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Determining the Number of Follicular Waves and the Differences in Estradiol and Progesterone Concentrations in Follicular Fluids of Different Sizes in Different Stages of the Estrous Cycle in Hybrid Dairy Cattles

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Abstract- In this study, non-pregnant and healthy reproductive devices related to 42 slaughtered hybrid cattle aged 2-3 years were collected in Ahwaz slaughterhouse and then transported to the laboratory in ice. Isolated ovaries were divided into four phases of Matt Strauss or days 1-4, earlier de-Strauss or days 5-10, late de-Strauss or days 11-17 and pro-Strauss and Strauss or days 18-21 Strauss cycle based on shape and color appearance of yellow material. Follicular fluid associated with the follicles of different sizes were independently extracted with insulin syringe and were stored at - 20 ° C until the analysis of steroid hormones (estrogen and progesterone). The number of small follicles (less than 5 mm), medium (6-9 mm) and large (10-20 mm) of ovaries related to different stages of estrous cycle were determined. The results achieved in this study indicated that the number of small follicles of the right ovaries at the stages of Matt Strauss and late de-Strauss of Strauss cycle were significantly higher than the other stages. Also, by growing and increasing the size of follicle, estrogen concentration of follicular fluid will be increased and a significant relationship was found between increasing the estrogen concentration and the amount of follicular fluid. The estrogen concentration of follicular fluid was often decreased by increasing the size of follicles.

Keywords- *follicular wave, estrogen, progesterone, Strauss cycle.*

I. INTRODUCTION

Proper understanding of reproduction physiology can have many benefits for improving the reproductive performance. One to four follicular waves were grown during a dating cycle (Strauss cycle) and the ovulation follicles were originated from the last follicle wave (Einspanier et al. 1993).

A follicular wave is known as the simultaneous initiation of a group of follicles (5-7 follicles) in the ovaries (Ginter et al. 2003). A group of small follicles are entered the growth phase (4mm diameter) in each wave, in which one follicle , so called dominant or higher follicle, have finally grown more (Ginter et al. 1989) and cause inhibiting the growth of other follicles (oppressed follicles). New follicular waves prevent being created until the dominant follicles are present in the ovary (Armstrong and Webb 1997).

Several steroid hormones are secreted by the ovaries, in that the most important of them are estrogen and progesterone. The main estrogen, that is biologically active, is 17 beta estradiol. There is a relationship between increasing the estradiol concentration and increasing the size of dominant ovular follicle. It seems that the source of estrogen, which is responsible for the symptoms of estrus behavior, is dominant follicle. A sudden increase in plasma estrogen, especially estradiol, can be seen just before the onset of Strauss. Its maximum value occurs at the onset of Strauss's behaviors and will be basically decreased until ovulation. Progesterone is secreted by yellow material cells, and the changes in its value fully depend on the physical state of yellow material. The concentration of progesterone plasma will be increased almost in day 4 of Strauss cycle and will be maximized at day 8 and will be constant at the same amount until day 17. The concentration of progesterone is decreased and the concentration of estrogen will be increased (Ali et al. 2003).

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II. Materials and Methods

To do this project, 42 healthy and non-pregnant genital tracts of slaughtered cattle in Ahwaz slaughterhouse were used. The left and right ovaries of each tackle were carefully cut at the slaughterhouse and then were placed in separate falcons. The pairs of collected ovaries in ice were transported to the laboratory. The ovaries were washed up in the laboratory and then were divided into 4 stages of Matt Strauss or days 1-4, earlier de-Strauss or days 5-10, late de-Strauss or days 11-17 and pro-Strauss and Strauss or days 18-21 Strauss cycle based on shape and color appearance of yellow material in the way proposed by Ali et al. (2003). After identifying the different stages of Strauss cycle, the size of follicles was determined by caliper and then were placed in three small groups (less than 5 mm), medium (between 6-9mm) and large (10-20 mm). The number of follicles related to each of these follicular groups was recorded at different stages of Strauss cycle. The follicular fluid of small, medium and large follicles of the pairs of cattle's ovaries were separately collected at different stages of Strauss cycle by separate insulin syringes and poured into the micotupe and finally were maintained at 20 - ° C after being scored until being sent to the laboratory and the assessment of estrogen and progesterone hormones. Data were analyzed using one-way analysis of variance (ANOVA) as well as Duncan's multiple range comparative Test using SPSS software and the data were presented as Mean±SE.

III. Results and Discussion

As is observed in Table (1), the number of small follicles (less than 5 mm) of the right ovary at stage one (Matt Strauss) and stage 3 (late de-Strauss) of Strauss cycle is more than that of the second stage (early de-Strauss) and the fourth stage (pro-Strauss and Strauss) ($p<0.05$). Stages 2 and 4 of Strauss cycle showed no significant difference in terms of the number of small follicles ($p>0.05$). the number of small follicles of the left ovary at stage one was more than that of other stages of Strauss cycle and the stages 2, 3 and 4 showed no significant difference in terms of the number of small follicles ($p>0.05$). However, the number of small follicles of the left ovary at stage three was numerically more than the stages 2 and 4 of Strauss cycle. The number of small follicles of total left and right ovaries at stage one was more than that of other stages ($p>0.05$), and the Strauss cycle in stage 3 was more than stages 2 and 4. There was no significant difference at the stages 2 and 4 of Strauss cycle in terms of small follicles of total ovaries ($p>0.05$). The number of medium follicles (between 6-9mm) related to left, right and total ovaries showed no difference at different stages of Strauss cycle ($p>0.05$). The number of large follicles (between 10-20 mm) at stage one of Strauss cycle for the right, left and total ovaries was zero. The number of large follicles at stage 4 of Strauss cycle for the right and total ovaries was more than other stages ($p<0.05$), which this indicates that the ovulation of the right ovary was more than that of the left one in the cattle under study. The number of large follicles of the right ovary between the stages 2 and 3 of Strauss cycle showed no significant difference ($p>0.05$). The difference of large follicles between the stages 2, 3 and 4 of Strauss cycle was not significant in the left ovary ($P>0.05$). the number of large follicles in the total ovaries at the stage 4 was more than that of other stages of Strauss cycle there was no significant difference at the stages 2 and 3 ($P>0.05$). As can be seen in the present study, the highest number if small follicles at stage one (Matt Strauss) and stage three (late de-Strauss) was seen in Strauss cycle, in which these studies indicate that the cattle under study had two follicular waves in their ovaries. The first wave was in days 1-4 of Strauss cycle and the second one in days 11-17 of Strauss cycle. The first wave was in days 1-4 and the second one was in days 11-17 of Strauss cycle. These results achieved in this study were compatible with the ones on buffalo in northern Iraq (Ali et al. 2003) and the ones in Egypt and India (Hooda and Yadav 2002, Azawi et al. 2009). Also, the results achieved in this study is compatible with the ones achieved in (Kulkarni et al. 1994) in this regard, in that they found two follicular waves at the Strauss cycle of cattle, one in days 3 and 4 and the second one in days 12 and 14 of Strauss cycle. As can be observed in table 2, the estradiol concentration of follicular fluids in large follicles was significantly larger than that of the smaller follicles ($P<0.05$) in most stages of Strauss cycle in that these results were compatible with the ones achieved in dairy cattle (Ginter et al. 1989, Mapletoft et al. 1994, Nishimoto et al. 2009, Renaville et al. 2007, Tabatabaei et al. 2011) and buffalos (Hooda and Yadav 2002, Ginter et al. 2003) respectively, which the estrogen concentration of follicular fluid was increased by the growth of follicles, and there is a direct relationship between increased concentration of estrogen and the value of follicular fluids. Also in another study (Kulkarni et al. 1994), the highest concentration of estrogen was reported in the follicles with 15 mm diameter. In another study, the sudden increase of estrogen was reported in the follicles larger than 8 mm in that this increase associated with the size of follicles (Klumpp 2004). Similar to these results, a significant difference was seen between the mean of estrogen concentration in different sizes in another study (Kulkarni et al. 1994). These researchers found that the mean of estrogen concentration with the increase of the size of follicle. It seems that granulose cells are mainly the place for the activity of androgen aromatase within the follicle in that FSH hormone stimulates the activity of granulose cells aromatase and thus the estrogen production is increased (Azawi et al. 2009). According to Table (1), there is an inverse relationship between the progesterone concentration of follicular fluid and the size of follicles at the stage of pro-Strauss as well as sexual cycle Strauss, as the progesterone concentration of follicular fluid is significantly decreased by the increase of the size of follicles ($P<0.05$) at this stage. The results of this study were compatible with the ones achieved by another researcher (Klumpp 2004) in this regard. This researcher reported different concentrations of progesterone in different sizes of follicles, but the concentration of progesterone was decreased by the increase of the size of follicles. A higher level of progesterone was seen in the smaller follicles in another study (Mapletoft et al. 1994). Also

consistent with the results of this study, a significant reduction was reported in the progesterone concentration of larger follicles (Kulkarni et al. 1994, Nishimoto et al. 2009). Therefore, the difference in the concentration of follicular steroids at different stage stages of follicular growth showed that the follicular cells have high ability to change the value of hormone production (Kulkarni et al. 1994).

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TABLE I. MEAN±SE NUMBER OF FOLLICLES DURING DIFFERENT STAGES OF THE ESTROUS CYCLE OF CATTLE

Stages of estrous cycle	Number of follicles (Mean±SE)								
	Small (≤ 5 mm)			*Medium (6-9 mm)*			*Large (10-20 mm)*		
	Right ovary	Left ovary	Total	Right ovary	Left ovary	Total	Right ovary	Left ovary	Total
Stage 1 (1-4 days) (metestrus) n=10	10.71±1.49b	13.14±1.65b	23.86±2.10c	1.83±0.91	0.83±0.31	2.67±0.71	0.00±0.00a	0.00±0.00a	0.00±0.00a
Stage 2 (5-10 days) (early diestrous) n=10	3.80±1.19a	3.80±0.98a	7.60±1.89a	1.70±0.37	1.40±0.27	3.10±0.35	0.80±0.25b	0.20±0.13ab	1.00±0.21b
Stage 3 (11-17 days) (late diestrous) n=10	9.38±1.16b	7.00±1.46a	16.38±1.69b	1.10±0.28	0.50±0.27	1.60±0.45	0.60±0.22ab	0.50±0.17ab	1.10±0.31b
Stage 4 (18-21days) (proestrous and estrous) n=12	5.00±0.67a	3.83±0.65a	8.83±1.12a	1.50±0.31	1.08±0.50	2.58±0.75	1.50±0.23c	0.83±0.30b	2.33±0.28c

a-c Columns with different superscripts differ significantly (P<0.05).

TABLE II. CONCENTRATIONS OF FOLLICULAR FLUID STRADIOL (PG/ML) AND PROGESTERONE (NG/ML) IN DIFFERENT STAGES OF ESTROUS CYCLE

Stages of estrous cycle	*Small follicle (≤ 5 mm)*		*Medium follicle (6-9 mm)*		*Large follicle (10-20 mm)*	
	Stradiol	Progesterone	Stradiol	Progesterone	Stradiol	Progesterone
Stage 1 (1-4 days) (metestrus) n=10	2212.8±425.35	58.23±8.46	11596±893.65b	41.68±13.94	---	---
Stage 2 (5-10 days) (early diestrous) n=10	11776±777.79b	42.82±5.12	6243.8±2607.2ab	34.68±8.13	13208±671.14	62.83±5.35
Stage 3 (11-17 days) (late diestrous) n=10	1680.5±312.30a	64.45±11.97	6703±3407.5ab	64±10.82	11968±803.63	61.53±10.06
Stage 4 (18-21days) (proestrous and estrous) n=12	2891±699.78a	57.18±9.38	1350.7±386.91a	32.20±7.67	13003±68.35	69.00±11.01

a-b Columns with different superscripts differ significantly (P<0.05).