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BIOPHYSICAL CHANGES IN CERVICAL MUCUS OF BUFFALOES IN RESPONSE TO PGF2α TREATMENT AND PLANE OF NUTRITION

(With 7 Tables and 4 Figures)

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التغييرات البيوفزيائية لمخاط عنق الرحم في الجاموس نتيجة المعاملة بالبروستاجلاندين ف٢ ألفا وطريقة التغذية

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أجريت هذه التجربة لتوضيح تأثير حقن البروستاجلاندين ف٢ ألفا وتأثير التغذية على الخواص الفيزيائية والكيميائية لمخاط عنق الرحم في الجاموس وذلك لأهمية مخاط عنق الرحم في التأثير على معدل التلقيح الإيجابي وبالتالي على الخصوبة ولقد أوضحت النتائج أن تركيز هرمون البروجسترون في الدم في منتصف فترة تكوين الجسم الأصفر لدورة الشبق يقل في الجاموس الذي تتم تغذيته بأقل من احتياجاته الغذائية وأوضحت أيضا أن الجاموس الذي تتم تغذيته بالإحتياجات الكافية له زاد معدل اكتناز جسمه حوالي ٠,٢٥ بينما الجاموس الذي تم تغذيته بأقل من احتياجاته من التغذية قل معدل اكتناز جسمه بحوالي ٠,٧٥ كما أوضحت نتائج الخواص الفيزيائية لمخاط عنق الرحم في أثناء الشبق للحيوانات في مختلف المجموعات عدم وجود فرق معنوي في درجة اللزوجة ودرجة الحموضة ولكن خاصية التشجر الفطري للسائل كانت بنسبة ٩% غير مثالية في مخاط الحيوانات التي تم تغذيتها بشكل طبيعي وحقنت بروستاجلاندين ف٢ ألفا وبدراسة الخواص الكيميائية لمخاط عنق الرحم وجد أن تركيز البروتين الكلي في المخاط قد قل معنويا في المجموعة التي تم تغذيتها بأقل من احتياجاتها الغذائية بينما حقن البروستاجلاندين ف٢ ألفا أدى إلى زيادة تركيز البروتين والكربوهيدرات في المخاط ز. إنزيم الفوسفات القلوي انخفض معنويا في مخاط الحيوانات التي غذيت بأقل من احتياجاتها الغذائية عن الحيوانات في المجموعة الأخرى حقن بروستاجلاندين ف٢ ألفا في الحيوانات التي تتغذى بكل احتياجاتها يقلل معنويا إنتريم الفوسفات القلوي في المخاط كما أن تركيز الصوديوم في مخاط عنق الرحم يقل معنويا في الحيوانات التي تتغذى بأقل من احتياجاتها الغذائية.

SUMMARY

It is established that cervical mucus has important functions in fertility through its relation with conception; therefore, this study was conducted to determine the effects of prostaglandin $F_2\alpha$ injection and feeding two levels of nutrition on the physical and chemical properties of cervical mucus in buffalo cows. The results showed that serum progesterone concentration at mid luteal phase of estrous cycle was significantly lower ($P< 0.05$) in buffaloes fed low level of nutrition. Buffaloes fed on normal plane of nutrition gained 0.25 score unit while those fed on low plan of nutrition lost about 0.75 score unit ($P< 0.05$). Physical properties of mucus in animals during different estrus phase showed no significant difference in the consistency and pH of mucus while there was atypical fern pattern in mucus appeared in 9% of cases of normal feeding synchronized group. Studying the chemical properties of cervical mucus showed that total protein concentration was significantly lower ($P< 0.05$) in the group fed on low plane of nutrition while synchronization by $PGF_2\alpha$ injection led to an increase in total protein and total carbohydrates concentrations. Alkaline phosphatase activity was significantly decreased in the group fed low plane of nutrition than the animals fed normal nutrition. Also synchronization by $PGF_2\alpha$ injection significantly decreased alkaline phosphatase activity in the group fed normal plane of nutrition. The level of sodium in the cervical mucus was significantly decreased ($P< 0.05$) by feeding animals low plane of nutrition.

Key words: *Biophysical changes, Cervical mucus, Buffaloes, $PGF_{2\alpha}$*

INTRODUCTION

Reproductive efficiency is a major factor necessary for high output in buffalo reproduction. One of obstacles facing productive and reproductive improvement in buffalo is heat detection. Synchronization of estrus in buffalo was used to solve this problem (Jainudeen, 1976; Pandey, 1979, Ibrahim, 1987 and Hassan, 1989). According to hormonal analysis, the quality of the synchronized estrus is similar to spontaneous one (Hassan and Essawy, 2002), however, conception rate are lower (Kaim *et al.*, 1990 and Hayat, 1996). On the other hand, it is well established that, inadequate nutrition especially low available energy and protein in the diet of buffaloes can have deleterious effects in

reproduction. Such effects including conception rate to first service, services per conception as well as calving to conception interval (Hegazy, 1993, Hegazy *et al.*,1995 and 2002). The mechanisms by which nutrition affects reproduction have not been clear until now. The chemical and physical properties of cervical mucus have important functions in fertility through its relation with conception as they constitute the environment that receive, maintain survival and help in the transport of semen through the genital tract (Hamana *et al.*, 1971, Bugalia and Sharma, 1988 and Hafez, 1993). Cyclic changes in the physical properties and chemical composition of cervical mucus in buffaloes have been described (Prasad *et al.*, 1981). Where wide variations in carbohydrates, protein and non-protein nitrogenous contents have been observed in cervical mucus at different stages of estrous cycle. During estrus phase, carbohydrate metabolism of cervix furnishes energy for spermatozoa during migration into the uterus, also during estrus phase glycogen utilization by spermatozoa is stimulated by alkaline phosphatase (Smith *et al.*, 1970). Meanwhile, Gibbon and Sellwood (1973) found a relationship between the protein content in cervical mucus and fertility. Deviations in these parameters coincided with lowered fertility (Zaaijer and Van der Horst, 1983)

The present study aimed to clarify some physical and chemical changes of cervical mucus of buffaloes in both spontaneous and synchronized estrus and its relation to plane of nutrition.

MATERIALS and METHODS

1) Experimental design: The present study was conducted on ten multiparious, non-pregnant; non-lactating buffalo-cows aged 4-5 years. Animals were kept in Buffalo farm of Animal Reproductive Research Institute. Estrus was observed twice daily at 8 A.M. and 8 P.M. Cyclicity of all animals was followed for three successive cycles before experimental design was imposed Animals were divided according to parity and body condition score into two equal groups (n=5). Buffalo-cows in group 1 (control) were fed a balanced ration supporting the nutrient requirements of buffalo-cows for maintenance plus 10% for activity (according to Ranjhan and Pathak, 1979). The animals in group 2 (Low) were fed on only 75% of the requirements. Ingredient, chemical analysis as well as daily nutrient intakes in the two tested groups are presented in Table 1.

Table 1: Ingredient, chemical analysis as well as daily nutrient intakes of buffalo-cows fed on normal and low plane of nutrition.

Items	Normal	Low
Ingredients (kg/head day on as fed basis):		
Lin seed meal	0.50	0.37
Ground corn	1.25	0.95
Wheat bran	1.30	1.00
Trace mineral Mix.	0.025	0.019
Vitamin Mix.	0.09	0.07
Wheat straw	3.70	2.75
Green Berseem (13%DM)	29.50	22.25
*Chemical analysis *(on DM basis):*		
Crude protein (%)	12.00	
Total Digestible Energy (%)	60.00	
Crude Fiber (%)	27.00	
Ether Extract (%)	2.90	
Calcium (%)	0.70	
Phosphorus (%)	0.35	
Sodium (%)	0.23	
Daily nutrient intake:		
Dry matter (kg)	10.00	7.50
Total digestible energy (kg)	6.00	4.50
Crude protein (kg)	1.20	0.90
Ether extract (kg)	0.29	0.21
Calcium (g)	70	52.5
Phosphorus (g)	35	26.25
Sodium (g)	23	17.25

* Analyzed according to A.O.A.C. (1984).

One month after the nutritional regimen was imposed, spontaneous estrus was detected in animals of the two groups (at least two cycles) by observation and confirmed by examination of the genital tract of cows rectally and also by measuring progesterone (P4) concentration. After that, all buffaloes were synchronized by injection of 5 ml (25mg) of synthetic $PGF_2\alpha$ (Lutalyse, Dinoprost. Tromethamin, Upjohn S.a Puurs, Belgium) intramuscularly on the seventh to ninth day after ovulation. Synchronized estrus was also detected and confirmed as spontaneous one with the same previous steps.

2) Sampling: Blood samples were collected twice weekly from each animal for P_4 profile. Cervical mucus was aspirated from around the

cervical os using clean plastic pipette according to Adams (1981) Mucus collected from both spontaneous and synchronized estrus in both nutritional groups.

3) Hormonal analysis: Progesterone hormone was measured by direct radioimmunoasaays using coat A-count kit (Diagnostic products corporation, Los Angles.
4) Body condition score: Body condition score was carried out for all buffaloes according to Surinder *et al.* (1987) by the same person at the start of the nutritional treatment and then every month until the end of the experiment.
5) Physical and chemical studies: The consistency of the mucus was determined (Shokeir, 1958) and the pH was measured by digital pH meter. Mucus samples were spread on a clean, dry glass slide and allowed to dry at room temperature then examined microscopically three times per sample for fern arborization. The degree of fern arborization was classified into typical and atypical according to Rout and Kadu, 1989). After that the rest of the mucus samples were kept at -20C for chemical analysis.

   Before chemical analysis was imposed, mucus samples were homogenized by heating at 60 C for 20 minutes (Frank & Gaio, 1982). Carbohydrates were determined in mucus samples by phenol Sulfuric acid procedure according to Dubois *et al.* (1956). Meanwhile levels of total proteins were measured by Dye Binding assay according to Bradford (1976). Acid and alkaline phosphatase were measured in mucus by using commercial kits from Quimica Clinica Aplicada for acid phosphatase and from Audit Diagnostics for alkaline phosphatase. Calcium, potassium and sodium were determined by using Atomic Absorption Spectrophotometer, Perkin Elmer, Mod. 3300, USA according to Kapito *et al.*, 1973.
6) Statistical analysis: Data were statistically analyzed using COSTAT computer program, ver. 3.03: copyright 1986 Cottort software.

RESULTS

Progesterone levels throughout spontaneous and synchronized cycles in both normal and low levels of nutrition follow the curve represented in Fig (1). The progesterone concentration in day 0 (day of estrus) was the lowest level (0.06 ng/ml; basal level). While in the mid-cycle (day 10), it reach to its highest level (1.75 ng /ml). However, serum progesterone level at mid luteal phase (d.7-12 of estrus cycle,

0=estrus) of estrus cycle (Table 2) was higher in buffaloes fed on normal plane of nutrition than those fed on low plane of nutrition. Meanwhile, through rectal examination of animals in both groups during estrus (spontaneous or synchronized), the contractility of uterus reached its maximum degree. While during the mid-cycle, CL was palpated in both groups.

Table 2: Serum progesterone level in buffaloes at mid luteal phase (ng/ml) in both normal and low levels of nutrition during spontaneous or synchronized estrus.

Items	Normal plane of nutrition		Low plane of nutrition	
	Spontaneous	Synchronized	Spontaneous	Synchronized
Progesterone (ng/ml)	1.88±0.28[a]	1.85±0.44[a]	1.18±0.14[b]	1.35±0.29[b]

[a, b, c] Means with different superscripts within raw are significantly different at (P<0.05). Data are presented as mean ± SE.

Body condition score changes during the experimental period are presented in Table 3. Buffaloes fed on normal plane of nutrition gained about 0.25 score unit during the experimental period, while those fed on low plane loss about 0.75 score unit.

Table 3: Effect of plane of nutrition on body condition score (BCS) of buffaloes under experiment.

Items	Plane of nutrition	
	Normal	Low
Initial BCS	3.00±0.22	3.25±0.51
Final BCS	3.25±0.40	2.50±0.31*
Net increase in BCS	+0.25	-0.75

Significant at P<0.05.

Mucus consistency of animals in both nutritional groups during the spontaneous or synchronized estrus was thin initially and become thicker at the end of estrus phase. Data analysis revealed non-significant differences (P<0.05) between normal or low plane of nutrition in one hand or between spontaneous and synchronized on the other hand. Similarly, pH of mucus samples of both types of estrus ranged from 7.2-7.7 in normal group and ranged from 7.6-7.58 in Low group. The difference was not significant.

Ferning in cervical mucus is palm leaf like and composed of crystals of sodium and potassium chloride arranged around a small amount of organic materials in typical (long, medium and mixed) and atypical shape (Table 4). Typical fern pattern (Fig. 2) appeared in most

cases of both groups in spontaneous & synchronized estrus. Meanwhile, atypical pattern (Fig.3) appeared in 9% of cases of normal feeding and synchronized groups. Fern pattern of most cases of Normal group showed long degree of arborization and crystallization (contained a crystal-like structure at its terminal) (Fig.2) while, the majority of cases of low nutrition group showed medium and mixed degree of arborization (Fig.4).

Total protein level and total carbohydrates concentration in mucus samples (μg/ml) of both nutritional groups (Normal or Low) during both spontaneous or synchronized estrus was tabulated in Table 4. Total proteins levels were lower in group fed on low plane of nutrition than those fed on normal one. However, synchronization in both nutritional groups led to an increase in total protein level when compared with spontaneous estrus. The difference within normal cycle was significant ($P<0.05$).

Table 5, also revealed that, total carbohydrate concentrations in cervical mucus in both nutritional groups tend to be lower in spontaneous estrous than that in synchronized one.

Both acid and alkaline phosphatase activities (U/L) in mucus samples of both nutritional groups during spontaneous or synchronized estrus were tabulated in Table 6. The activity of acid phosphatase enzymes was higher in spontaneous estrus than that in synchronized estrus in both normal and low groups, respectively. However, the difference was not significant ($P<0.05$). Meanwhile, alkaline phosphatase activity was significantly decreased in synchronized estrus in normal group than that in spontaneous one. In low nutritional group, the activity of alkaline phosphatase increased in synchronized estrus than that in spontaneous estrus, although, this increase was not significant.

Levels of sodium, potassium and calcium (mg %) in mucus samples of both nutritional groups during spontaneous and synchronized estrus were shown in Table 6. Levels of sodium were significantly increased by feeding normal plane of nutrition in both spontaneous and synchronized estrus than those in low plane of nutrition. Table 7 also showed that neither nutritional level nor synchronization has a significant effect on potassium or calcium level on cervical mucus.

Table 4: Type of fern patterns in cervical mucus of buffaloes subjected to normal or low planes of nutrition during spontaneous or synchronized estrous.

Group	Atypical Fern pattern	Typical Medium Fern pattern	Typical Long Fern pattern	Both long and medium
Low level of nutrition				
Spontaneous	0^{a}	$33.33\%^{a}$	$22.22\%^{a}$	$44.44\%^{a}$
Synchronized	0^{a}	0^{b}	$9.09\%^{b}$	$90.9\%^{b}$
Normal level of nutrition				
Spontaneous	0^{a}	33.33^{a}	$58.33\%^{c}$	$8.33\%^{b}$
Synchronized	$9.09\%^{b}$	0^{b}	$45.45\%^{c}$	$45.45\%^{a}$

^{a,b,c} Percentages within column that have the same superscripts are non significantly different ($P<0.05$).

Table 5: Total protein and carbohydrates concentrations (ug /ml) in cervical mucus of buffaloes subjected to normal or low planes of nutrition during spontaneous or synchronized estrous.

Items	Normal plane of nutrition		Low plane of nutrition	
	Spontaneous	Synchronized	Spontaneous	Synchronized
Total protein (ug/ml)	127.0 ± 11.84^{a}	178.38 ± 30.3^{b}	94.07 ± 5.38^{c}	122.47 ± 8.19^{a}
Carbohydrates (ug/m)	179.38 ± 19.9^{b}	227.44 ± 23.0^{a}	182.91 ± 9.72^{b}	246.84 ± 34.93^{a}

$^{a, b, c}$ Means with different superscripts within raw are significantly different at ($P<0.05$). Data are presented as mean ± SE.

Table 6: Acid and alkaline phosphatase activity (u/L) in cervical mucus of buffaloes subjected to normal or low planes of nutrition during spontaneous or synchronized estrous.

Items	Normal plane of nutrition		Low plane of nutrition	
	Spontaneous	Synchronized	Spontaneous	Synchronized
Acid phosphatase (u/L)	12.08 ± 0.68	11.35 ± 0.67	12.88 ± 0.63	11.08 ± 0.79
Alkaline phosphatase (u/L)	458.8 ± 4.30^{a}	436.5 ± 5.7^{b}	443.6 ± 3.8^{b}	449.38 ± 3.7^{ab}

$^{a, b, c}$ Means with different superscripts within raw are significantly different at ($P<0.05$). Data are presented as mean ± SE.

Table 7: Sodium, Potassium and Calcium concentrations (mg%) in cervical mucus of buffaloes subjected to normal or low levels of nutrition during spontaneous or synchronized estrus.

Items	Normal plane of nutrition		Low plane of nutrition	
	Spontaneous	Synchronized	Spontaneous	Synchronized
Sodium (mg%)	311.31±6.62[b]	309.3±14.31[b]	285.46±8.93[a]	280.0±7.10[a]
Potassium (mg%)	16.06±2.64	14.5±4.68	17.02±1.22	16.41±1.36
Calcium (mg%)	13.38±1.00	15.01±1.56	16.22±2.67	11.75±0.72

[a, b, c] Means with different superscripts within raw are significantly different at ($P<0.05$). Data are presented as mean ± SE.

DISCUSSION

Through out the estrus phase the secretory cells of the cervix, columnar epithelial cells, undergo changes which governed largely by the hormones; estrogen and progesterone to induce this response (Adams and Tang, 1979 and Adams, 1981).

Progesterone level was a valuable aid in the assessment of ovarian function in both cattle and buffaloes (Kamonpatona, *et al.*, 1979 and Hegazy *et al.*, 1995). There are no differences in the pattern of progesterone hormone in both spontaneous and synchronized groups. These results are in agreement with that reported by Jainudeen (1976); Bachlaus *et al.* (1979) and Pandey (1979). However, the reported higher progesterone level at mid-luteal phase in buffaloes fed on normal plane of nutrition compared with those fed on low plane of nutrition was previously reported by Hegazy *et al.* (1995) in buffaloes. Also he found a relationship between milk progesterone level during the estrus cycle preceding insemination and occurrence of conception.

There was no difference in the consistency of mucus in both spontaneous and synchronized heat in both nutritional groups and this was in case similarly to that reported by (Rout and Kadu, 1989).

The pH of cervical mucus was considered to be a critical factor affecting spermatozoa motility and viability (Hartwig, 1959). The present work revealed that the pH of cervical mucus of both spontaneous and synchronized heat averaged 7.3. This is in agreement with that reported by El-Azab (1980), Nasser (1992) and Mohamed (1995) while it was lower than that reported by Rout and Kadu (1989) and Bhosrekar *et al.* (1995).

In the present work, typical fern pattern arborization was appear in majority cases at spontaneous and synchronized heat in both normal

and low plane of nutrition. Meanwhile a typical type was appeared in only 9% of cases of synchronized heat of normal group. This may be attributed to the disturbance in the level of estrogens and progesterone hormones (Nooran *et al.*, 1975) and/ or due to the disturbance in electrolyte levels (Ghannam and Sorensen, 1964).

The results showed significant increase in protein content of estrus cervical mucus in synchronized estrus in buffaloes of both nutritional groups. The same results were obtained by Tsiligianni *et al.* (2001). Higher protein content in the cervical mucus in buffaloes may be due to higher estrogen profile (Bugalia and Sharma, 1988). Meanwhile, identical treatments of $PGF_2\alpha$ in buffaloes enhance plasma estradiol-17 B levels (Bachlous *et al.*, 1979).

Synchronization of estrus in both planes of nutrition significantly increases the total carbohydrate contents of the cervical mucus. Recorded observation is in accordance with the finding of Fayez *et al.* (1992). Such increase may due to high estrogen profile during estrus as estrogen stimulates glycogen secretion in cervical mucus (Gregoire *et al.*, 1968). Moreover, Zaayer and Vander Horst (1984) stated that $PGF_2\alpha$ injection into cows at days 10, 12 or 14, 15 affected endometrium especially at day 12 as observed in the formation of sorbitol (when P_4 was present) and glucuronic acid (when estrogen present) when assayed in cervical mucus

The activity of acid phosphatase enzymes was reduced in synchronized estrus in both nutritional groups with non-significant differences between them. These findings were agreed with that reported by Bhosrekar *et al.* (1995). The reported higher pre treatment (spontaneous) activity of alkaline phosphatase is in agreement with that reported by Vanlinken berg (1953), Smith *et al.* (1970) and Prasad *et al.* (1981). Smith *et al.* (1970) reported that the activity of alkaline phosphatase in cervical mucus has been reported to be consistency low under the influence of both estrogen and progesterone administration. The high estradiol level during estrus synchronization may exert some inhibitory effect on the secretion of alkaline phosphatase. Moreover, feeding of buffaloes on normal plane of nutrition in the present experiment significantly increased alkaline phosphatase values. The influence of food ingestion on alkaline phosphatase values appears to dependent on the type of meal ingested. Walker *et al.* (1971) and Longman *et al.* (1969) reported that, ingestion of a high fat meal caused a 17 % increase in the alkaline phosphatase activity. The amount of fat (Ether Extract, table 1) intake in normal plane of nutrition was 27.59 %

higher than that in low plane of nutrition. Moreover, David *et al.* (1999) demonstrated that, restriction of nutrition induced a decrease in the growth rate and maximum diameters of successive dominant follicles and eventually results in suppressed estradiol concentration which may affect the cervical concentration of total protein, total carbohydrates as well as alkaline phosphatase activity.

Statistically our results showed significant decrease in the concentration of sodium in cervical mucus in both spontaneous and synchronized groups of buffaloes fed low plane of nutrition compared with those fed on normal plane of nutrition. This result was evidenced by the microscopic examination of cervical mucus smear and its fern pattern as the sodium was decisive for the process of ferning (Cohen, 1968). Meanwhile, Sharma and Tripath, (1989) reported a significant decrease in the sodium concentration of the cervical mucus in repeat breeder buffaloes. Such effect may be related to the physiological imbalance of estrogen and progesterone that are essential to maintain optimal cervical constituent (Bugalia and Sharma, 1988).

From this study, it was suggested that, buffaloes fed low plane of nutrition attained low BCS and low progesterone level during luteal that led to negative changes in cervical mucus that may explain the lower pregnancy rate reported in many researches associated with under nutrition. Meanwhile, synchronization of estrus using $PGF_2\alpha$ revealed different changes in the constituent of cervical mucus between buffaloes fed normal or low plane of nutrition which may explain the variation in its effect on pregnancy rate reported in many researches.

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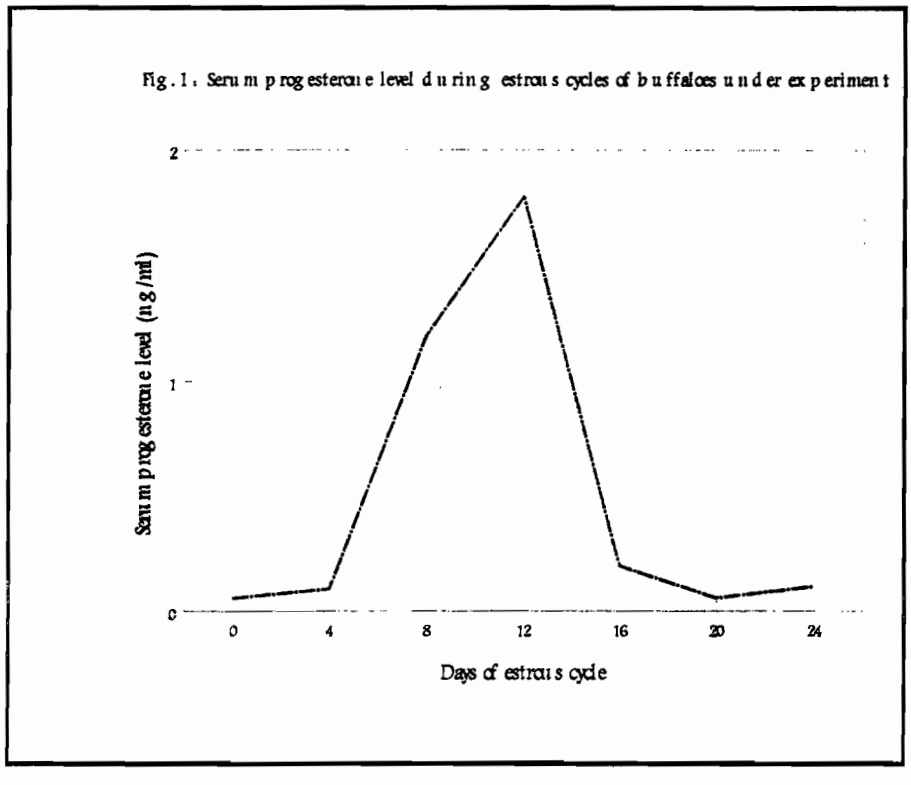

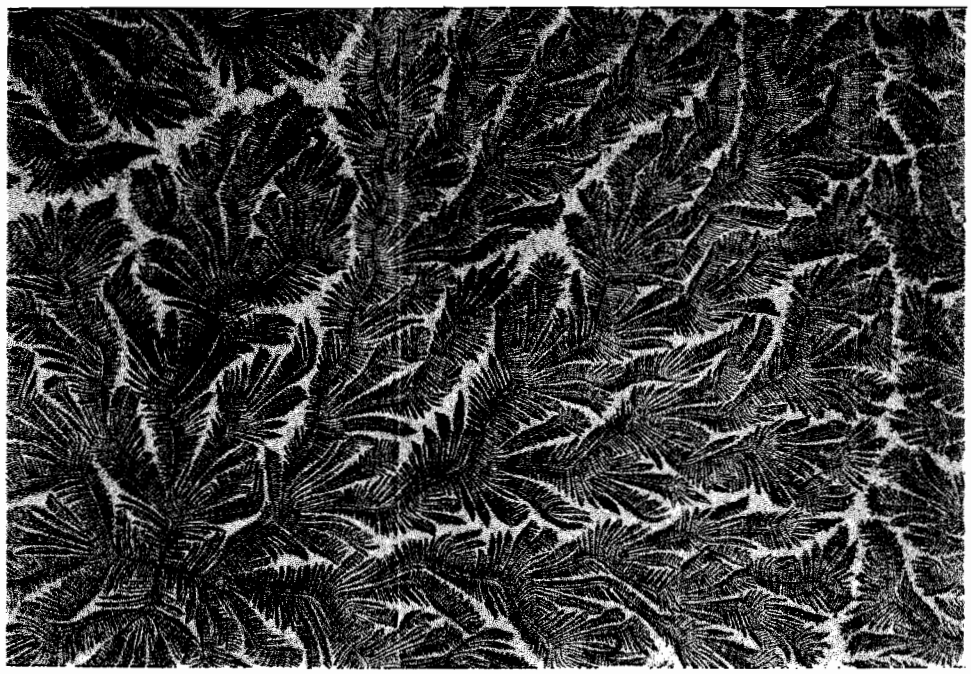

Fig. 2: Cervical mucus showing typical fern pattern during estrous cycle. Note the highly arborization of the fern (40x)

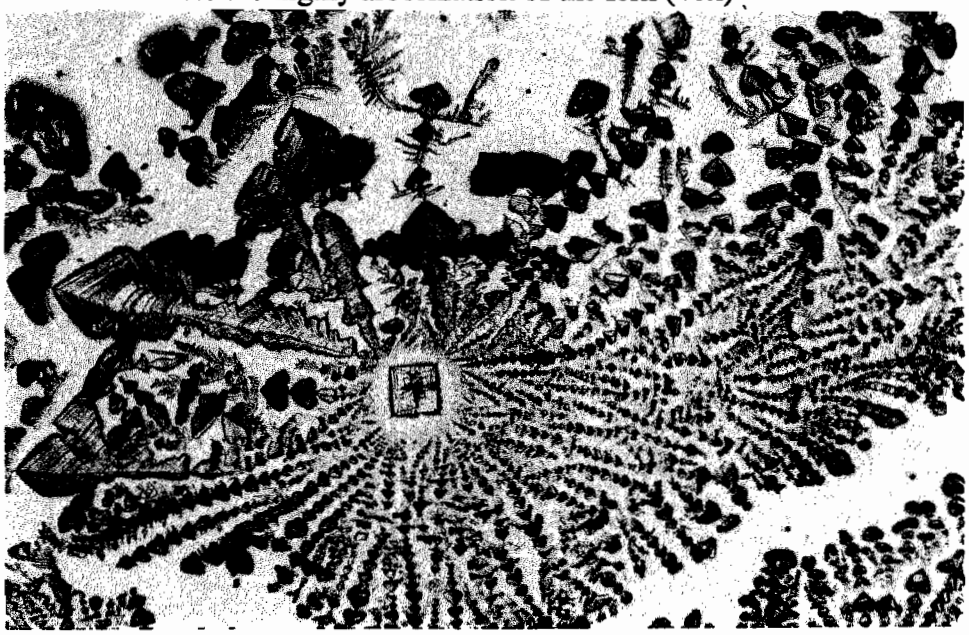

Fig. 3: Cervical mucus showing atypical fern pattern during estrus. Note the terminal crystal like structure (40x).

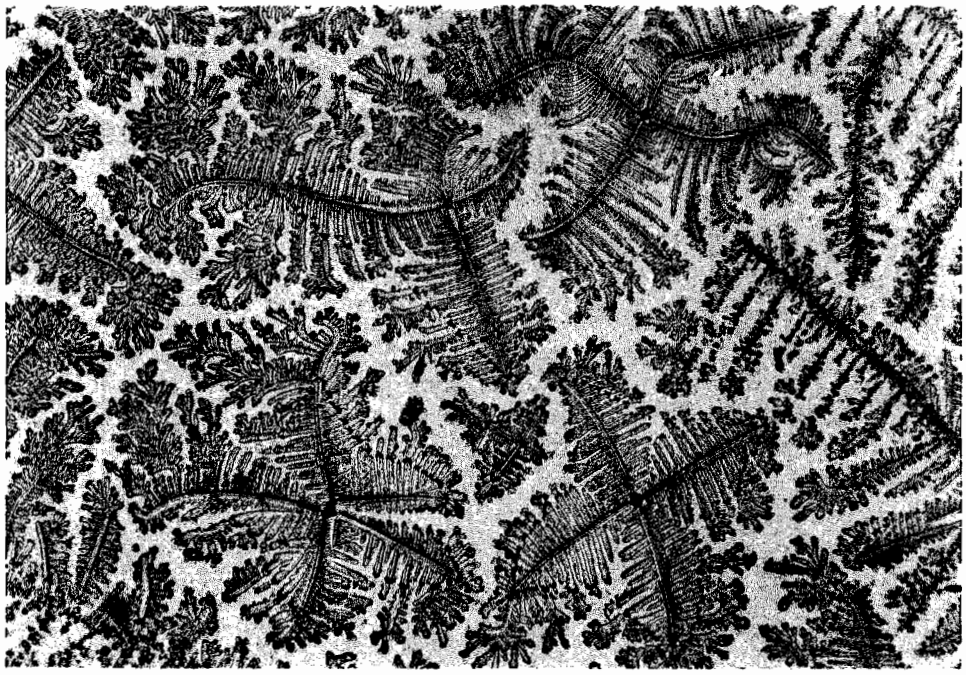

Fig. 4: Cervical mucus showing a medium-size fern pattern during estrus (40x)