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Relationship of Luteinizing Hormone Concentration With Reproductive and Productive Performance in Holstein Cows Bred In Iraq

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Abstract: *This research briefly reviews the effect of Luteinizing hormone (LH) with the reproductive and productive performance of German Holstein cows bred in Iraq. Blood samples were collected for 100 cows to measure the concentration of LH in August 2019 .Cows so .The reproductive characteristics included the following: the number of services per conception (SPC), Day opens (DO), Calving interval (CI), the rate of abortion for reproductive reasons (AB), and the Age at the first calving (AFC). Also, Its effect on milk production , which is determined by milk yield characteristics, Total milk yields (TMY), daily milk yields (DMY) and Length lactation (LL).An increase in the concentration of LH positively affected cows fertility, as (UP) cows needed SPC 0.11 ± 1.37 service less compared to (IN) and (LO) cows which needed 3.17 ± 0.34 and 4.12 ± 0.30 service respectively, also achieved a shorter (UP) The DO duration was 149.71 ± 4.88 days which outperformed (IN) and (LO) which had 155.32 ± 3.35 days, respectively. (UP) achieved the shortest CI 457.39 ± 14.08 days. (UP) was characterized by having less AB 2.05 ± 0.19%. The (UP) achieved the youngest age at first birth 34.31 ± 0.39 months compared to (IN) and (LO) which were 35.10 ± 0.50 and 36.24 ± 0.75 months, respectively. The increased LH concentration positively affected DMY and (TMY) as UP outperformed IN and LO and negatively in (LL) as (LO) overtook (UP)*

Keywords: *Luteinizing hormone; Holstein cows; milk; Total milk yield; daily milk yield; length lactation.*

1. INTRODUCTION

Reproductive competence is a measure of a cow's ability to conceive and produce viable calves. Fertility is usually assessed at an economic level. By birth period, that is, the period between successive (CI) calves [1]. The number of services per conception (SPC) is an indicator of fertility, and the normal range from 1.6-1.8 services [2]. The reproductive competence of animals decreases with the increase in the number of services per conception [3]. The period from calving to the next pregnancy (DO) is one of the most important measures of reproductive competence, as shortening this period reduces the period between two consecutive calves (CI), which extends from 12 to 13 months, and increases the total yield milk produced, which reduces the rate of exclusion Replacement and reduction in health care and services costs per pregnancy [4,42,43], and increased financial profits by increasing lifetime milk production and annual production [5]. The period from birth to fruitful fertilization varies according to the breed and origin, and this period ranges in the country of origin from 60 to 90 days for Holstein cows [6,7], which determines the period between two births, as the duration of pregnancy is constant (280-285) days in Milk cows [8]. Cattle miscarriage is the loss of a fetus between the ages of 42-260 days [9]. Pregnancy lost before 42 days is called early abortion, while a calf that is stillborn between 260 days and the full term is known as fetal

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death, and the normal rate of abortion on farms is 3-5% for every 100 pregnancies per year [10,11] . Age at first calving (AFC) is the age at which a cow gives its first birth, which is the date of the beginning of production [43], and it is considered one of the most important factors that have a significant impact on cows 'milk productivity in different seasons and their productive life [44,45]. Total milk yield (TMY) is the milk production during a specific period of lactation, and total milk production varies during successful production cycles in the same cow [46]. Daily milk production (DMY) is the 24-hour milk production [47], and the length of the lactation period (LL) is important because it affects the total milk production. In most modern dairy cows, the 305 days milk season is accepted as standard [12].
The aim of current study is to investigate the effect of LH hormone concentration on the important reproductive traits and milk production characteristics and use the obtained results to improve the local cattle performance under the farming condition.

2. MATERIALS AND METHODS

The study was conducted at Taj Al-Nahrain station for milk cows located in the northeast of Al-Diwaniyah. The station includes semi-open pens built to raise dairy cows, pens for pregnant cows and closed pens for the care of newborns until the age of weaning, and others for taking care of the wheels from weaning to the age of one year, the wheels are inseminated at the age of at least 22 months, the cows are mechanically milked twice a day (Six in the morning and five in the evening), and the station currently owns about 1000 cows. The cows at the station receive vaccinations on a seasonal basis for infectious diseases that are common in the region, including vaccinations against: foot and mouth disease, hemorrhagic septicemia, yellow fever, anthrax, rinderpest, and tuberculosis. Animals are sprayed with pesticides for ectoparasites, and the animals are periodically checked. Cows, fever, febrile disease, and positive counts were tested.

Blood samples

Blood samples (8ml) from each cow (100 cows choosing randomly) from jugular vein and placed into ~~a~~ gel tubes and then centrifuged for 10 minutes at 3000 rpm to separate the serum from the red blood cells and then the serum was collected and placed in Eppendrof Tube (0.5 ml) .It was then placed in a cooled container and transferred to the laboratory for further analyses.Data were analyzed using SAS (2012) computer program by general linear model procedure (GLM) according to the following model:

$$Yijk = \mu + Ti + Eijk \qquad (1)$$

Where:
μ: the overall mean
Ti: effect of LH levels (minimum , moderate and maximum) on reproductive traits(abortion rate, calving interval, days open, Age at the first calving(AFC) and service per conception) and milk production characteristics (Total milk yield (TMY)daily milk yields (DMY) length lactation (LL).
Eijk: the random error.
Duncan's multiple range test (Duncan, 1955) was used to compare differences among means. The concentrations of Luteinizing hormone (LH) were analyzed using commercially available ELISA kits specific for the bovine species* which is used enzymatic immunoassay method, according to the manufacturer's instruction.

Procedure

1. Dilution of Standards: Ten wells are set for standards in a Microelisa stripplate. In Well I and Well 2, 100ul Standard solution and 50μl Standard Dilution butfer are added and mixed well. In Well 3 and

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Well 4, 100µl solution from Well I and Well 2 are added respectively. Then 50ul Standard Dilution buffer are added and mixed well. 50ul solution is discarded from Well 3 and Well 4. In Well 5 and Well 6 50ul solution from Well 3 and Well 4 are added respectively. Then 50ul Standard Dilution buffer are added and mixed well. In Well 7 and Well 8, 50ul solution from Well 5 and Well 6 are added respectively. Then 50ul Standard Dilution buffer are added and mixed well. In Well 9 and Well 10, 50ul solution from Well 7 and Well 8 are added respectively. Then 50ul Standard Dilution buffer are added and mixed well. 50ul solution is discarded from Well 9 and Well 10. After dilution, the total volume in all the wells are 50µl and the concentrations are 6 ng/ml, 4 ng/ml, 2 ng/ml, Ing/ml and 0.5 ng/ml, respectively.

2. In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10ul sample are added (dilution factor is 5).
3. Samples should be loaded onto the bottom without touching the well wall Mix well with gentle shaking 3. Incubation:. incubate 30 min at 37 C after sealed.with Closure plate membrane. .
4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 µl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Add 50 pil Chromogen Solution A and 50 ul Chromogen Solution B to each well, mix with gently shaking and incubate at 37 C for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 ul stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

3. RESULTS AND DISCUSSION

Table (1) shows the general averages and the standard line of reproductive characteristics for each of the number of services per conception (SPC), open day (DO), Calving interval (CI), the rate of abortion for reproductive reasons (AB) , and the age at the first birth (AFC) , as the average SPC was 2.88±0.25 service, which is higher than the global rates of the strain 1.6-1.8 service in the country of origin in Europe [2] and within the range 1.8-4.45 service, which was reached by most studies in Iraq on Holstein and Friesian cows [13]. The average DO was 161.74±4.53 days, which is higher than the normal limit for the strain 60-90 days [6],[7]. The average CI was 531.31±17.07 days, which is higher than the general average for the breed 360-390 days [14]. The average AB was 3.62±0.20% and it is within the normal limits of 3-5% [10],[11]. The mean age at first calving was 35.21±0.54 months, which is less than what found [15], which was 43.4±13.8 months, and higher than [16], which was 25.97 months.

The effect of LH concentrations increasing in this station can be considered positively on the fertility of cows, as the LH concentration had a highly significant effect ($p<0.01$) on the SPC and the CI, and a significant effect ($p<0.05$) on the DO., AB and AFC. The results indicated that the UP group was the most fertile among the three groups. (UP) required 1.37±0.11 service, while (LO) required 4.12±0.30 service. The reason for this large discrepancy between the groups (UP) and (LO) is the difference in the

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concentration of LH, which ranged between 3.29-2.34 ng/ml in bovine serum (UP) and 1.37-0.41 ng/ml in bovine serum (LO), and our results are consistent with the findings of [17] who found that cases with a high LH concentration in their blood serum are fertile and those with a low LH concentration are less fertile. This result is consistent with [18] who pointed out that the first appearance of estrus after calving and the period between estrus and ovulation is influenced by the concentration of LH and the intensity of its pulses, as the low concentration leads to the weakness of the ovaries and the prolonged period between calving and the first estrus, meaning an increase in the period of DO. Since there is a close correlation between LH concentration, the return of ovarian activity and the period of ovulation, especially after calving [19], the DO which leads to an increase in the CI and an increase in the SPC through the timing difference between ovulation and the optimal time for fertilization, thus the fertilization process fails and the SPC increases, which leads to an increase in the CI [20].

This result is in agreement with [21] who found that the concentration of LH stimulates the development of ovarian follicles, as a low level of LH concentration leads to a decrease in the quality of the oocytes resulting from its heartbeat and a decrease in the growth capacity of the fetus resulting from these oocytes and increased concentration leads to strength. The impulses that result in increased development of ovarian follicles in the same period of the estrous cycle also agreed with [22] who stated that the dominant follicle continues to grow as a result of stimulation of the LH, which leads to ovulation which is the most important event in reproduction process, so the low concentration of LH is one of the most important reproductive causes that lead to abortion.

Our findings are also in agreement with [23] and [24] who indicated that increasing the concentration of LH before puberty represents a critical event that determines the age of sexual maturity and that the occurrence of puberty is the result of increased secretion of LH, which regulates the onset of puberty in the heifers, and the increased release of its impulses leads to the promotion of the growth of ovarian follicles that produce enough estrogen to stimulate the behavior of estrus. Age at first service and first pregnancy is one of the main determinants of female productivity, and age at puberty is the main factor that determines the efficiency of the female in the first breeding season. This result is in agreement with [25] who found that suppressing the increase in the concentration of LH necessary for the growth of ovarian follicles to the pre-ovulation stage disrupts the estrus cycle in sexually mature animals, and weak LH pulses lead to delayed sexual puberty that precedes sexual maturity with a period and delay of fertilization. The first that determines the age at first birth.

Table (1): The effect of LH concentration on reproductive characteristics

LH concentration ng/ml	Number of observations	Mean ± standard error				
		Number of services per conception (service)	Day Opens (day)	Calving interval (day)	Abortion %	Age at First Calving (Month)
UP (2.34 – 3.29)	33	c 1.37 ±0.11	b 149.71 4.88±	c 457.39 14.08±	c 2.05 0.19±	b 34.31 0.39±
IN (1.38 -2.33)	46	b 3.17 ±0.34	b 155.32 3.35±	b 537.45 11.67±	b 3.66 0.17±	ab35.10 0.50±

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LO (0.41 – 1.37)	21	a 4.12 ±0.30	a 180.15 5.37±	a 599.10 25.46±	a 5.17 0.25±	a 36.24 0.75±
Mean ± standard error		2.88±0.25	161.74±4.53	531.31± 17.07	3.62±0.20 %	35.21 ±0.54
Significance		**	*	**	*	*

**(P<0.01) , *(P<0.05)

The results of Table (2) showed that there are significant differences between the three groups in total milk yield, as (UP) cows that produced the highest Total milk yield (TMY), which amounted to 2445.75±24.7 kg, were significantly (p<0.01) superior to cows of the two groups (IN) and (LO) that produced 1930.18±18.5 and 17.9 ± 1759.63 kg respectively. The average daily milk yields (DMY) was significantly affected (p<0.05) by the change in the concentration of LH, and the average (DMY) of the three groups (UP), (IN) and (LO) were 10.81±1.5, 8.17±1.1 and 7.24±0.98 kg, respectively. UP affects significantly on the groups (IN) and (LO), which had no significant difference between them. This result is in agreement with [26] who found that increasing age at first calving AFC (one of the determinants of reproductive efficiency that affects on fertility) [27] leads to decreased daily and total milk production. Also, this result is consistent with [28] who found that reducing (AFC) has a significant effect on daily milk production, taking into account that it does not reach 21 months. It also agrees with [29] who found that reducing the AFC led to an increase in the daily and total production (total yield) of milk, and the reason for the difference in results in terms of optimal AFC from the results of the current study is due to the effect of heat stress. It negatively affects cows and leads to reduced reproductive and productive performance [30],[31],[32]. This result differs with [33],[34],[35],[36] who indicated that increased LH concentration positively affects fertility that is inversely related with milk production. The difference in the concentration of LH significantly affected (p<0.05) on the length lactation LL, as (LO), whose LL was 4.2±242.75 days, was significantly superior to (UP), whose cows averaged 226.33±10.8 days, and there was no difference. Significant between (LO) and (IN) whose cows averaged 236.11±9.4 days. This result is in agreement with [37] who found that increasing the AFC to 36 months leads to a decrease in milk production, as the AFC (one of the determinants of reproductive efficiency) is affected by the concentration of LH. The superiority of (LO) cows in the LL is a result of the negative relationship between milk production and the reproductive performance of cows, i.e. fertility [38], which its decrease leads to a decrease in the daily production rate and an increase in the length of the LL [39]. This finding is also in agreement with [40] who found that lower DMY leads to longer LL. These results disagree with [41] who found no effect of fertility on milk productivity in Holstein.

Table (2):effect of LH concentration on milk production traits

Mean ± standard error			**Number of observations**	**LH concentration ng/ml**
length lactation (day)	**daily milk yields (kg)**	**Total milk yield (kg)**		
226.33b ±10.8	10.81a ±1.5	2445.75a ±24.7	21	UP (2.34 – 3.29)
236.11a ±9.4	8.17ab ±1.1	1930.18b ±18.5	46	IN (1.38 -2.33)

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242.75a ±4.2	7.24b ±0.98	1759.63c ±17.9	33	LO (0.41 – 1.37)
235.06±8.13	8.74±1.19	2045.18±20.13	Mean ± standard error	
*	*	**	Significance	

**(P<0.01) , *(P<0.05)

4. CONCLUSIONS

The increased or decreased LH concentration affects fertility and yield of Holstein cows, and the effect of increased LH concentration in this station was positive on fertility and daily and total milk yield.

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