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Effect of Vaginal and Uterine pH on Ovarian Structures of Anestrum Dairy Friesian Cows Using Cosynch + Progesterone Protocol to Induce Estrus

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Abstract

THE PRESENTED study was conducted to investigated the effect of vaginal and uterine pH on the growth of ovarian follicles of dairy Friesian cows. Thirty Friesian anestrus cows (Postpartum ovarian inactivity) were used and were divided into three groups (10 each). The uterus of cows in the two groups was washing with pH buffer solution, 6 for 1st group, 8 for 2nd group, and 3rd group not washing and kept as a control group. All groups were subjected to a Cosynch+progesterone protocol to induce estrus, and ultrasound scanning, and blood samples were taken at various intervals. The study found that adjusting the pH level had no significant effect on ovarian follicle development, but did have a significant effect on the rate and growth of dominant follicles in the acidic groups. Finally, adjusting the pH level to 6 increased the ovulation rate and decreased ovulation time due to its effect on the growth of dominant follicles.

Keywords: Friesian dairy cows; Ovarian follicles; Synchronization; Vaginal and uterus pH.

Introduction

Maintaining a pH range of 6-8 was found to prevent microbial venereal diseases, [2]. The bacterial communities largely interact with the uterine environment by assisting in maintaining the proper pH [3]. The high glycogen concentrations in the vagina have increased the abundance of Lactobacillus and lowered vaginal pH [4]. The produced hydrogen peroxide and lactic acid play a role in maintaining a low pH (<4.5) of the environment which is considered a defense mechanism against pathogens [4, 5, 6]. Lactobacillus species were at very low abundances (<1%) corresponding with the higher pH (>6) in the cattle vagina. The Lactobacillus prevalence insignificantly differs between the bovine and human reproductive [7, 8, 9, 10, 11]. Extra dietary protein (especially non-protein nitrogen) cannot all be utilized by the rumen microbes, leading to increasing plasma urea and ammonia that can travel to the uterus and increase acidity [12]. Increasing uterus acidity inhibits pregnancy via its impact on sperm motility and survival, [13, 14]. However, no impact of extra protein on the uterine pH was concluded, reproductive functions, or fertility [15].

Double ovulation protocols rely on Gonadotropins and Follicle Stimulating Hormones to form, grow, and select follicles for ovulation via ovarian and pituitary feedback [19, 17]. Blood FSH levels rise, causing a new wave of follicle growth that requires gonadotropins to form the dominant follicle that secretes epoetin and estradiol for hypothalamic and pituitary hormonal regulation [20, 16]. Lower FSH concentrations allow for the growth of the ovulated follicle, while corpus luteum secretion of progesterone lowers LH impulses [22, 18]. The absence of corpus luteum results in a wave of ovulation, and dominant follicles ovulate after LH influx [23].

Due to advancements in artificial insemination, including ovulation synchronization programs, semen sorting, and embryo transfer, the study aimed to optimize the fertility efficiency of sexed semen in cows through pH adjustment of the vagina and uterus [1]

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Material and Methods

This study was carried out at El-Karada Experimental Station, Kafr El-Sheikh province belonging to the Animal Production Research Institute (APRI), Agriculture Research Center, Egypt during the period from December to July.

The study was performed on 30 multiparous medium lactating Friesian cows of different ages (4±1.5 years) and weights (500±25 kg). The experimental animals (n=30) were divided after waiting voluntary period into 3 groups (10 animals in each group) as follows: First group: Animals were washing vaginally and uterine with slightly acidic (pH 6|) buffer solution (Bedaya Center of Fertility and IVF, Giza, Egypt). Second group: Animals were washing vaginally and uterine with a slightly alkaline (pH 8) buffer solution (Bedaya Center of Fertility and IVF, Giza, Egypt). The third group: the uterus and vagina of the animal were not washing and kept as a control. All groups were given a Cosynch + progesterone synchronization protocol: Day 0: received an injection of 100 µg GnRH (Receptal; Intervet MSD Animal Health Ltd, Egypt SAE) given intramuscular and an intra-vaginal Controlled Intravaginal Drug Release(CIDR) device insert (1.38 g progesterone, CIDR Cattle Insert; Zoetis New Zealand Limited, Auckland, New Zealand) beginning of the experiment, Day 3: received an injection of 800 IU eCG (Folligon; MSD Animal Health Ltd) intramuscular, Day 6: received an injection of 500 µg cloprostenol (PG, Estrumate; MSD Animal Health Ltd., Wellington, New Zealand) intramuscular, in conjunction with CIDR removal. Day 8: a second 100 µg GnRH injection is given intramuscularly in conjunction with insemination (Figure 1).

Ovarian monitoring: Ovarian structures of cows were monitored at 9:00 a.m. on the third, sixth and eighth days of the program by ultrasonography (Advanced Digital Ultrasonic Diagnostic Imaging System Model: Dus-6000.Anti-electro-shock Type: Class I 100V-240V ~50HZ/60HZ 150VA; Advanced Inc. Miami, U.S.A) once daily at d 3, d 6 and d 8 (ovulation and insemination day). The development of growing follicles at injected eCG at day 3 in the protocol, the diameter of the growing follicle of both ovaries (mm), the onset of the follicular wave before injection eCG (h), the onset of the follicular wave of injection GnRH, development in the dominant follicle at injected PG at day 6 in the protocol: diameter of the dominant follicle of both ovaries (mm), the Growing rate at injected eCG at day 3 until injected PG at day 6 in the protocol, Growing rate (mm/d), time ovulation from injected PG (h), count mature follicles were recorded.

The characteristics of the reproductive tract mucus

During estrus before insemination, the external genitalia were cleaned with a mild antiseptic solution and dried with cotton, mucus samples were collected by aspiration using a sterilized glass pipette (10 ml), whose pointed end was connected to a syringe with a rubber junction. The glass pipette was followed per rectally to pass into the cervix or near the vaginal fold. The characteristics of mucus including the color (clear or cloudy), consistency (thin or thick), and pH were recorded. The collected mucus samples were placed on clean glass slides covered with transparent coverslips, and examined by microscope to determine the fern pattern (typical, atypical, or nil).

Serum progesterone and estradiol levels

Blood samples were collected (10 ml) from the jugular vein once daily of all experimental cows at 12:00 a.m on Day 0, Day 5 and Day 10 of the program, serum was separated by centrifugation and stored at -20 °C until analysis to measure the level of progesterone and estradiol using Enzyme-linked immunosorbent assay (Elisa Test).

Statistical analysis

Analyzed by Completely Randomized Design (CRD) in the case of replicates are equal - General linear models, univariate. Data were represented in mean ± standard deviation values. (Least Significant Difference – LSD) the test was performed for comparing values among the groups - General linear model, univariate. $P<0.05$ was considered to be significant. The obtained data were statistically analyzed according to [25] using a computer program of IBM Corp [26].

Mathematical Model $Yij = \mu + Ti + eij$

Yij: the value of j observation pertaining to the transaction i

µ: The general average of the studied trait.

Ti: The effect of the treatment. I

eij: The random error is normally distributed with a mean of zero and a variance of σ2e

Results

The effect of changing the pH of the vagina and uterus on the development and growth of follicles at eCG injection on the third day of the study protocol were presented Table (1) and Figure (2) illustrated that adjusting the pH levels pH in the vagina and uterus hadn't significantly affect follicle growth. CONTROL records largest size at eCG injection and shortest onset time. The right ovary has a mean diameter (mm) of 5.17 ± 0.32, 4.99 ± 0.52, 5.22 ± 0.43 while the left ovary has a mean of 4.79 ± 0.41, 4.74 ± 0.50, 5.08 ± 0.36 mm respectively. Mean onset (h) of follicular wave from GnRH injection is right ovary: 9.96 ± 3.707, 12.78 ± 6.19, 9.38 ± 5.13 and left ovary: 14.45 ± 4.89, 15.84 ± 6.43, 10.99 ± 4.31 h, respectively.

The effect of changing the pH of the vagina and uterus on the growth of dominant follicles at PG

injection on the sixth day, and the increase rate from eCG injection to PG injection from the third day to the sixth day of the study protocol were presented in Table (2) and Figure (3) demonstrate that acidic pH (AC) had significantly enhances the growth rate of dominant follicles compared to (AK and CONTROL). This is due to increased FSH receptors on the ovary surface. AC exhibited the highest growth rate and the largest size for dominant follicles. The mean diameter (mm) of dominant follicles from eCG to PG injection for the right ovary was 16.78 ± 2.03 (AC), 12.16 ± 3.01 (AK), and 10.93 ± 2.13 (CONTROL); for left ovary, 17.08 ± 1.78 (AC), 13.30 ± 1.40 (AK), and 11.57 ± 1.66 (CONTROL). The average increase rate for dominant follicle (mm) from eCG to PG injection for the right ovary was 11.80 ± 2.03 (AC), 7.06 ± 3.12 (AK), and 5.76 ± 1.92 (CONTROL); for left ovary, 12.22 ± 2.05 (AC), 5.12 ± 4.78 (AK), and 5.32 ± 3.30 (CONTROL). The daily increase rate (mm/d) for both ovaries was 3.99 ± 0.48 (AC), 2.43 ± 0.81 (AK), and 2.00 ± 0.35 (CONTROL).

The effect of changing the pH of the vagina and uterus on Ovulation time and the number of mature ovum per ovary from PG injections and the number of days from GnRH injection of the study protocol were presented in Table (3) shown hadn't significant effect of pH levels in the uterus and vagina on ovulation rate. AC had the highest ovulation rate (2.6 ± 0.5d), while AK had the lowest (1.8 ± 0.8d) and CONTROL had an average of 2.00 ± 0.7d. However, there had a highly significant effect of uterine and vaginal acidity on ovulation time (h) from prostaglandin injection. AC had the least time (36 ± 12h), followed by AK (83.2 ± 18.4h) and CONTROL (86.4 ± 13.1h).

Effect of altered pH of the vagina and uterus on the physical properties of cervical mucus were presented in Table 4, it showed significant differences in pH between treatments. AC had an average of 5.9±0.12 due to long-term 6% pH buffer uterine wash, AK had an average of 8.4±0.19 due to long-term 8% pH buffer uterine wash, and CONTROL had an average of 7.3±0.37 due to long-term 7% pH buffer uterine wash.

Fern pattern of mucus was observed at 100% in all groups (Table 5): AC (60%), AK (20%), and control (20%) showed 85% fern pattern, indicating one ovum ovulation per ovary. AC and AK (40%) and control (60%) showed a 75% fern pattern, indicating ovulation of one ovum from each ovary. AK (40%) and control (20%) showed ovulation of two ovaries with ovum diameter at 14-16.

The effect of changing the pH of the vagina and uterus on the level of the hormones progesterone and estradiol during the ovarian cycle using the study protocol were presented in Table (6) shows that the pH of the vagina and uterus hadn't significantly affect the levels of progesterone and estradiol hormones in the early follicular phase, late follicular phase, and early luteal phase, but there had significant differences between the two hormones in these phases. These variations in hormone levels can be attributed to the different patterns of ovarian follicles in the ovarian cycle. In the late follicular phase, there was an increase in both progesterone and estradiol levels, with an average of 9±1.1, 10.6±1.3, and 9.4±1.2 for progesterone and 1415.6±156.4, 1166.2±198.6, and 917.2±130.9 for estradiol, respectively.

Discussion

Estrus in cows occurs in 2-5 waves and consists of follicular and luteal phases. Gonadotropin-dependent follicle growth begins on day 0 and ends on day 4 with follicle diameter around 4-5mm. The early follicular phase is from day 0 to day 4 for a double ovulation protocol to induce estrus [31]. Adjusting the pH levels pH in the vagina and uterus hadn't significantly affect follicle growth due to unaffected GnRH receptors on the ovary surface [31]. Follicle growth on either ovary is influenced by progesterone in vaginal suppository at a rate of 2 ng/ml or higher, inhibiting the ovarian rotation factor [20, 32]. Progesterone plays a major role in stimulating GnRH receptors and increasing the response to GnRH can lead to higher double ovulation rates up to 96% [21, 33]. With a new follicular wave occurring within 2 days after treatment only in treated animals. The diameter of developing follicles in the early follicular phase of cows can vary depending on numerous factors including breed, age, and reproductive history. However, a study found that the average diameter of follicles in cows during the early follicular phase was 4.63 ± 0.19 mm [34]. Similarly, another study reported an average diameter of 5.5 ± 0.3 mm for developing follicles in the early follicular phase of dairy cows [35]. During the dominant follicle stage, granulosa cells secrete and form an antral vacuole, which grows due to fluid accumulation until a follicle is selected. A reduction in eCG concentration and an increase in LH during the development of the dominant follicle cause granulosa cells to form a cumulus pile. The ovary and oocyte separate from the follicle wall to mature in the antrum [31]. These results align with the Angus cow study [20] with a similar protocol, which found average dominant follicle diameter (mm) at PG injection was 11.6 ± 0.7 and increased rate (mm/d) 1.9 ± 0. Individual ovulation ranged from 10-17.9 mm and double/triple ovulation ranged from 8-8.9 mm [40].

With the infusion of high amounts of estrogen from the mature dominant follicle and its rise in the blood, the LH level rises and the FSH level decreases, Causing an enzymatic breakdown of the wall of the dominant mature follicle and releasing the egg in the process of follicular atresia to be called

ovulation [31, 36]. Acidic pH stimulated the growth rate of dominant follicles, thus increasing LH receptor sensitivity, which caused progesterone levels to rise, leading to a feedback mechanism with decreased FSH and LH secretion [23].

In addition, eCG administration in a d Cosynch + progesterone synchronization protocol on day 3 may induce more than 1 mature ovum in cows [34]. Co-administration of eCG with low progesterone concentrations can rapidly mature dominant follicles and reduce the time required for ovulation [37, 24, 38]. These results approximate a study on Angus cows using the same protocol, the average ovulation time (H) from PG Two ovaries was 88.0 ± 5.3 for the control and 82.7 ± 3.7 for the experimental group [22].

Physiochemical properties of cervical mucus reflect female reproductive health and fertility. These properties vary based on diet quality and hormonal status [31]. All treated cows had clean, non-cloudy mucus indicating a lack of microbial infections [2]. This is due to cows being examined and treated with antibacterial and antifungal agents before estrus and insemination [27]. This is consistent with literatureshowing that most naturally breeding cows have thin mucus consistency [28, 29]. Variations in estrogen levels and ovulation rates in cows account for these findings. As it became clear from this work that in cases of double ovulation, there is an increase in the values of estradiol when menstruation [30]. Finally, it was clear from Tables (4, 5) that the change in the pH of the vagina and uterus had no significant effect on the physiological characteristics of cervical mucus.

Consistent with the findings of reference [39]. In the late follicular phase, there was an increase in both progesterone and estradiol levels. Conversely, in the early luteal phase and the other two phases, the levels were slightly closer, tending to decrease. Low estrogen and progesterone in the early follicular phase lead to a slight increase in FSH level, promoting the growth of multiple follicles in the ovaries. As the late follicular phase arrives, FSH level decreases until the dominant follicle continue to secrete estrogen, resulting in a high level of estrogen. This high level of estrogen is detected by the hypothalamus, triggering the release of GnRH, which stimulates the pituitary gland to produce high levels of luteinizing hormone (LH) and follicle-stimulating hormone. In the early luteal phase, there is a low level of progesterone and estradiol [19]. Convergently, with what the reference values were determined for LH, FSH, estradiol, and progesterone using the Abbott ARCHITECT® Analyzer during various menstrual cycle phases. For the early follicular phase, the progesterone level is 4.7 nmol/L, the late follicular phase is 12.3 nmol/L, and the early luteal phase is 4.1 nmol/L. The estradiol level during the early follicular phase is 358.93 pmol/L, in the late follicular phase, it is 1568 pmol/L, and during the early luteal phase, it is 313 pmol/L [40].

Conclusion

Adjusting the vaginal and uterine pH to 5.9 ± 0.12 increased ovulation rate, reduced ovulation time, and stimulated the growth and rate of dominant follicles. No significant changes were observed in the characteristics of cervical mucus or the hormonal level of the ovarian cycle.

Acknowledgments

Not applicable.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This experiment was conducted according to the guide-lines of Kafrelsheikh University and approved by the local experimental animal care committee, Faculty of Agriculture, Kafrelsheikh University, Egypt (id 4/2016 Ec).All precautions were taken to decrease the risk of injury and disease throughout the trial period.

TABLE 1. Average diameter (mm) for the growth rate of developing follicles at ECG injection, average time (H) for the onset of the follicular wave after GnRH injection in the experimental groups.

Item		AC	AK	CONTROL
Diameter of the growing follicles at eCG injection	**Right ovary**	5.17±0.32[a]	4.99±0.52[a]	5.22±0.43[a]
	left ovary	4.79±0.41[a]	4.74±0.50[a]	5.08±0.36[a]
	Two ovaries	4.98±0.29[a]	4.97±0.53[a]	5.15±0.28[a]
Follicular wave onset (h) pre eCG injection	**Right ovary**	62.04±3.79[a]	59.93±6.19[a]	62.61±5.14a
	left ovary	57.55±4.89[a]	56.91±6.04[a]	61.01±4.31[a]
Follicular wave onset (h) from GnRH injection	**Right ovary**	9.96±3.78[a]	12.07±6.19[a]	9.38±5.13[a]
	left ovary	14.45±4.89[a]	15.84±6.43[a]	10.99±4.31[a]
	Two ovaries	12.2±3.49[a]	12.37±7.01[a]	11.58±4.68[a]

TABLE 2. Average diameter (mm) for the development rate of dominates follicles at PG injection, and average diameter for increased rate (mm/d) from eCG injection to PG injection in the experimental groups.

Item		AC	AK	Control
Diameter of the dominant follicles (mm) at PG injection	Right ovary	16.78±2.03[a]	12.16±3.01[b]	10.93±2.13[b]
	left ovary	17.08±1.78[a]	13.30±1.40[b]	11.57±1.66[b]
	Two ovaries	16.93±1.35[a]	12.32±2.5[b]	11.1±1.12[b]
Dominant follicle rate (mm) from eCG injection to PG injection	Right ovary	11.80±2.03[a]	7.06±3.12[b]	5.76±1.92[b]
	left ovary	12.22±2.05[a]	5.12±4.78[b]	5.32±3.30[b]
Increased rate (mm/d)	Two ovaries	3.99±.48[a]	2.43±.81[b]	2.00±.35[b]

a, b: Different letters on the same row indicate significance ($P < 0.05$), analyzed by Completely Randomized Design (CRD) in the case of replicates are equal – General linear models, univariate.

TABLE 3. Ovulation time and number of mature follicles per ovary from PG injections and number of days from GnRH injections.

Item		AC	AK	Control
Number of follicles	Right ovary	1.4±0.5[a]	1.0±0.0[a]	1.0±0.0[a]
	left ovary	1.2±0.4[a]	0.8±0.8[a]	1.0±0.7[a]
	Two ovaries	2.6±0.5[a]	1.8±0.8[a]	2.00±0.7[a]
Ovulation time (h) from PG injections	Right ovary	36±12[b]	83.2±18.4[a]	86.4±13.1[a]
	left ovary	36±12[b]	74.6±20.1[a]	90±12[a]
	Two ovaries	36.±12[b]	83.2±18.4[a]	86.4±13.1[a]
Period (d) from	Two ovaries	7.5±0.5[b]	9.5±0.7[a]	9.6±0.5[a]

a, b: Different letters on the same row indicate significance ($P < 0.05$).

TABLE 4. Percentage of physical characteristics for cervical mucus (n= 5):

Physical characteristics		AC		AK		Control	
		F	%	F	%	F	%
pH		5.9±0.12c		8.4±0.19a		7.3±0.37b	
Colour	Clean	5	100	5	100	4	80
	Cloudy	-	-	-	-	1	20
Shape consistency	Thin	4	80	4	80	3	60
	Thick	1	20	1	20	2	40
Fern pattern		3	100	1	100	1	100
	Typical	2	85	2	85	3	85
		-	75	2	75	1	75
	Atypical	-	-	-	-	-	-
	Nil	-	-	-	-	-	-

N, number simple to each group F, frequency physical characteristics %, percent physical characteristics analyzed by statistical descriptive frequencies.

TABLE 5. The significance and shape of the Fern pattern of cervical mucus in the experimental groups.

Significance	AC	AK	Control
100% of the fern plant, which means higher ovulation than an ovum for ovaries	3	1	1
75% of the fern plant indicates that one ovum is ovulated from both ovaries	-	2	1
85% of the fern plant, which means that one ovum ovulates for each ovary	2	2	3
Ovulation of two ovaries, the diameter of the ovum is 14-16 at ovulation	5	3	2

TABLE 6. Average serum concentrations of progesterone (ng/ml) and estradiol (pmol/L) in the experimental groups.

Item	AC	AK	CONTROL
Progesterone ng/ml			
Early follicular (d0)	5.3±.5[c]	5.7±1.1[c]	5.8±.4[c]
Late follicular (d5)	9.0±1.1[b]	10.6±1.3[a]	9.4±1.2[b]
Early luteal (d10)	2.6±.6[d]	2.5±.7[d]	2.9±.8[d]
Estradiol, pmol/L			
Early follicular (d0)	308.8±15.5[d]	299.8±26.8[d]	222.4±28.9[d]
Late follicular (d5)	1415.6±156.4[a]	1166.2±198.6[b]	917.2±130.9[c]
Early luteal (d10)	211.6±23.5[e]	128.2±54.9[e]	127.2±63.1[e]

a, b, c, d: Different letters within the same hormone indicate significance ($P < 0.05$

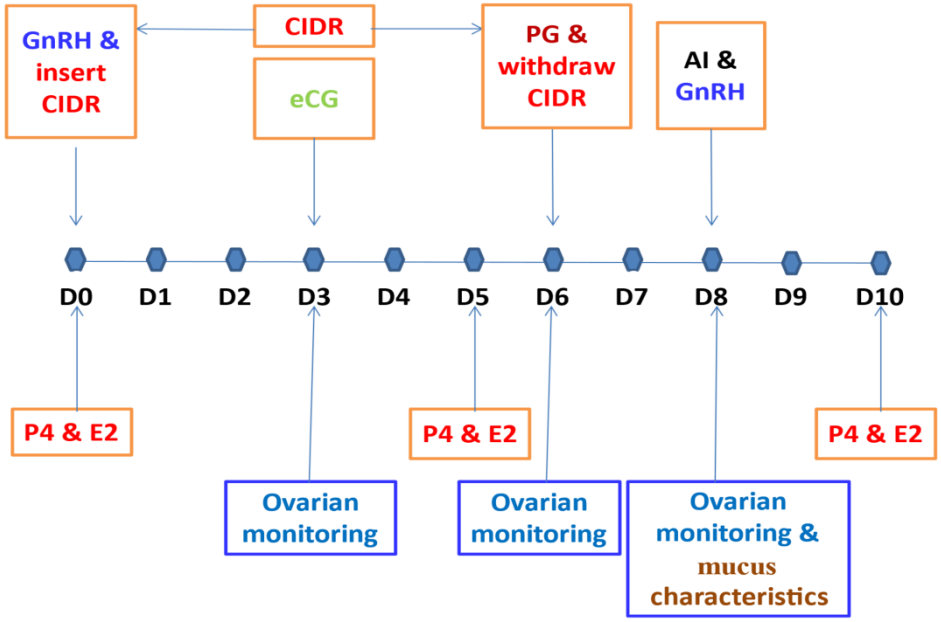

Fig. 1. Hormonal treatment protocol and timing of samples collection and ovarian monitoring

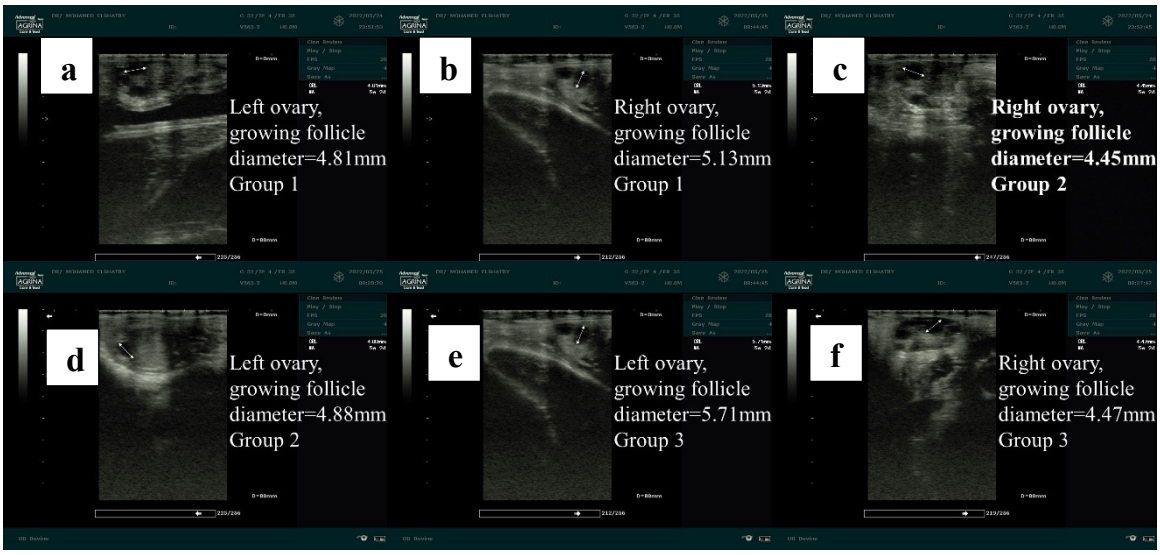

Fig. 2. Average diameter for the growing follicles (mm) at eCG injection, d 3 after GnRH injection.

a, ultrasound snapshot showing the developing follicle dimeter (mm) for left ovary at eCG injection, d3 after GnRH injection for 1st group ; b, ultrasound snapshot showing the developing follicle dimeter (mm) for right ovary at eCG injection, d3 after GnRH injection for 1st group ; c, ultrasound snapshot showing the developing follicle dimeter (mm) for right ovary at eCG injection, d3 after GnRH injection for 2nd group; d, ultrasound snapshot showing the developing follicle dimeter (mm) for left ovary at eCG injection, d3 after GnRH injection for 2nd group; e, ultrasound snapshot showing the developing follicle dimeter (mm) for left ovary at eCG injection, d3 after GnRH injection for 3rd group; f, ultrasound snapshot showing the developing follicle dimeter (mm) for right ovary at eCG injection, d3 after GnRH injection for 3rd group.

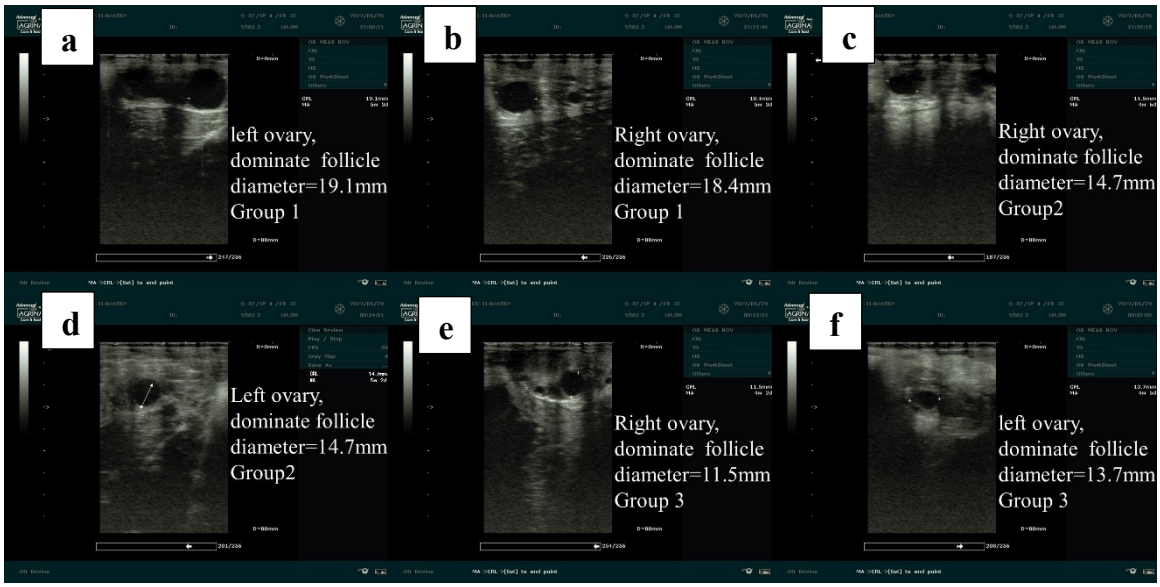

Fig. 3. Average diameter of dominate follicles (mm) during PG injection.

a, ultrasound snapshot showing the dominate follicle dimeter (mm) for left ovary during PG injection, d6 after GnRH injection for 1st group ; b, ultrasound snapshot showing the dominate follicle dimeter (mm) for right ovary during PG injection, d6 after GnRH injection for 1st group ; c, ultrasound snapshot showing the dominate follicle dimeter (mm) for right ovary during PG injection, d6 after GnRH injection for 2nd group; d, ultrasound snapshot showing the dominate follicle dimeter (mm) for left ovary during PG injection, d6 after GnRH injection for 2nd group; e, ultrasound snapshot showing the dominate follicle dimeter (mm) for right ovary during PG injection, d6 after GnRH injection for 3rd group; f, ultrasound snapshot showing the dominate follicle dimeter (mm) for right ovary during PG injection, d6 after GnRH injection for 3rd group.

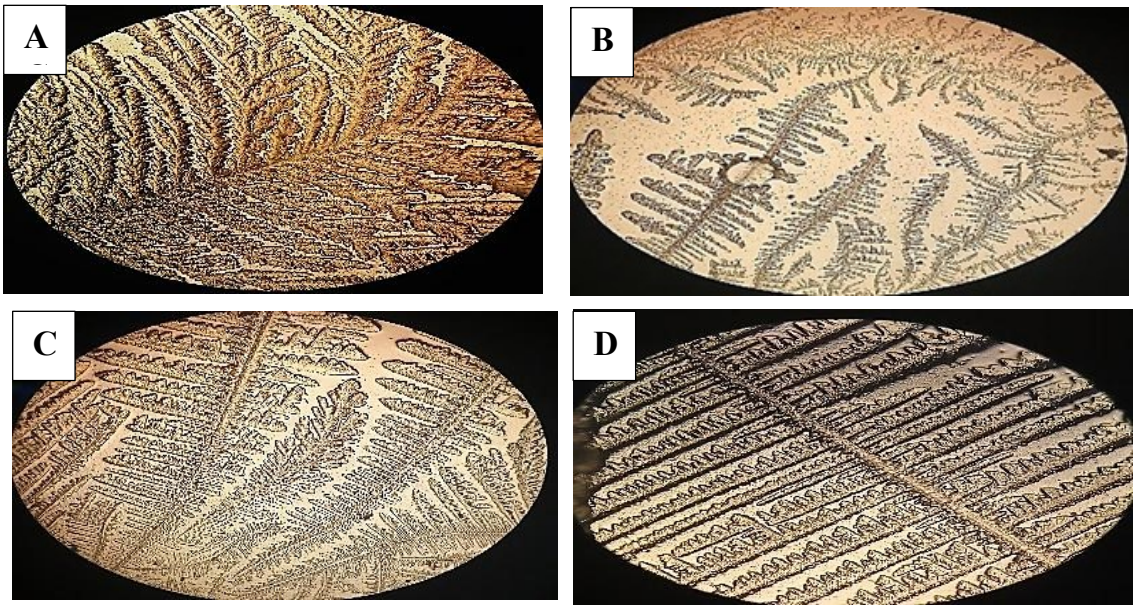

Fig. 4. Fern pattern of cervical mucus in the experimental groups.

A: microscopic snapshot illustrated mucus crystallite, 100% of the fern plant, which means higher ovulation than an ovum for ovaries B: microscopic snapshot illustrated mucus crystallite, 75% of the fern plant indicates that one ovum is ovulated from both ovaries. C: microscopic snapshot illustrated mucus crystallite, 85% of the fern plant, which means that one ovum ovulates for each ovary. D: microscopic snapshot illustrated mucus crystallite, Ovulation of two ovaries, the diameter of the ovum is 14-16 at ovulation.

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تأثير الأس الهيدروجينى للمهبل والرحم على التراكيب المبيضية لابقار الفريزيان الحلوب خارج الشياع باستخدام برتوكول Cosynch + progesterone لإحداث الشياع

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الملخص

تناولت هذه الدراسة تأثير درجة الأس الهيدروجيني للمهبل والرحم على تراكيب المبيض لدى أبقار الفريزيان الحلابة. وقد تم استخدام ثلاثين بقرة فريزيان عديمة الشبق وتم تقسيمها إلى ثلاث مجموعات (10 لكل مجموعة). تم غسل أرحام الأبقار في المجموعتين بمحلول منظم لدرجة الأس الهيدروجينى، 6 درجة في المجموعة الأولى، و8 درجة في المجموعة الثانية، والمجموعة الثالثة لم يتم غسلها وتم الاحتفاظ بها كمجموعة تحكم. خضعت جميع المجموعات لبروتوكول Cosynch + progesterone لتحفيز الشبق، والمسح بالموجات فوق الصوتية، وتم أخذ عينات الدم على فترات مختلفة. وجدت الدراسة أن تعديل مستوى الأس الهيدروجيني لم يكن له تأثير كبير على نمو الجريبات المبيضية، ولكن كان له تأثير كبير على معدل ونمو الجريبات السائدة في المجموعات الحمضية. أخيرًا، أدى تعديل مستوى الأس الهيدروجيني إلى 5,9 درجة إلى زيادة معدل التبويض وتقليل وقت التبويض بسبب تأثيره على نمو الجريبات السائدة.

الكلمات الدالة: درجة الأس الهيدروجيني للمهبل والرحم ، الحويصلات المبيضية ، تنظيم الشياع ، أبقار الفريزيان الحلابة.