate of stimulation of ovarian follicles on growth, development, and ovulation in sheep and goats (Blache and Martin, 2009; Zabuli *et al*, 2010). The cows that were used in the study were non-pregnant and that the decrease in the level of pregnancy hormone in the absence of the corpus luteum and the effect of feedback to the hypothalamus increased the release of gonad-releasing hormones (GnRH). These releasing factors stimulate the release of gonadal hormones, which are follicle-stimulating and ovulatory, for the growth and development of follicles (Khan *et al*, 2012).

When the size of the follicle reaches 0.1 mm, the granulosa cells in the primary follicles begin to form and develop receptors for FSH (Vlckova *et al*, 2008). The follicle grows and develops to become in the antral follicle stage. It continues in filamentous division with the increase in the size of the follicle and under the influence of the FSH, the theca cells secrete a large amount of the androgen hormone, which is converted by the granulosa cells into the luteinizing hormone, so its concentration begins to rise inside the follicle with the progression of its size, and then its concentration increases in the blood plasma.

Therefore, it will affect the secretion of FSH by negative feedback and cause inhibition and reduction of its production in the pituitary gland (Campbell, 2009), as well as a decrease in FSH receptors on granulosa and thecal cells with an increase in receptors for ovulation follicle before ovulation (Findlay *et al*, 2009), and the reason for the variation in the level of FSH with the size of the follicle can be attributed to the growth and division of cells at the beginning of follicle formation is fast, due to the completion of the different layers of the follicle that protect and nourish the egg, and the formation of these layers depends on the FSH, and after the completion of the growth and development of the follicle, the dependence on the FSH will decrease, so its level decreases with the increase in the size of the follicle consistent with the studies of Al-Jubouri (2017) on goats and Nandi *et al* (2006) on Buffalo and Nishimoto *et al* (2009) on cows.

In the same context, Scheetz *et al* (2012) reported that high levels of FSH can have negative effects on the size and quality of eggs in cows, and therefore it is not possible to determine the ideal level of FSH exactly through which to achieve the best activity of the ovaries, due to the interaction of many Among the factors affecting ovarian activity such as nutrition, management and health care, as well as age and the number of reproductive cycles. Other studies showed that high concentrations of glucose contribute to the generation of high levels of FSH, and thus increase the rate of ovulation (Viñoles *et al*, 2010).

4. Conclusion

It can be concluded from the study that the level of TNF-α was highest among the large follicles greater than (8 mm). Medium follicles (3-8 mm) recorded the highest levels of FSH at the expense of the size of the other follicles, and an increase in the size of the ovarian follicle was accompanied by an increase in the level of TNF-α as well as an increase in FSH.

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