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DIAGNOSTIC VALUE OF SOME CHEMICAL MILK CONSTITUENTS IN MONITORING OF OVARIAN CYCLICITY AND EARLY PREGNANCY DIAGNOSIS IN BUFFALOES

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SUMMARY

The objective of this study was to investigate the correlation between the plasma progesterone and some chemical milk constituents (fat%, lactose% and chlorine%) during the different phases of estrous cycle and early pregnancy in buffaloes.

Thirteen buffaloes aged 4-8 years were included in this study, 5 animals were used to study the correlation between milk progesterone and some chemical milk constituents during estrous cycle which was induced by using Cloprostenol (Estrumate-ICI). Another 8 animals with known date of mating were used to study the same correlation during the early pregnancy. Blood and milk samples were collected for plasma progesterone assay and determination of some chemical milk constituents (fat%, lactose% and chlorine%).

A strong negative correlation was observed between plasma progesterone and lactose % during estrous cycle and early pregnancy (r = -0.84 + 0.02 and r = -0.87 + 0.03, respectively). Meanwhile, a strong positive correlation was observed between plasma progesterone and chlorine% during estrous cycle and early pregnancy (r = 0.87+0.03 and r = 0.93 + 0.02, respectively). On the other hand, no significant correlation was observed between milk progesterone and fat%.

This study concluded that measuring of milk lactose and or chlorine could be of value in monitoring the ovarian cyclicity and early pregnancy diagnosis in buffaloes.

INTRODUCTION

In an effort to increase the reproductive profitability of buffalo; diagnostic laboratory methods should be involved in the evaluation of the reproductive state . Improved reproductive efficiency and treatment of infertility are

dependent upon the proper diagnosis of the stage of the reproductive and differentiation between the pregnant and the non-pregnant cows as early as possible

Rectal palpation is still, for economic reasons , the preferred technique for monitoning the ovarian cyclicity and pregnancy diagnosis in buffalo. The rely upon the rectal palpation technique in the diagnosis of early pregnancy is still dangerous for its disadvantageous effect on the survival of the embryo at the early stages of pregnancy. Accuracy of this technique is however, totally dependent upon the palpator's skill and experience (Sharifuddin and Jainudeen, 1983; El-wishy and Ghoniem, 1995). Waheed, (1996) revealed an overall correct rectal diagnosis of ovarian condition in only 60.% of the palpated buffaloes.

Moreover, the accuracy of diagnosis of early pregnancy in buffalo (21-25 days) by progesterone was as high as 71.4%-73% (Nanda et al., 1984). By measuring milk progesterone, the accuracy ranged between 66% -88% for pregnancy and 90%- 91% in the non-pregnant condition (Dionysuis, 1991). By using ultrasound device in pregnancy diagnosis, Rowbinson, (1984) reported that the overall accuracy of pregnancy testing was 86.5% in pregnant cows while it was 67.4% in the non-pregnant cows. Other pregnancy diagnosis tests including measuring pregnancy specific Ag (with overall accuracy of 77%) and counting of the thrombocytes were also applied (Noble, 1982).

Although hormonal changes during the different reproductive phases imposed a great effect on lactogenesis and milk composition, no precise data could be traced concerning the association between shemical constituents of milk and plasma progesterone. The objective of this study is to investigate the correlation between the plasma progesterone and some chemical milk constituents during the different phases of estrous cycle and early pregnancy aiming to develop a new method which may helpful in estrus detection and/or early pregnancy diagnosis in buffalo.

MATERIAL AND METHODS

This study was carried out on 13 buffalo aged 4-8 years and kept at the Faculty of Veterinary Medicine and Animal Reproduction Research Institute, to determine the exact time of the cycle, 5 animals were selected on the basis of the presence of CLs on their ovaries. The animals were then injected with 0.5mg Cloprostenol (Estrumate, ICI). Behavioral estrus was checked daily with the help of a buffalo-bull and depending on the estrus signs observed. Rectal palpation was used to ensure estrus in all 5 animals . The day of estrus was considered as day 0.

Blood samples (10ml) and milk samples (50ml) were collected between 8:00 and 11:00 AM daily starting on the day of Clorprostenol injection through a complete estrus cycle.

Another 8 animals with known date of mating were used to study the correlation between the plasma progesterone level and some chemical

milk constituents (fat% , lactose% and chlorine%) during early pregnancy (5 of them which were proved to be pregnant later were included in the study). Blood samples (10 ml) and milk samples (30 ml) were collected every 3 days during the 1st month after mating and then every 5 days during the 2nd month after mating . The blood samples were immediately centrifuged at 3000 r.p.m. for 10 minutes at 4C. The plasma was separated and stored at 20C until assay. The milk samples were kept at -20C until analysis.

Radioimmunossay of plasma progesterone was carried out according to the methods previously described by Dobeli, (1980).. Antiserum to progesterone was produced at the clinic for Andrology and Gynaceology, Zurich University by the immunization of rabbits with progesterone 11B-hemisuccinate HAS. The antiserum crossreacted 1.0 with progesterone, 0.0005 with 5 pregnene-3B01-20-one, 0.29 with 11B Hydroxy progesterone, 0.0054 with 11 Hydroxyprogesterone, 0.013 with hydroxyprogesterome, 0.0004 with testesterone, <0.0001 with 4 Androstene -3,17- dione, <0.0001 with Estrone, 17B Estradio, Estraiol and <0.0001 with cholesterol. The sensitivity of the the assay (n=6) was a level of 0.12+0.07) ng/ml plasma. The intra and inter-assay coefficients of variation were 6.4% and 8.8% respectively.

For the determination of milk fat%, lactose% and chlorine%, milk samples were collected in dry, clean and labeled bottles following the before mentioned schedule. Milk fat% was determined by Gerber's method as described by A.O.A.C. (1990). Lactose % and chlorine % were determined using method described by A.O.A.C. (1990).

RESULTS

*** Correlation between plasma progesterone and some chemical milk constituents during the estrous cycle:**

The Correlation between plasma progesterone and some chemical milk constituents (fat%, lactose% and chlorine%) during the different phases of estrous cycle are presented in table 1 and Fig. 1,2&3. There was a strong negative correlation between plasma progesterone and milk lactose% (r= -0.84+0.02). Moreover, a strong positive correlation was observed between plasma progesterone and milk chlorine% in all animals (r = 0.87+0.03). On the other hand, it was noticed that there was no correlation between plasma progesterone and milk fat% (r = -0.1+0.3).

*** Correlation between plasma progesterone and some chemicel milk constituents during early pregnancy:**

The Correlation between plasma progesterone and some chemical milk constituents (fat%, locatose% and chlorine%) during early pregnancy are presented in table 2 and Fig.4,5 & 6. Nearly, the same abovementioned correlations (during the estrous cycle) were observed also during early pregnancy. Plasma progesterone was positively correlated with chlorine% (r= 0.93+0.02) and negatively correlated with lactose% (r=-0.87+0.03) while it was not correlated with fat% (r=9.4+2.1).

Table1:Plasma progesterone and some chemical milk constituents (fat %, lactose % and chlorine %) during estrous cycle.(Mean+SD)

	Fat %	Lactose %	Chlorine %	Progesterone(ng/ml)
-3	7.0+0.3	4.0+0.3	0.12+0.02	1.21+0.2
-2	6.1+0.5	4.4+0.2	0.1+0.03	0.58+0.4
-1	6.3+0.4	4.6+0.09	0.1+0.03	0.46+0.3
0(day of estrus)	6.5+0.6	4.8+0.07	0.08+0.02	0.3+0.2
1	6.5+0.7	4.2+0.08	0.1+0.03	0.78+0.3
2	6.8+0.2	3.84+0.07	0.11+0.02	0.91+0.2
3	6.8+0.3	3.58+0.1	0.12+0.02	1.14+0.4
4	6.1+0.5	3.52+0.09	0.128+0.01	1.33+0.6
5	5.7+1.1	3.34+0.06	0.134+0.01	1.88+0.5
6	6.3+0.7	3.2+0.1	0.13+0.02	1.89+0.3
8	5.7+1.3	3.0+0.2	0.13+0.02	2.0+0.8
10	6.2+0.6	3.0+0.1	0.134+0.01	2.2+0.2
12	6.3+0.4	2.9+0.2	0.138+0.01	2.6+0.6
14	6.5+0.2	2.8+0.2	0.165+0.02	3.08+0.3
16	6.0+0.8	2.8+0.3	0.175+0.01	3.53+0.2
18	6.5+0.3	3.76+0.1	0.127+0.02	2.9+0.3
20	6.0+0.7	4.2+0.2	0.1+0.03	1.6+0.4
22	5.5+1.2	4.5+0.3	0.06+0.03	0.6+0.2

Table 2:Plasma progesterone and some chemical milk constituents (fat %, lactose % and chlorine %) during early pregnancy.(Mean+SD)

	Fat %	Lactose %	Chlorine %	Progesterone(ng/ml)
1	6.3+0.9	4.4+0.2	0.07+0.02	0.2+0.2
3	7.0+0.3	4.12+0.1	0.1+0.03	0.3+0.2
6	6.5+0.8	3.84+0.2	0.1+0.04	0.7+0.4
9	6.8+0.4	3.8+0.3	0.12+0.02	1.2+0.6
12	6.0+1.0	3.61+0.2	0.13+0.06	1.85+0.4
15	6.5+0.5	3.45+0.1	0.144+0.01	2.76+0.3
18	6.5+0.3	3.33+0.1	0.145+0.02	2.94+0.2
21	6.2+0.5	3.2+0.2	0.17+0.03	3.35+0.2
24	6.2+0.7	3.1+0.1	0.18+0.02	3.6+0.3
27	6.0+1.2	3.55+0.2	0.2+0.05	3.47+0.1
30	6.8+0.5	3.8+0.3	0.21+0.02	3.54+0.3
35	6.5+0.6	3.24+0.1	0.226+0.01	3.58+0.2
40	7.0+0.2	3.24+0.2	0.22+0.01	3.65+0.4
45	6.5+0.5	3.2+0.08	0.24+0.05	3.85+0.2
50	7.0+0.2	3.04+0.09	0.236+0.01	3.73+0.3
55	7.0+0.2	3.93+0.2	0.24+0.02	3.9+0.4
60	6.5+0.4	2.9+0.3	0.24+0.03	3.87+0.2

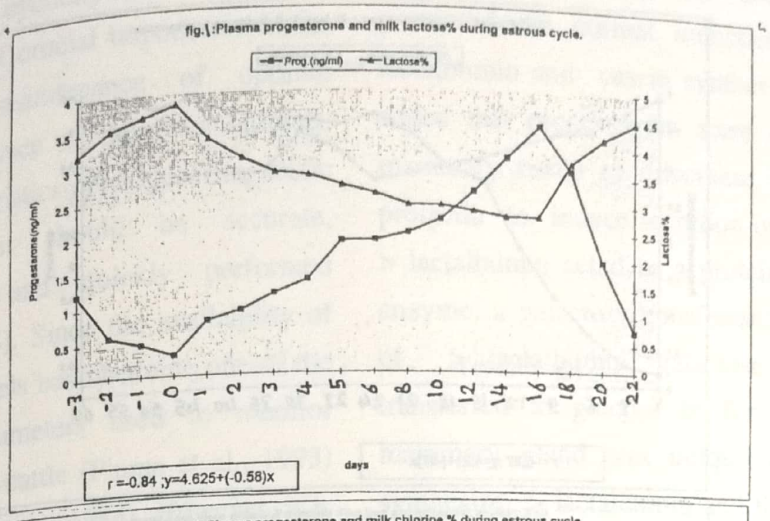


fig.1:Plasma progesterone and milk lactose% during estrous cycle.

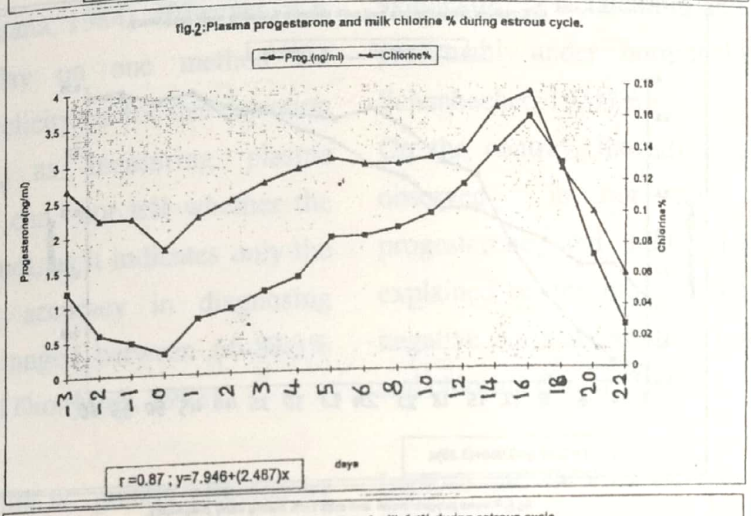


fig.2:Plasma progesterone and milk chlorine % during estrous cycle.

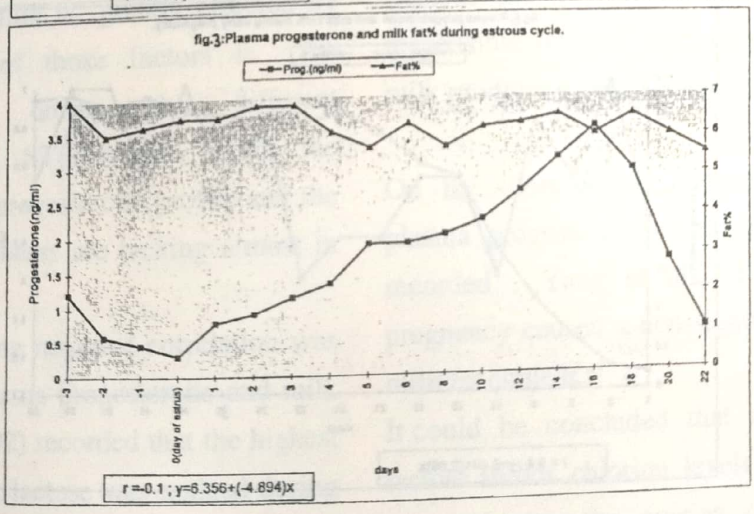
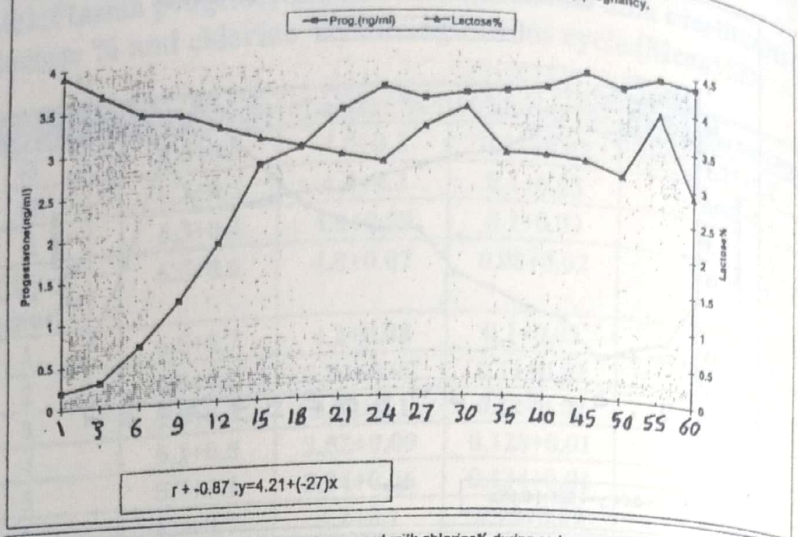


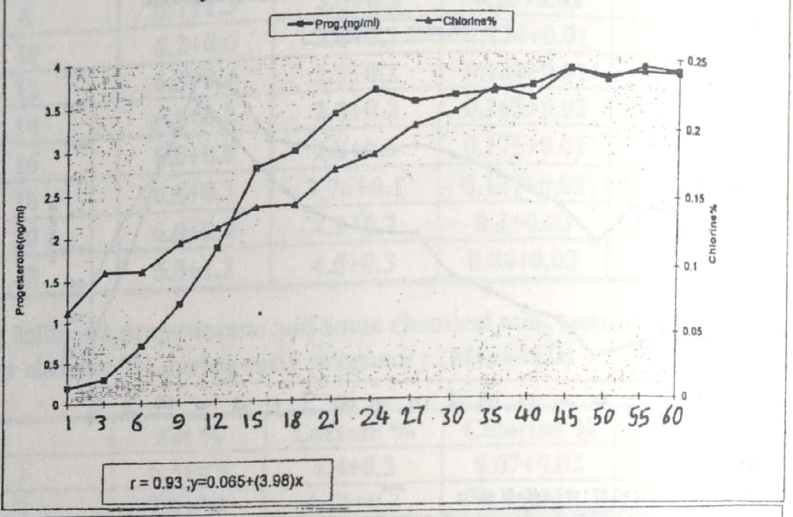
fig.3:Plasma progesterone and milk fat% during estrous cycle.

fig.4:Plasma progesterone and milk lactose% during early pregnancy.



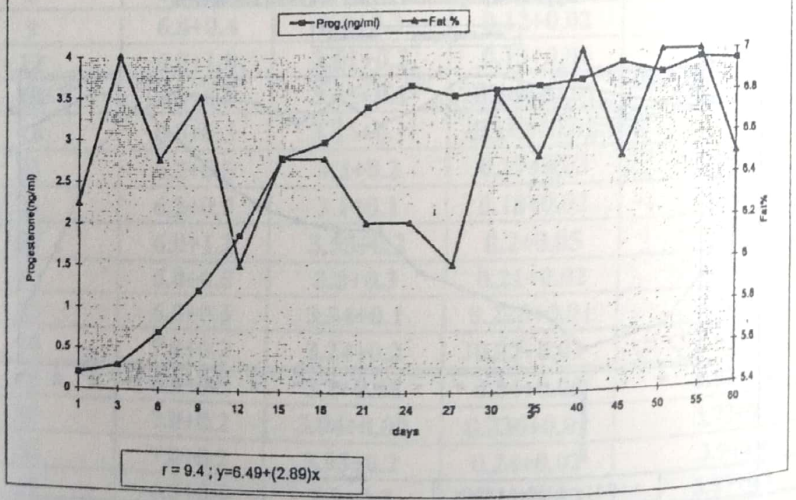
r + -0.87 ;y=4.21+(-27)x

fig5:Plasma progesterone and milk chlorine% during early pregnancy.



r = 0.93 ;y=0.065+(3.98)x

fig.6:Plasma progesterone and milk fat% during early pregnancy.



r = 9.4 ; y=6.49+(2.89)x

DISCUSSION

Monitoring of avrian cyclicity and diagnosis of early pregnancy are of crucial importance in the establishment and maintenance of optimal reproductive performance of the farm animals. The principal characteristics of an ideal diagnostic method are that it should be accurate, inexpensive, easily and quickly performed (Ghoniem et al., 1994). Since the availability of RIA, progesterone levels have become one of the major hormonal parameters used to monitor reproductive status in cattle (Plame et al., 1993) and buffalo (Kamonpatana, 1984). However, it is not advisable to relay on one method for monitoring ovarian cyclicity or for the detection of early pregnancy, as measuring plasma progesterone by itself can not tell whether the animal is pregnant or not, as it indicates only the presence of CL. Its accuracy in diagnosing pregnancy, however, ranged between 66-83.3% (Nanda et al., 1984 and Dionysius, 1991).

The composition of milk is greatly affected by many factors, one of those factors is the hormonal changes during the different reproductive phases . STudies that handle the relationship between progesterone profile and the chemical milk constituents are lacking almost in all farm animals.

In our study , a strong negative correlation was observed between plasma progesterone and milk lactose%. SMith, (1992) recorded that the highest concentration of milk lactose was noticed during estrus where progesterone was minimal and found that estrogens directly stimlated bovine mammary tissue cultured in vitro to synthesize> lactalbumin. Our finding could be aslo explained according to Bruce , (1985) who reported that injection of progesterone during pregnancy prevented the normal induction of lactose, > lactalbumin and casein synthesis. Moreover, he added that progesterone acted directly on the mammary tissue to decrease the ability of prolactin to induce secretion of > lactalbumin ; > lactalbumin acted as a protein modifier of an enzyme, a galactosyltransferase; in the presence of > lactalbumin, galactose might also be transferred to glucose to form lactose . The mammary gland was unique in its ability to synthesize > lactalbumin and this synthesis was presumably under hormonal control (Ebner and Schanbacher, 1974).

On the contray, the strong positive correlation observed in our study between plasma progesterone and milk chlorine% could be explained on the basis of the highly significant negative correlation observed between lactose and chlorine (Bruce, 1985).

Lactose and chlorine were the major constitents responsible for the osmotic pressure inside the milk producing units (Noble, 1988).

On the other hand, no association between plasma progesterone and milk fat% could be recorded . Yang et al., (1989) reported that pregnancy caused a non-significant decrement in milk fat content.

It could be concluded that measuring of milk lactose and/or chlorine levels could be of value in monitoring the ovarian cyclicity and early pregnancy diagnosis in buffalo and may provide an inexpensive , easily and quickly

diagnostic method for both cyclicity and early pregnancy in buffalo. However , further studies are still needed to determine the accuracy of this diagnostic method.

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