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RESEARCH ARTICLE

Hormonal And Chemical Study of Cervical Mucus in Normal and Repeat Breeder Cows

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ABSTRACT

The aim of the study is to compare the hormonal levels of Luteinizing Hormone, Testosterone and 17-beta-estradiol in the blood of reproductively healthy cows with their levels in the blood of repeat breeder cows, in also to comparing the levels of minerals represented by (chloride, magnesium, potassium, phosphorous, sodium, Calcium, zinc and selenium) in cervical fluids of reproductively healthy cows with frequent estrus during estrus. The results of the hormonal study in the blood serum of the reproductively healthy cows showed that the levels of LH were 42.29 mIU/ml +-3.49 mIU/ml and testosterone was ng/ml 11.02 +- 0.54 and estradiol was 97.46 pg/ml +-4.14, while the hormonal levels of cows were Suffering from repeat breeder 60.55 mIU/ml +-2.86 pg/ml for LH, 14.86 +- 0.43 ng/ml for testosterone, and 149.9 +- 2.8 pg/ml for beta-estradiol -17, respectively. The results of the current study showed that the chloride concentration was 95.03 +- 1.38 mEq/l, the magnesium concentration was 0.305 +- 0.02 µg/dl, the potassium concentration was 4.36 +- 0.22 mEq/l, and the phosphorous concentration was 4.36 +- 0.22 mEq/l. It was 6.17 +- 0.20 mg/dl, the sodium concentration was 120.8 +- 1.31 mEq/l, the calcium concentration was 10.73 +- 0.53 mg/dl, the zinc concentration was 15.23 +- 0.37 µg/dl, and the selenium concentration was 145.4 +- 0.85 µg/L. While their concentrations were 128.58 +- 0.86 mEq/l for chloride, 0.124 µg/dl +- 0.01 for magnesium, 6.66 mEq/l +- 0.25 for potassium, 4.35 mg/dl +- 0.18 for phosphorous, and 141.8 mEq/l +- 1.80 for sodium, 6.21 mg/dl +- 0.21 for calcium, 21.9 µg/dl +- 0.99 for zinc, and 127.73 +- 2.57 µg/dl for selenium. There is also a Positive correlation between the levels of testosterone, beta-17-estradiol in the blood serum of cows and the concentrations of elements (chloride, potassium, sodium and zinc) in the estrous mucus of the study cows. The study also showed a positive correlation between the concentration of the element chloride and the concentration of (potassium, sodium and zinc) in the estrus mucus of the study cows.

KEYWORDS:
Testestrone, Beta-17- Estradiol, Chloride, Potassium, Sodium And Zinc.

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INTRODUCTION

Cows are among the mammalia animals and the age of sexual puberty is 7-18 months, While the age of sexual maturity is at 30 months. It is the age at which the female is able to conceive and give birth, and the insemination takes place during the time of estrus, which has a duration of 24 hours, and ovulation occurs 12 hours after the end of the stage of estrus. The period of estrus is accompanied by many physiological and morphological changes to prepare the uterus for fertilization and cleans it from microbes. These changes include the uterine glands secretion , Cervical mucus (CM), uterine relaxation and the opening of the cervix. The Cervical mucus (CM) is continuously produced by the secretory cells of the endcervix, and its quality and quantity vary depending upon the hormonal status in the estrous cycle .(Lopez et al ,. 1993). Variations of cervical mucus rheology , flow and deformation properties , during the estrous cycle reflect, in part, the response of the cervical epithelium to steroid hormones .(Carlstedt & Sheehan ,1989). Two types of CM are estrogenic , recognized and gestagenic (Vigil . 2009) constituted by different subtypes. Biophysical and biochemical characteristics in CM change during menstrual cycle (Vigil . 2009- Morales et al ,.1993.) during estrous cycle in cows (Lopez et al ,. 1993- Carlstedt & Sheehan ,1989). These changes are believed to be driven by steroid hormone levels and are somehow associated with ovulation .(Roth,1993.) The Cervical mucus plays an essential role in the reproductive process of all mammals. The primary function of cervical mucus is that of a mechanical barrier against microbial infection of the uterus. Cervical mucus contain about 92-95% water along with low molecular weight substances such as electrolytes carbohydrates , amino acids ,

lipids and soluble macromolecular compounds such as proteins and polysaccharides dissolved in it , during estrus , The modification of physical and biochemical properties of cervical mucus takes place under influence if sex steroid hormones in order to facilitate the transport of spermatozoa .(Schumacher,1970.) Sufficient intakes of nutrients during the transition period of piriparturient will support the reproduction success (Leblanc, 2005.) ,Selenium (Se), zinc (Zn), vitamin A and E as well as several other important minerals are essential source for health and reproductive performance of dairy cows. (Meglia etal,. 2004) The objective of the study is to determine the content of certain ions (sodium , potassium , calcium , magnesium , and chlorine) in the cervical mucus of cows at the time of inseminating . The presence of estrogen greatly affects animal physiology during the estrus phase and causes the animal to be able to copulate. (Tsiligianni, 2011.) reported that super ovulated cows in the follicular phase had a minimum estrogen level of 30.95 pg/mL and a maximum level of 54.77 pg / mL (picogram pg=10−12 gram) (Bernardi et al,.2016) . The increase in estrogen concentration in blood circulation reaches the anterior pituitary and stimulates L.H. release. Moreover, estrogen also affects the nervous system, causing animals to be restless and to mount other cows. Estrogen also causes the uterus to contract gently, which allows spermatozoa to be transported to the female reproductive genitals after insemination and natural copulation. Other effects of high estrogen concentration include dilatation of blood vessels in genital organs and mucus secretion by cervical and vaginal glands. (Ramli,2016.) In cows, a recurrence of normal estrus occurs after more two successive insemination without pregnancy and with no clinical abnormalities, and it is expected that it is due to pathological changes or incorrect time of insemination or that it is due to uterine infection.

MATERIALS AND METHODS

Animals

120 adult female Iraq cross breed cows, aged between 3 - 7 years and weighing 100 to 200 kg which determine by teeth formula according to the techniques which described by Dyce and Sack (2010) , had already given birth and had gone through at least two regular cycles. The cows were divided into two groups of 60 cows, 60 female cows were normal reproduction state and 60 female cows were repeat breeders, all cows were come to the artificial insemination center in the province of AL- Qadisiyah.

Blood Serum Collecting

Following the mucus collection, a 10 mL blood sample was collected from the jugular vein. To separate blood cells and serum into two layers, the sample was placed in a thermos and left for many hours. The serum was then transferred into a micro tube after centrifuging the tube for 10 minutes at 3000 rpm. Finally, serum was frozen at -20 degrees Celsius until hormone analysis could be performed.

ELISA Profile

Luteinizing hormone (LH) ELISA kit, Testosterone (T) ELISA Kit, Estradiol (E2) ELISA Kit, all these kits were obtained from a company CUSABIO of Chinese origin.

Statistical analysis

Statistical analysis Data are presented as mean ± SD. The comparison between control and repeat breeder group were performed by using T test. The 0.05 level of probability was used as the criterion for significance. All statistical analyses were performed using SPSS software version 27 (Ozdamar,2005).

RESULTS

Concentration of Hormones in serum of estrus cows

In table1. shows the concentration levels of hormones (Luteal hormone, estradiol -17B hormone and testosterone hormone) in the reproductively normal cows (control) and the repeat breeder cows. The study showed that there is a statistical difference between the reproductively healthy (control) cows and the repeat breeder cows. The concentration of hormones in the serum of normal cows (control) was, Luteal hormone (42.29 +- 3.49) mIU/ml, testosterone hormone (11.02 +- 0.54) ng/ml and estradiol-17B hormone (97.46 +- 4.14) pg/ml. While in cows of repeat breeder, the concentrations in the serum were, Luteal hormone (60.55 +- 2.86) mIU/ml, testosterone hormone (14.86 +- 0.43) ng/ml and estradiol-17B hormone (149.9 +- 2.8) pg/ml. Table1.

Table 1: level of luteal hormone, testosterone hormone and estradiol hormone concentration in the serum of estrus cows.

Hormone	Control cows	Repeat breeder
Luteal hormone (L.H) mIU/ml	42.29 +- 3.49 A	60.55 +- 2.86 B
Testosterone hormone (ng/ml)	11.02 +- 0.54 A	14.86 +- 0.43 B
Estradiol hormone (pg/ml)	97.46 +- 4.14 A	149.9 +- 2.8 B

*The different capital

Levels of mineral concentrations in estrus mucus.

Levels of chloride, magnesium and potassium concentrations in estrus mucus.

Table2. shows the concentration levels of minerals (chloride, magnesium and potassium) in the reproductively healthy cows and the repeat breeder cows. The study showed that there is a statistical difference between the reproductively healthy

(control) cows and the repeat breeder cows. The concentration of minerals in the estrus mucus of healthy (control) cows was chloride (95.03+-1.38) mEq/L, magnesium (0.305 +- 0.02) µg/dl and potassium (4.36 +-0.22) mEq/L. While in cows of repeat breeder, the concentrations in the estrus mucus were, chloride (128.58 +- 0.86) mEq/L, magnesium (0.124 +- 0.01) µg/dl and potassium (6.66 +- 0.25) mEq/L . Table 2.

Table 2: level of chloride, magnesium and potassium concentration in the serum of estrus cows.

minerals	Control cows	Repeat breeder
Chloride (CL)mEq/L	95.03 +- 1.38 A	128.58 +- 0.86 B
Magnesium (Mg) µg/dl	0.305 +- 0.02 A	0.124 +- 0.01 B
Potassium (K)mEq/L	4.36 +- 0.22 A	6.66 +- 0.25 B

The different capital letters represent significant differences at level in the same row.

Levels of phosphor, sodium and calcium concentrations in estrus mucus.

Table3. shows the concentration levels of minerals (phosphor, sodium and calcium) in the reproductively healthy cows and the repeat breeder cows. The study showed that there is a statistical difference between the reproductively healthy (control) cows and the repeat breeder cows. The concentration of minerals in the estrus mucus of healthy (control) cows was phosphor (6.17 +- 0.20) mg/dl, sodium (120.8 +- 1.31) mEq/L and calcium (10.73 +- 0.53) mg/dl. While in cows of repeat breeder, the concentrations in the estrus mucus were, phosphor (4.35 +- 0.18) mg/dl , sodium (141.8 +- 1.80) mEq/L and calcium (6.21 +- 0.21) mg/dl . Table 3.

Table 3: level of phosphor , sodium and calcium concentration in the serum of estrus cows.

Minerals	Control cows	Repeat breeder
phosphor (P)mg/dl	6.17 +- 0.20 A	4.35 +- 0.18 B
sodium (Na) mEq/L	120.8 +- 1.31 A	141.8 +- 1.80 B
calcium (Ca)mg/dl	10.73 +- 0.53 A	6.21 +- 0.21 B

The different capital letters represent significant differences at level in the same row

Levels of zinc and selenium concentrations in estrus mucus.

The table 4. shows the concentration levels of minerals (zinc and selenium) in the reproductively healthy cows and the repeat breeder cows. The study showed that there is a statistical difference between the reproductively healthy (control) cows and the repeat breeder cows. The concentration of minerals in the estrus mucus of healthy (control) cows was zinc (15.23 +- 0.37) µg/dl, selenium (145.4 +- 0.85) µg/L . While in cows of repeat breeder, the concentrations in the estrus mucus were, zinc (21.9 +- 0.99) µg/dl and selenium (127.73 +- 2.57) µg/L .Table 4.4. , figure 4.4.

Table 4: level of Zinc and selenium concentration in the serum of estrus cows.

Minerals	Control cows	Repeat breeder
zinc (Zn) µg/dl	15.23 +- 0.37 A	21.9 +- 0.99 B
selenium (Se) µg/L	145.4 +- 0.85 A	127.73 +- 2.57 B

The different capital letters represent significant differences at level in the same row.

Correlation between hormones and minerals un normal and repeat breeder cows.

The correlation between luteal hormone, testosterone hormone and estradiol -17B hormone was positive correlation and the correlation between hormones and minerals was a positive (+v) correlation between luteal hormone and (chloride, sodium and zinc); Testosterone hormone and (chloride, potassium, sodium and zinc); Estradiol -17B and (chloride, potassium, sodium and zinc), under signification at $P < 0.01$ (table5). The correlation between minerals was positive correlation between chloride and (potassium, sodium and zinc); Magnesium and (phosphor, calcium and selenium); Potassium and (sodium and zinc); Phosphor and (calcium and selenium) ; Sodium and (zinc) ; Calcium and (selenium) , under signification at $P < 0.01$ (table5).

Table 5: correlation between hormones and minerals in normal and repeat breeder cows.

	L.H	TESTO.H.	ESTR.H.	CL	MG	K	P	Na	Ca	Zn	Se
L.H	I										
TESTO.H.	0.924	I									
ESTR.H.	0.940	0.970	I								
CHLORIDE	0.946	0.962	0.989	I							
MAGNSIUOM	-0.923	-0.956	-0.973	-0.976	I						
POTASSIUM	0.914	0.949	0.965	0.975	-0.958	I					
PHOSPHOR	-0.931	-0.937	-0.975	-0.980	0.950	-0.958	I				
SODIUM	0.934	0.975	0.976	0.986	-0.971	0.971	-0.960	I			
CALCIUM	-0.926	-0.956	-0.976	-0.977	0.965	-0.965	0.952	-0.976	I		
ZINC	0.917	0.943	0.969	0.971	-0.954	0.963	-0.949	0.965	-0.970	I	
SELENIUM	-0.911	-0.949	-0.976	-0.976	0.960	-0.958	0.945	-0.969	0.967	-0.970	I

* Positive value (+) mean positive correlation, negative value (-) mean negative correlation under significant at P < 0.01.

DISCUSSION

The results of the study indicated that the level of the luteinizing hormone in serum of healthy cows ware (42.29 +- 3.49 mIU\ml). This result was accordance with the findings of Abhijit Barui et al., (2015)1 which reported that the level of the luteinizing hormone were (0.3644±0.05449 mIU\ml) in plasma . While the results of the study indicated that the level of luteinizing hormone in the repeat breeder cows were (60.55+- 2.86 mIU\ml) and this was very high then the results of Abhijit Barui et al.,(2015) which recorded that the level of the luteinizing hormone in serum was (0.3190±0.041 mIU\ml) in repeat breeder cows . The changing of that may be due to affected of exogenous (environment and nutrition) and endogenous (weight of animals and breed) factors, or it may be due to the method of hormone analyzer from (serum or plasma). The current study indicated that the level of testosterone hormone in healthy cows were (11.02+- 0.45ng\ml) and (14.86+-0.43 ng\ml) in repeat breeder cows, These results contradict with the results that shown by (Nessan and King1981), which were (0.046 +- 0.042 ng/ml) in normal cows, This difference may be due to the difference in the environment or breed, feeding , management and time and type of test. The recent study showed that the concentration of Estradiol 17b in the serum were (97.46+-4.14pg/ml) in healthy cows and (149.9+-2.8 pg/ml) in repeat breeder cows respectively, This was agreed with the results of (Asghar Mogheiseh et al. , 2019.) whom recorded (75.70 ± 24.34 pg/ml) in healthy cows , but differs in repeat breeder cows (36.00 ± 11.27 pg/ml), This difference may be due to the difference between the breeds , management , nutrition.(Table1).The recent study results showed that the level of Chloride in cervical mucus in healthy cows were (95.03+-1.38 mEq/L) and (128.58+-0.86 mEq/L) in repeat breeder cows, These finding was near to the mean value which recorded by (Asghar Mogheiseh et al. , 2019) which were (97.90 ± 3.73 mEq/L) in healthy cows and(113.00 ± 11.27 mEq/L) in repeat breeder cows respectively , The results of Magnesium level in our study were (0.305+-0.02µg/dl) in healthy cows and (0.124+- 0.01µg/dl) in repeat breeder cows , these results not agreement with the results of (KADOOM1 AND M.A.A. EL-HENAWY 2017) which shown that the concentration of Magnesium in cervical mucus in healthy cows were (1.6±0.41 mg/dl) and (1.2±0.29 mg/dl) in repeat breeder cows. The study also showed that the Potassium concentration in cervical mucus in the healthy cows were (4.36 +-0-22 mEq/L) and (6.66+-0.25 mEq/L) in repeat breeder cows, this result basically was near with these findings that shown by (Asghar Mogheiseh et al. , 2019) which were (4.35 ± 0.46 mEq/L) in healthy cows and (3.96 ± 0.12mEq/L) in repeat breeder cows are.(Table 2). The recent study showed that the concentration of Phosphorous in cervical mucus in healthy cows is (6.17+- 0.02 mg/dl) and (4.34+-0.18 mg/dl) in repeat breeder cows respectively, this result does not correspond with the results of the Gohel , et al.,(2020)5 whose found that Phosphorous concentration in healthy cows were (2.78 ± 0.16 mg/dl) and (2.24 ± 0.25 mg/dl) in repeat breeder cows , These difference is likely to be due to many factors, including the nature and type of food, the time of sampling, and the difference in the soil of the field. The results indicated that the concentration of Sodium in the healthy cows were (120.8+-0.20 mEq/L) and(141.8+-1.80 mEq/L) in repeat breeder cows , these results did not agree with the results that which recorded by Kadoom and EL-henawy(2017)4 in healthy cows which were (139.87 ± 1.83 mEq/L) and agreement with the results in the repeat breeder cows which were (138.60 ± 1.75 Meq/L) . The current study indicated that the results of the calcium concentration in healthy cows were (10.73+-0.53 mg/dl) and (6.21+-0.21 mg/dl) in repeat breeder cows , these results in the healthy cows is close to the results that recorded by (Gohel , et al. , 2020.) which were (13.29 ± 0.40 mq/dl) and does not agree with the results of the repeat breeder cows which were (16.50 ± 0.75 mg/dl) , this difference may be due to the type of feeding and the type of breeding for cows. (Table3). The results illustrated that the levels of zinc concentration in cervical mucus during estrous were in healthy cows (15.23+- 0.73µg/dl) and (21.9+-0.99 µg/dl) in repeat breeder cows, these results agreement with the results that shown by (Sayed Mortaza et al. 2012), whose showed that the concentration of Zinc in healthy cows were (216.9 ± 61.3 µg/dl-1) while not agree with results that showed by (KADOOM1 and EL-HENAWY 2017) in repeat breeder cows which were (0.2±0.02 µg/dl)

, This may be due to the breed of the cows , type of feeding or the management of the herd. The result showed The correlation between luteal hormone , Testosterone hormone and estradiol -17B hormone was positive correlation ,this result agree with (Arthur , et.al.,2019) which reported that there is correlation between Luteal hormone , Testosterone hormone and Estrogen hormone in the regulation of cyclical activity in the female cows , the results also showed that the correlation between hormones and minerals was positive (+v) correlation between luteal hormone and (chloride , sodium and zinc) ; Testosterone hormone and (chloride , potassium , sodium and zinc) ; Estradiol -17B and (chloride , potassium , sodium and zinc) , under signification at $P < 0.01$ (table 5), There were no other studies done to investigate the correlation between hormones and minerals but this results agree with the results that refer to the increase minerals in the estrous mucous during estrous and can used that increase in the minerals in the estrous mucous for estrous detection in the cows (Gordon,2002). The results of the study have shown that there is correlation between minerals was positive correlation between chloride and (potassium , sodium and zinc) ; Magnesium and (phosphor , calcium and selenium) ; Potassium and (sodium and zinc) ; Phosphor and (calcium and selenium) ; Sodium and (zinc) ; Calcium and (selenium) , under signification at $P < 0.01$ (table 5),There were no other studies done to investigate the correlation between minerals in the estrous mucous but this correlation may be due to the hormonal effect on the estrous mucous during estrous which lead to increase estrous mucous during estrous (Hafez and Hafez,2002; Squires,2003; Ball and Peters,2004).

REFERENCES

1. Lopez-Gatius F, Miro J, Sebastian I, Ibarz A, Labernia J.(1993). Rheological properties of the anterior vaginal fluid from super-ovulated dairy heifers at estrus. Theriogenology. . 40:167–180.
2. Carlstedt I, Sheehan JK.(1989). Structure and macromolecular properties of cervical mucus glycoproeitns. In: Chantler E, Ratcliffe NA, editors. Mucus and Related Topics. Society for experimental biology. Cambridge, UK: Cambridge Univ Pr; .pp. 289–316
3. Vigil, M. E. Cortes, A. Zuniga, J. Riquelme, F. Ceric. (2009). Scanning electron and light microscopy study of the cervical mucus in women with polycystic ovary syndrome. J Electron Microsc (Tokyo) 58, 21-7.
4. Morales P, Roco M, Vigil P. (1993) . Human cervical mucus: Relationship between biochemical characteristis and ability to allow migration of spermatozoa. Hum Reprod. 8:78–83.
5. Roth B.(1993). Fertility awareness as a component of sexuality education. Preliminary research findings with adolescents. Nurse Pract. 18:40–48
6. Schumacher, G.F.B. (1970). Biochemistry of cervical mucus . Fertility and Sterility., 21: 697-700.
7. Leblanc, S. (2005). Overall reproductive performance of canadian dairy cows: challenges we are facing. Advances in Dairy Technology, 17.
8. Tsiligianni TH, Karagiannidis IA, Brikas P, Saratsis PH (2011). Physical properties of bovine cervical mucus during normal and induced (progesterone and/or PGF2ά) estrus. Theriogenology. 55(2): 629-640.
9. Ramli M, Siregar TN, Thasmi CN, Dasrul, Wahyuni S, Sayuti A (2016). Hubungan antara intensitas estrus dengan konsentrasi estradiol pada sapi aceh pada saat inseminasi. J. Med. Vet. 1(10): 27-30.
10. Ozdamar, K. (2005). Biostatistics with SPSS. Kann press Eskisehir
11. Abhijit Barui1, Subhasis Batabyal1, Sarbaswarup Ghosh2, Debjani Saha1 and Saibal Chattopadhyay1, 2015. Plasma mineral profiles and hormonal activities of normal cycling and repeat breeding crossbred cows: A comparative study. Veterinary World, EISSN: 2231-0916.
12. Nessan and G. J. King . 1981. Sexual behaviour in ovariectomized cows treated with oestradiol benzoate and testosterone propionate. Department ofAnimal and Poultry Science, University ofGuelph, Guelph, Ontario, Canada NIG 2 Wl;61,171-178.
13. Asghar Mogheiseh1, Mojtaba Kafi1 , Navid Golestani1 , Abbas Roshan-Ghasrodashti2 , Saeed Nazifi1 , Abdollah Mirzaei. 2019. Follicular fluid composition of ovulatory follicles in repeat breeder Holstein dairy cows. Asghar Mogheiseh et al. Asian Pacific Journal of Reproduction (2019)124-131.
14. kadoom1 and m.a.a. el-henawy. 2017. Serum and estrus mucus enzymes activities and chemistry of normal and repeat breeder friesian cattle. Indian journal of animal reproduction 39 (1).
15. Gohel, M.M., Kavani, F.S., Dhami, A.J. & Hadiya, K.K. (2020). Electrolytes Profile of Estrual Mucus of Gir Cows with Reference to Body Condition Score and Fertility. Ind J Vet Sci and Biotech, 16(1):7-10.
16. Sayed Mortaza Alavi-Shoushtari*, Siamak Asri Rezaie, Mozhgan Pak, Sajad Alizadeh, Roya Abedizadeh, Amir Khaki . 2021. Copper and zinc concentrations in the uterine fluid and blood serum during the bovine estrous cycle . Veterinary Research Forum ; 3 (3) 199 – 203 .
17. Arthur, G.H., Noakes, D.E. and Pearson, H. (2019). Veterinary Reproduction and Obstetrics. 8 th edition, Bailliere Tindall, London, p. 212.