

PHYSICAL AND CHEMICAL PROPERTIES OF EWES CERVICAL MUCUS DURING NORMAL ESTRUS AND ESTRUS INDUCED BY INTRAVAGINAL SPONGES

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SUMMARY

This study was planned to determine the physical and chemical properties of cervical mucus collected during normal and induced estrus of Ossimi ewes. Estrus of 14 Ossimi ewes were induced by intravaginal sponges for 14 days. Estrus was observed one to two times in ewes after estrus induction with a range of 17-21 days. Cervical mucus samples were collected from all ewes (14 each for induced and normal estrus) at the first and three days post estrus.

Results revealed that the physical properties of cervical mucus: volume, viscosity, density and elasticity of cervical mucus in the normal estrus group were higher ($P < 0.05$) than those in the induced one, while, the pH value of cervical mucus for normal estrus was similar to that for induced estrus. There were significant differences in chemical properties, glucose, fructose, total protein and cholesterol concentrations between ewes cervical mucus at different days of estrus during normal estrus. In contrast there were no significant differences in chemical properties of cervical mucus at different days of estrus except glucose concentration during induced estrus. Overall mean of glucose and total protein concentrations were higher in normal than in induced estrus, while no significant differences were found between ewes in normal and induced estrus for fructose and cholesterol concentrations. In conclusion, the physical properties (viscosity, density, elasticity and volume) and biochemical properties (total protein and glucose) of cervical mucus were higher ($P < 0.05$) in the normal estrus group than in the induced-estrus group. Such changes in the mucus may be used as an indicator of days of estrus and the time of mating.

Keywords: Cervical mucus, physical properties, chemical properties, ewes, estrus, normal, induced

INTRODUCTION

Through natural service, semen in sheep is deposited in the vagina. Thus, the physical and chemical properties of the cervical mucus determine the capability of the spermatozoa to reach the site of fertilization (Linford, 1974, Tsiligianni *et al.*, 2001a,b and Predojevic *et al.*, 2007). Changes in the physical and chemical properties of cervical mucus are response to the hormonal state of the female. Such changes can be used as an indicator of ovulation in various species (Predojevic *et al.*, 2007).

The most important physical characteristics of cervical mucus are pH, viscosity, elasticity and crystallization (Tsiligianni *et al.*, 2001a).

Changes in the physical properties of cervical mucus are usually corresponded to the different reproductive stages (Nasir-ud-Din *et al.* 1979). At the time of ovulation, cervical mucus becomes profuse, thin, elastic and viscid, while in the post-ovulatory phase, the mucus is turned into thick and cheesy material (Hafez, 1987, Nasir-ud-Din *et al.*, 2003 and Mahmoud, 2009)

Cervical mucus is oligosaccharide side chains linkage polypeptide chain (Elstein *et al.*, 1971). Tsiligianni *et al.* (2001a) showed that the physical properties of cervical mucus

during three consecutive normal estruses after calving did not change.

Low molecular weight such as electrolytes, carbohydrates, amino acids, lipids and soluble macromolecular compounds such as soluble and locally produced proteins and polysaccharides are dissolved in cervical mucus (Schumacher, 1970). Proteins in cervical mucus derive partly from the blood serum and are partly produced somewhere in the genital tract (Schumacher, 1970; Elstein, 1974 and Yildiz and Aydin, 2005).

A number of carbohydrates have been identified in cervical mucus, e.g. glucose and fructose. These compounds appear to be the major free sugars in uterine flushing from the non-pregnant rat, rabbit, cow, ewe and pig (Suga and Masaki, 1973, Zavy *et al.*, 1982 and Tsiligianni *et al.*, 2001b).

Lipids may play a nutritive role for spermatozoa, and there is speculation that they may also protect spermatozoa from temperature changes (Marcus, 1969). Cholesterol, which is the precursor of the steroid hormones (Trakatellis, 1986) is related to the stages of the estrous cycle (Zaaijer *et al.*, 1993 and Yildiz and Aydin, 2005).

The physical and chemical properties of cervical mucus vary with state of ovarian function (Predojevic *et al.*, 2007).

The phenomenon described as arborization, is one of remarkable properties of cervical mucus in estrus (Mahmoud, 2009). In fact, the presence of arborization in the cervical mucus depends on the action of estrogen (Elstein, 1974). Fawzi (1976) indicated that the fern-like patterns had been observed in the cervical mucus of cow and buffalo during the follicular phase, but not in the luteal phase.

The purpose of the present work was to study compare the physical and chemical properties of cervical mucus in normal estrus or an induced estrus of ewes.

MATERIALS AND METHODS

Experimental animal and management:

The present study was carried out in the Experimental farm of Animal Production Department, Faculty of Agriculture, Assiut University. This study was conducted during February and March 2011. A total number of 14 Ossimi ewes of about 3-4 years of age and average 46.5 ± 1.5 kg body weight were used. The ewes were fed on the experimental diet which formulated according to NRC (1985) for sheep. Water was made available all day time.

The ewes were synchronized using intra-vaginal sponges (Chronogest, Laboratorios Intervet SA, Madrid, Spain) impregnated with 20 mg of fluorogestone acetate. After 14 days, the sponges were removed (Martínez-Rodríguez *et al.*, 2012). Estrus was observed one to two times in ewes after estrus induction with a range of 17-21 days since 14 estrous periods were detected.

Estrous detection:

Estrus was detected by exposing a fertile ram to ewes two times (8.0 a.m. and 4.0 p.m.) a day for half an hour in each time. The onset of estrus was considered as the time when the female stood to be mounted with the observation of arborization (fern like pattern) in the smear under the microscope. Signs of estrus behaviour (vaginal discharge; vulva swelling and tail twitching) have been considered as estrous signs to recognize ewes in estrus (Cerbito *et al.*, 1995).

Cervical mucus collection:

Mucus was withdrawn (at the first and three days post estrus) by entering a plastic pipette into the vagina (Mahmoud, 2009 and Martínez-Rodríguez *et al.*, 2012) for observing arborization (fern-like pattern) smear and to determine the physical and chemical properties of mucus.

Physical properties of cervical mucus:

After mucus collection, samples were examined immediately for volume, pH, density, viscosity, and elasticity. Mucus volume (ml) was measured by a graded tube to the nearest 0.1 ml at the first hour of the onset of estrus (The onset of estrus was considered as the time when the female stood to be mounted with the observation of full arborization (fern like pattern) in the smear under the microscope (Mahmoud, 2009).

Initial pH of cervical mucus was measured using nitrating pH paper. Mucus density, viscosity and elasticity were evaluated using a scale of 1-3; where, 1= low, 2= medium and 3= high. Small drop of mucus was placed onto the microscopic slide and the thickness or density of mass was recorded. Thumb and forefinger were used in determination of mucus viscosity. Mucus elasticity was evaluated by the tube collection and glass slide (Mahmoud, 2009).

Fore observing arborization, a small drop of cervical mucus was placed on a microscopic glass slide to make smear. The smear was done by touching another slide to push the drop from back to forward and leaving it to dry in air. Dried smear was examined under the microscope for observing arborization (Mahmoud, 2009). Mucus specimens were stored at -20°C until the chemical analysis of total protein, glucose, fructose and cholesterol.

Chemical properties of cervical mucus:

Total protein, glucose and cholesterol analysis were carried out by spectrophotometer using commercial test (Spinreact, Spain). Fructose concentration was measured by the method of Tsiligianni *et al.* (2001b).

Statistical Analysis:

Data were statistically analyzed using General linear model (GLM) and Chi-Square test procedure of SAS. (1996). Differences between means were tested using Duncan's multiple range test (Duncan, 1955). The following model was used for analysis of variance: $Y_{ij} = \mu + A_i + e_{ij}$.

Where: Y_{ij} = observation, μ = overall mean, A_i = estrus type effect (normal and induced estrus), e_{ij} = experimental errors. Physical properties were statistically analyzed by Chi-square analysis.

RESULTS AND DISCUSSION

Low viscosity, low density and low elasticity were significantly higher in the cervical mucus of ewes in the induced estrus (IE) group compared with ewes in the normal estrus (NE) group (Table 1).

Table 1. Physical properties of cervical mucus in the normal and in the induced estrus ewes

| Items | Estrus type | Low % (1) | Medium % (2) | High % (3) |
|--------------|-------------|--------------|-----------------|---------------|
| Viscosity** | Induced | 68.42 | 35.00 | 15.38 |
| | Normal | 31.58 | 65.00 | 84.62 |
| Density* | Induced | 72.22 | 54.55 | 20.00 |
| | Normal | 27.78 | 45.45 | 80.00 |
| Elasticity** | Induced | 68.42 | 35.00 | 15.38 |
| | Normal | 31.58 | 65.00 | 84.62 |

*P < 0.05 ** P < 0.001

Mucus viscosity, density and elasticity were evaluated using a scale of 1-3; where, 1= low, 2= medium and 3= high

These results are in agreement with those obtained by Boyd *et al.* (1972) observed that most watery cervical mucus (low viscosity and low elasticity) on the day of estrus in cows that received megestrol acetate (MGA). Moghissi and Marks (1971), who showed that the sheep treatment with exogenous progestagen caused a reduction in the secretion of cervical mucus and a loss of elasticity. Moreover, Rexroad and Barb (1977) found lower elasticity in cervical mucus of ewes after induction of estrus with progestogens (MAP, FGA) compared to ewes with normal estrus.

This is also supported by the findings of Tsiligianni *et al.* (2001a and 2002) reporting that viscosity was significantly lower in the cervical mucus of cows in the spontaneous-oestrus group compared with cows in the induced-oestrus groups. On the other hand, cervical mucus in the PGF2a-induced estrus group was significantly more viscous than that in the normal estrus but less viscous than that in the P4-induced estrus group (Lopez-Gatius *et al.*, 1993).

Cervical mucus volume was higher (P<0.05) of ewes of NE compared with ewes

of IE. Also, there was no difference between cervical mucus pH of ewes of IE and NE (Table 2).

These results are in agreement with Moghissi and Marks (1971) and Smith and Allison (1971), who showed that the sheep treatment with exogenous progestagen caused significant reduction in the secretion of cervical mucus at estrus than untreated sheep.

pH values for mucous of ewes of NE were similar to those of IE, which agrees with the finding of Tsiligianni *et al.* (2001a).

Glucose concentration was significantly lower in the cervical mucus of ewes of NE and IE at the first and second days of estrus (Table 3). There was no a significant difference between glucose concentrations in the cervical mucus of ewes in NE and IE at the first day of estrus, while there was a significant difference at the second and third days of estrus. Additionally the overall mean of glucose concentration was higher (P<0.05) in the cervical mucus of ewes in NE compared with in IE (Table 4).

Table 2. Values (LSM± SE) of volume and pH of cervical mucus as affected by normal and induced estrus of Ossimi ewes

| Items | Induced estrus | Normal estrus |
|------------------|----------------|---------------|
| Volume (ml)* | 1.11 ± 0.31 | 2.29 ± 0.24 |
| PH ^{ns} | 6.74 ± 0.09 | 6.80 ± 0.12 |

*Difference is significant at P < 0.05 , ns (non significance)

Table 3. Chemical properties of cervical mucus (LSM± SE) in the normal and in the induced estrus during days of estrus in ewes

| Items | Estrus type | Days of estrus | | | |
|---------------|-------------|------------------------------|-----------------------------|------------------------------|-----------------------------|
| | | 1 | 2 | 3 | 4 |
| Glucose | Induced | 15.10 ± 4.20 ^{bA} | 16.09 ± 2.44 ^{bB} | 17.20 ± 3.56 ^{abB} | 33.69 ± 5.45 ^{aA} |
| | Normal | 19.95 ± 3.32 ^{bA} | 27.98 ± 3.68 ^{abA} | 56.29 ± 11.88 ^{aA} | 48.58 ± 3.76 ^{abA} |
| Fructose | Induced | 35.73 ± 8.48 ^{aA} | 51.46 ± 16.99 ^{aA} | 55.73 ± 16.13 ^{aA} | 43.82 ± 0.00 ^{aA} |
| | Normal | 11.91 ± 8.90 ^{bA} | 28.09 ± 8.64 ^{abA} | 110.34 ± 1.24 ^{aA} | 81.91 ± 7.53 ^{abA} |
| Total protein | Induced | 1.18 ± 0.41 ^{aA} | 1.30 ± 0.31 ^{aA} | 1.32 ± 0.23 ^{aB} | 1.81 ± 0.51 ^{aA} |
| | Normal | 1.94 ± 1.02 ^{abA} | 0.91 ± 0.17 ^{bA} | 2.27 ± 0.35 ^{abA} | 4.90 ± 1.57 ^{aA} |
| Cholesterol | Induced | 37.30 ± 33.54 ^{aA} | 19.49 ± 7.07 ^{aA} | 77.65 ± 17.92 ^{aA} | 79.98 ± 53.74 ^{aA} |
| | Normal | 55.11 ± 10.75 ^{abA} | 33.05 ± 10.08 ^{bA} | 123.11 ± 2.85 ^{abA} | 143.64 ± 7.21 ^{aA} |

^{a and b} Means within the same row with different superscripts are significantly different (P<0.05).

^{A and B} Means within the same column with different superscripts are significantly different (P<0.05).

Table 4. Overall means (LSM± SE) of chemical properties of cervical mucus in the normal and in the induced estrus during days of estrus in ewes

| Estrous type | Glucose mg/dL | Fructose mg/dL | Total protein g/dL | Cholesterol mg/dL |
|--------------|---------------------------|----------------------------|--------------------------|----------------------------|
| Induced | 18.65 ± 2.03 ^b | 46.95 ± 6.34 ^a | 1.32 ± 0.17 ^b | 53.37 ± 13.34 ^a |
| Normal | 36.58 ± 4.64 ^a | 55.89 ± 16.42 ^a | 2.37 ± 0.49 ^a | 82.40 ± 13.01 ^a |

^a and ^b Means in the same column with different superscripts are significantly different (P<0.05).

These results are in agreement with those obtained by Zaaijer and Van der Horst (1983), who found that in the cervical mucus of cows the glucose content was very low at day 0 of estrus. Also, Tsiligianni *et al.* (2001b) indicated that the glucose concentration of cervical mucus in cows with normal estrus was higher compared with induced estrus, but not significant at the first day of estrus. Glucose concentrations recorded in this study were significantly less in a high percentage of ewes with IE.

Fructose concentration was significantly lower in the cervical mucus of ewes of NE at the first day of estrus (Table 3). But, no difference was observed in fructose concentration between days of estrus of IE ewes. There were no significant differences between days of estrus and overall mean of fructose concentrations in the cervical mucus of ewes in NE and IE (Table 3 and 4). Fructose concentration was higher at the first and the second days of estrus in the cervical mucus of ewes in IE, while was higher at the third and the fourth days of estrus in NE.

These results are in agreement with those obtained by Zaaijer and Van der Horst (1983), who found that in the cervical mucus of cows the fructose content was very low at day 0 of normal estrus. Also, Tsiligianni *et al.* (2001b) found that fructose concentrations of cervical mucus do not differ significantly in cows with normal and induced estrus. On the contrary, El-Naggar and Hornath (1971) found higher fructose concentration in the cervical mucus of cows with normal than that obtained in the induced estrus. Furthermore, fructose concentrations significantly decreased in cows with induced estrus on the first day of estrus, but the overall mean of fructose concentrations were similar in the normal and in the induced estrus groups (Tsiligianni *et al.*, 2001b)

Total protein concentration was significantly lower in the cervical mucus of ewes in NE at the first and the second days of estrus. But, no difference of total protein concentration was detected between days of estrus in IE ewes. There was no significant differences between total protein concentration in the cervical mucus of ewes in NE and in IE at the first and the second days of estrus, while, there was significant difference at the third day of estrus. Additionally the overall mean of total

protein concentration was higher (P<0.05) in the cervical mucus of ewes in NE compared with IE (Table 3 and 4).

These results are in agreement with those obtained by Prasad *et al.* (1981) and Tsiligianni *et al.* (2001b), who observed that the biochemical properties that mostly differ is the total protein concentration in the cervical mucus. The minimal levels of protein in cervical mucus at the first day of estrus have already been established (Predojevic *et al.*, 2007). They also observed increased proteins in cervical mucus collected from cows in the luteal phase of estrus. On the other hand, total protein concentration in the PGF2 α -induced estrus group was similar to that in normal estrus for cows at the first day of estrus. Furthermore, High concentrations of protein in mucus were negatively associated with elasticity of mucus (Rush and Elstein, 1974). These results are in agreement with Nasir-ud-Din *et al.* (2003) and Mahmoud (2009), who found that at the time of ovulation, cervical mucus becomes profuse, thin, elastic and viscid, while in the post-ovulatory phase, the mucus is turned into thick and cheesy material. Finally, total protein was lower in cervical mucus of ewes that had received (MAP) intravaginal sponges than it was in the control group (Rexroad and Barb, 1977). There was a significant decline (P<0.01) in the concentration of protein in the cervical mucus following induction of estrus (Linford, 1974 and Prasad *et al.*, 1981). On contrary, total protein concentration was significantly lower in the normal estrus group compared to those in the induced estrus groups (Tsiligianni *et al.*, 2001b).

Cholesterol concentration was significantly lower in the cervical mucus of ewes in NE at the first and the second days of estrus, but, no differences between days of estrus in IE ewes. There were no significant differences between days of estrus and overall mean of cholesterol concentrations in the cervical mucus of ewes in the NE and IE (Table 3 and 4). But it is usually higher in ewes in NE.

These results are in agreement with those obtained by Zaaijer and Van der Horst (1983) found that in the cervical mucus of cows the cholesterol content was very low at day 0 of estrus. Also, Zaaijer *et al.* (1993) and Yildiz and Aydin (2005) found a similar cholesterol

concentration in cows with NE and IE. Additionally, 60 % of cows with P4+PGF2 α induced estrus had cholesterol concentrations similar to those of cows with normal estrus (Tsiligianni *et al.*, 2001b). On the contrary, cholesterol concentration was significantly lower in the normal estrus group compared to those in the induced estrus group (Tsiligianni *et al.*, 2001b).

CONCLUSION

In conclusion, the results from the present study showed that the physical and chemical of cervical mucus were significantly higher in the normal estrus group than in the induced-estrus group.

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الصفات الفيزيائية والكيميائية لمخاط عنق الرحم أثناء أطوار الشياح الطبيعية والمستحثة بواسطة الإسفنجيات المهبلية

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أجريت هذه الدراسة على أربعة عشر من إناث الأغنام الأوسيمي (28 دورة شياح) بغرض دراسة الصفات الفيزيائية والكيميائية لمخاط عنق الرحم لدورة الشياح المستحثة بواسطة الإسفنجيات المهبلية ودورة الشياح الطبيعية. وتم تجميع إفرازات عنق الرحم في اليوم الأول من تقبل الأنثى للذكر وأستمر تجميع العينات لمدة ثلاث أيام بعد أول يوم من حدوث الشياح. أظهرت النتائج المتحصل عليها أن الصفات الفيزيائية لمخاط عنق الرحم مثل الحجم، اللزوجة، الكثافة والمطاطية زادت زيادة معنوية ($P < 0.05$) في مخاط النعاج ذات الدورة الطبيعية مقارنة بمخاط الدورة المستحثة. ووجد أن درجة الحموضة متشابهة في كل من مخاط الدورة الطبيعية والمستحثة.

ووجد أيضا أن الصفات الكيميائية لمخاط عنق الرحم مثل الجلوكوز، فركتوز، البروتين الكلى والكوليسترول أظهرت اختلافا معنويا بين أيام دورة الشياح في مخاط النعاج ذات الدورة الطبيعية. وعلى عكس ذلك لم يوجد اختلاف معنوي بين الصفات الكيميائية لمخاط عنق الرحم بين أيام دورة الشياح في النعاج ذات الدورة المستحثة ما عدا الجلوكوز. كما وجد أن متوسط تركيز الجلوكوز والبروتين الكلى زاد زيادة معنوية في مخاط دورة الشياح الطبيعية مقارنة بمخاط دورة الشياح المستحثة، ولم يوجد اختلاف معنوي في متوسط تركيز الفركتوز والكوليسترول في مخاط دورات الشياح الطبيعية والمستحثة، ومثل هذه التغيرات في الصفات الفيزيائية والكيميائية لمخاط عنق الرحم يمكن استخدامها دلالة لأيام الشياح ووقت التلقيح في النعاج.