PHYSICAL TRAITS OF VAGINAL MUCUS DISCHARGE AND THEIR RELATIONS TO CONCEPTION RATE OF EGYPTAIN BALADI COWS

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SUMMARY

The current research aimed to study the conception rate of Egyptian native cows as influenced by vaginal mucus discharge (VMD). Forty cows were used in the experiment and were followed up after two-week post-partum. Vaginal mucus discharges were divided into three categories according to transparency (transparent, cloudy and dirty), viscosity (thin and thick) or pH (7-7.5, 7.5-8 and >8). The results indicated that the percentage of cows that conceived which have transparent vaginal mucus discharge (VMD) during estrus were significantly (P <0.05) higher (73.3%) compared with cows which have cloudy and dirty vaginal mucus (40 and 20%), respectively. The percentage of cows that conceived which have thin vaginal mucus during estrus was significantly (P <0.05) higher (87.5%) compared to those cows which have thick vaginal mucus (25%). The conception rate was significantly (P <0.05) higher (78.6%) in cows which have vaginal mucus pH level at estrus >8 compared with those cows which had vaginal mucus pH value ranging from 7 to 8. No significant difference in plasma progesterone concentrations during estrus in cows which have different vaginal mucus pH in pregnant and non-pregnant baladi cows. However, concentrations of estradiol-17βduring estrus in pregnant cows were significantly (P <0.05) higher than non-pregnant cows. Concentration of estradiol-17β at estrus was significantly (P <0.05) higher in cows which have strong estrus intensity expression compared with cows having weak estrus intensity expression. ***In conclusion,*** *the results clarified that the cows, which had vaginal mucus discharge (VMD): transparent, thin, and pH value at estrus >8 recorded higher conception rate. Estradiol-17β concentration was significantly (P <0.05) higher during estrus period in cows, which displayed high estrus intensity.*

Keywords: Physical traits, vaginal mucus discharge, Egyptian Baladi cows

INTRODUCTION

Vaginal mucus discharge is considered a good indicator of reproductive health and potential fertility status of livestock (Tsiligianni *et al*., 2001 and Jeong *et al*., 2010). Kumar *et al*. (2017) stated that the color of vaginal mucus discharge was associated with several reproductive problems in cattle and buffalo. Physical properties of vaginal mucus discharge have direct bearing on the fertility status of the animals (Panchal *et al*., 1994 and Rangnekar *et al*., 2002). Many researchers investigated the relationships between properties of cervical mucus during estrus phase and conception rate in cattle (Madkar *et al*., 2015, Mellado *et al*., 2015 and Bernardi *et al*., 2016). Cervical mucus properties as an indicator of the female hormonal condition also play a vital role in conception rate (Benbia *et al*., 2011). Several authors suggested a high association between color of vaginal mucus and conception rate in cows (Lim *et al*., 2014, Bhat *et al*., 2015 and Parikh *et al*., 2018). Recently Hay *et al*. (2019) showed that abnormal cervical mucus discharge was associated with significantly decreased pregnancy rate in cows. In addition, Gautam *et al*. (2010) found that cows, which had cloudy vaginal mucus at mating, had lower conception rate compared with other cows. Bhat *et al*. (2015), Bernardi *et al*. (2016) and Siregar *et al*. (2019) reported that there was a relationship between viscosity of vaginal mucus discharge and conception rate in cows. Ningwal *et al*. (2018), Kumar *et al*. (2019) and Hanumant *et al*. (2019) stated that pH of vaginal mucus discharge affected the conception rate in cows. Atkins *et al*. (2010), Perry *et al*. (2014) and Mogheiseh *et al*. (2019) found a positive relationship between estradiol-17β concentrations and intensity of estrus sings expression in cows. The current investigation was carried out to determine the effect of vaginal mucus discharge characteristics during estrus phase on conception rate of Baladi cows.

MATERIALS AND METHODS

Climatic conditions and farm location:

The study was carried out in animal farm located in Kom Ombou area (32º, 31' 23" East and 22º, 28' 09" North), Aswan Governorate. The animals were kept in traditional farm condition under semi shade system. Aswan Governorate climate is famous for higher ambient temperature and lower relative humidity especially during the hot season. Table (1) illustrates the average of the ambient temperature, relative humidity and temperature humidity index

during the experimental period. The temperature humidity index (THI) was estimated according to the formula proposed by Mader *et al*. (2006):

Temperature-humidity index (THI) = 0.8 x ambient temperature + [(% relative humidity) 100) x (ambient temperature -14.4)] + 46.4

Table 1. The ambient temperature (^{o}C), relative humidity (RH %) and temperature humidity index (THI) during the experimental period

Months of calving	Average Ambient Temperature (^{o} C)		Average Relative humidity (RH %)	THI
	Min	Max		
November	20.4	31.3	18	74.5
December	16.1	28.3	21	70.5
January	13.2	21.2	22	64.9
February	15.9	24.2	23	68.1
March	18.3	32.2	20	75.8
April	20.1	34.0	17	76.9

Management and feeding of herd:

Forty Baladi cows were used in the current work. Cow's parity ranged between 2nd and 6th. The averages of age 5.98±2.31, years and body weight were 371.72±13.91, kg, respectively, at calving. Animals were fed on Barseem Higaze (*Alfa- Alfa)* alongside the concentrate ration and hay wheat was offered during the experimental period.

Forty Baladi cows were divided according to transparency (transparent, cloudy and dirty), viscosity (thin and thick) or pH (7-7.5, 7.5-8 and >8) of vaginal mucus. The experimental animals calved during the interval from November to April and were followed up for two-week post-partum. Table (2) clarifies the classification of zones based on THI values in cattle with THI model according to Samal (2013).

Experiment design:

Table 2.Classification of zones based on THI values in cattle with THI model according to (Samal, 2013)

THI	Stress level	Response of cattle
<72	Non	Non-noticeable
72-79	Mild	Dairy cows will adjust by seeking shade, increasing respiration rate and dilation of the blood vessels. The effect on milk production will be minimal.

Heat detection and diagnosis of pregnancy:

Daily visual observation for cows were performed for heat detection in the morning at 6:0 am. and 6:0 pm, for sexual behavior signs display. The cows were naturally mated when exhibited standing symptoms of heat. Rectal palpation was used (60 days post-mating), to diagnose the established pregnancy as described by Arthur (1964).

Estrus intensity:

According to display, the estrus sings during the estrus phase, the cows were classified into two categories: strong estrus intensity expression, these cows, which appeared ≥3 estrus signs at least during the estrus and weak estrus intensity expression, these cows, which appeared one estrus sign only during the estrus phase.

Conception rate: calculated as the percentage of cows, which conceived from the first mating post-partum

Conception rate = (Number of pregnant cows/Total number of mated cows) x 100

Collection of vaginal mucus:

Samples of vaginal mucus were collected from all cows immediately before mating. Vulva of the cow was washed by water and iodine solution 10% concentration before the collection. Plastic syringe (10ml) was used to withdraw the mucus from the vagina. The mucus was placed in transparent glass tubes.

Measurement of vaginal mucus traits:

Immediately, after the collection of vaginal mucus, they were examined according to Lim *et al*. (2014) classification as follows: vaginal mucus color (transparent, cloudy and dirty), and consistency (thin and thick). The pH was estimated using pH Merck paper by 0-14 indicators. The pH paper was dipped into the vaginal mucus. The alteration in the color of the paper is compared to the attached standard value.

Blood samples collection and hormones assay:

Blood samples (10 ml) were collected from cows at estrus, after mating in heparinized tubes from the jugular vein. The blood samples were centrifuged at 3000 rpm for 20 minutes for plasma harvesting. Plasma was separated and stored at -18ºC until the time of analysis. Progesterone (P4) and estradiol-17β (E_2) hormone were determined using radioimmunoassay kit (Immunotech, France). Progesterone and estradiol-17β sensitivity values were reported to be 0.03 ng/ml and 4.0 pg/ml, respectively according to manufacturer information. Intra assay variation coefficient was 7.4% and 12.7%

for progesterone (P4) and estradiol-17β (E_2), respectively.

Statistical analysis:

The statistical design included one factor (effect of color (transparent, cloudy and dirty), consistency (thin and thick) or pH (7-7.5, 7.5-8 and >8) of vaginal mucus on conception rate). Chi Squire was used to test the significance of the percentage values. Statistical analysis was carried using software (SAS, 2002). The following model was used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = the observation trait

μ = overall mean

T_i = the fixed effect of color (transparent =1, turbid =2 and dirty =3) or consistency (thin=1 and thick=2) or pH (7-7.5=1, 7.5-8=2 and >8=3)

e_{ij} = experimental error

Duncan's Multiple Range test (Duncan, 1955) was used to test the significance of the differences between means.

RESULTS AND DISCUSSION

Vaginal mucus transparency during estrus and conception rate in Egyptian Baladi cows

Table (3) indicated that the percentage of cows that conceived had transparent vaginal mucus (VM) during estrus increased by 73.3% (*P <0.05*) compared with those cows which had cloudy and dirty vaginal mucus. Similar results were reported by Lim *et al*. (2014) who found that the conception rate was 67.3% for dairy cows that had transparent vaginal mucus and Bhat *et al*. (2015) found that the conception rate was 94.1% for cows that had transparent vaginal mucus and reported similar trend. In addition, Mellado *et al*. (2015) suggested that the conception rate from the first service with transparent mucus was higher (31.9%) than turbid mucus (22.3%) in cows. Parikh *et al*. (2018) reported that cows with clear mucus had higher conception rate. Senosy *et al*. (2012) found that postpartum first service conception rate in dairy cows having abnormal vaginal mucus discharge was significantly ($p < 0.01$) lower than those cows having normal vaginal mucus discharge (2.4% *vs*. 30.2%) during the early weeks postpartum. Loeffler *et al*. (1999) reported that clear cervical mucus discharge at artificial insemination was positively, correlated with increased first service conception rate. LeBlanc *et al*. (2002) reported that the first service conception rate was higher in dairy cows with normal vaginal discharge (37%) compared to those with abnormal vaginal contents (29%) during postpartum period. Mahmoudzadeh *et al*. (2001) showed that the conception rate was significantly *(P < 0·05)* higher (38·3%) in the normal vaginal discharge than that of the abnormal group (27·9%) in cows. Sharma and Tripathi (1987) suggested that there was a great influence of the nature of cervical mucus on spermatozoa activity in the female reproductive duct. Verma *et al*. (2014) showed that higher conception rate of 41.3% in animals, which had clear cervical mucus than cloudy 14.3% in buffaloes. Bernardi *et al*. (2016) suggested that the conception rate was higher (70%) in Holstein cows, which had transparent cervical mucus than opaque cervical mucus (30%). Lopez-Gaius *et al*. (1993) stated that color of cervical mucus is considered one of the important factors which affects on sperm penetration and conception however, turbid mucus arrested sperm motility in cows. Hay *et al*. (2019) showed that abnormal cervical mucus discharge associated with significantly decreased in the pregnancy rate in cows. Murugavel and López-Gatius (2009) reported that cervical mucus appearance indicated good sexual health and associated with a higher conception rate in cows. Gautam *et al*. (2010) found that cows, which had cloudy vaginal mucus at artificial insemination, had lower conception rate compared with another cows.

Table 3. Effect of vaginal mucus (VM) transparency during estrus on the conception rate of Egyptian Baladi cows

Items	Vaginal mucus transparency		
	transparent	cloudy	dirty
Total number of cows	(30)	(5)	(5)
No. of pregnant cows	22	2	1
% of pregnant cows[1]	73.3[a]	40[b]	20[c]
No. of non-pregnant cows	8	3	4
% of non-pregnant cows[1]	26.7[a]	60[b]	80[c]

1-Conception rate calculated from post-partum first service

a,b,c: values within the same row having different superscripts are significantly at ($P < 0.05$).

Vaginal mucus viscosity during estrus and conception rate in Egyptian Baladi cows:

Table (4) illustrated the percentage of cows that conceived which had thin vaginal mucus during estrus was significantly (*P <0.05*) higher 87.5% compared to those with thick vaginal mucus. Similar results were found by Lim *et al*. (2014) they found that higher (*P*<0.10) conception rates 81.8% in cows had thin cervical mucus than thick 18.2% in dairy cattle. Similar trend showed by Bhat *et al*. (2015) who found the conception rate was 88.2% of cows, which had thin vaginal mucus compared with cows, which had thick vaginal mucus 11.8%. Bernardi *et al*. (2016) stated that cows with thin and clear vaginal mucus had higher (65%) pregnancy rate compared with those had thick vaginal mucus (40%). Verma *et*

al. (2014) reported that the conception rate in Murrah buffaloes, which had thin and moderate cervical mucus was higher 74.2% compared with thick cervical mucus (11.1%). Siregar *et al*. (2019) stated that the conception rate was higher (100%) in aceh cattle, which had thin cervical mucus compared with thick cervical mucus 20%. Rutllant *et al*. (2005) suggested that during follicular phase the rheological properties of cervical mucus were more copious, watery and less viscous which assisted the transport of spermatozoa in the female reproductive tract. Bernardi *et al*. (2016) showed that the success rates of artificial insemination in Holstein cows can be classified according to cervical mucus viscosity (9, 30 and 61%) for cows with thick, moderate and thin cervical mucus respectively. Rangnekar *et al*. (2002) and Layek *et al*. (2013) stated that coiled muco-proteins and reduced penetration and progressive movement of sperm maybe one of vital causes of lower conception rate in thick cervical mucus in Holstein Friesian and Zebu cattle. Siregar *et al*. (2019) showed that total protein content was 8.2, 9.75 and 9.48 (g/dL) in thin, moderate and thick cervical mucus. The mucoprotein leads to reduce sperm penetration and prevent progressive movement of sperm, which causes a lower conception rate in cows with thick cervical mucus.

Table 4. Effect of vaginal mucus viscosity during estrus on the conception rate of Egyptian Baladi cows

Items	Vaginal mucus viscosity	
	Thin	thick
Total number of cows	(32)	(8)
No. of pregnant cows	28	2
% of pregnant cows[1]	87.5[a]	25[b]
No. of non-pregnant cows	4	6
% of non-pregnant cows[1]	12.5[a]	75[b]

1-Conception rate calculated from post-partum first service,
a,b: values within the same row having different superscripts are significantly at ($P < 0.05$).

Vaginal mucus pH level during estrus and conception rate in Egyptian Baladi cows

Table (5) clarified that the conception rate was significantly (*P <0.05*) higher (78.6%) in cows which had vaginal mucus pH level at estrus >8 compared with those cows with vaginal mucus pH level ranging between 7 to 8. The current result corresponds with that reported by Ningwal *et al*. (2018) who found that the conception rate was higher 67.5% in crossbred cows that had vaginal mucus pH of 8.14 than those cows with vaginal mucus pH of 7.46.Verma *et al*. (2014) reported that conception rate was higher 42.2% in Murrah buffaloes, which had vaginal mucus pH level at estrus ranging between (7.5 to 8) compared with those cows with vaginal mucus pH value ranging between (7 to 7.5) 25%. Siregar *et al*. (2019) reported that pregnancy rate was 100% in Aceh cattle when pH level of cervical mucus was ranged from 7 to 8. Kumar *et al*. (2019) suggested that the vaginal mucus pH was 8.42±0.09 and 8.72±0.22 in conceived and non-conceived crossbred cows respectively. Dodamani *et al*. (2010) suggested that pH levels of cervical mucus during the estrus were around (8.83-8.91) in Deoni cows. Modi *et al*. (2011) found that the pH levels of cervical mucus of fertile cows ranged from (7.3 to 9.1) in Kankrej cows. Bennur *et al*. (2004) and Rathod (2016) reported that the mean pH value of cervical-vaginal mucus in conceived cows was (8.13±0.07) and (8.45±0.11), respectively. Hafez and Hafez (2000) suggested that the increased acidity or alkalinity in cervical mucus could weak sperm motility and lead to fertilization failure. Predojevic *et al*. (2007) suggested that the pH of cervical mucus is considered one of the main factors to determine the success of pregnancy on livestock because cervical mucus is the transport medium for sperm in the reproductive tract in female. Branigan and Larry (2008) and Agarwal *et al*. (2008) stated that the pH of 7.0 – 8.5 is optimal level, which supports sperm viability and motility, while pH below six lead to the weak motility of sperm. Siddiquee (2006) and Zaman *et al*. (2013) found that the mean pH value of cervical-vaginal mucus in non- conceived cows was (7.40±0.09 and 7.95±0.09). In a recent report, Hanumant *et al*. (2019) suggested that mean pH values in cervical mucus of conceived and non-conceived cows were (8.30±0.11) and (7.76±0.12), respectively.

Table 5. Effect of vaginal mucus pH level during estrus on the conception rate of Egyptian Baladi cows

Items	pH level		
	7-7.5	7.5-8	>8
Total number of cows	(4)	(8)	(28)
No. of pregnant cows	1	5	22
% of pregnant cows[1]	25[a]	62.5[b]	78.6[c]
No. of non-pregnant cows	3	3	6
% of non-pregnant cows[1]	75[a]	37.5[b]	21.4[c]

1-Conception rate calculated from post-partum first service
a,b,c: values within the same row having different superscripts are significantly at ($P < 0.05$).

Progesterone, estradiol-17βconcentrations in blood plasma and pH of vaginal mucus during estrus of pregnant and non-pregnant Egyptian Baladi cows

Table (6) indicated no significant *(P <0.05)* difference in plasma progesterone concentrations during estrus in the cows which had different pH in pregnant and non-pregnant Baladi cows. However, concentrations of estradiol-17β in pregnant cows were significant *(P <0.05)* higher than in non-pregnant Baladi cows Table (6). The present finding agreed with that stated by Bernardi *et al*. (2016) who found lower concentrations of progesterone and higher concentrations of estradiol-17β in pregnant cows which had cervical mucus pH (7.6) compared to non- pregnant cows which had cervical mucus pH (7.2) in Holstein cows. Rolfs *et al*. (2005) reported that the hormonal alteration during the per-estrus period affected on the estrus behavior expression. Tsiligianni *et al*. (2011) and Layek *et al*. (2013) suggested that traits of cervical mucus were converting according to ovarian hormones level secreted during estrus, this cervical mucus can act either as a mechanical barrier to sperm motion, or as a facilitator of sperm to arrive the site of fertilization in reproductive tract in the female. Predojevic *et al*. (2007) reported that the cow onset estrus, a time when there is lack or recession of the corpus luteum, concentration of progesterone are in lower levels and full follicular development. Mullins and Saacke (1989) and Pluta *et al*. (2011) stated that physical and chemical changes in cervical mucus during the estrus cycle related to fluctuations in sex steroid hormones.

Table 6. Progesterone (ng /ml), estradiol-17β (pg/ml) concentrations (mean ± SE), in blood plasma and pH of vaginal mucus during estrus of pregnant and non-pregnant Egyptian Baladi cows

Items	pH	P4 (ng /ml)	E2 (pg/ml)
Pregnant cows	7-7.5	0.22±0.02[a]	32.4 ± 4.3[a]
	7.5-8	0.24±0.03[a]	33.2 ± 3.1[a]
	>8	0.27±0.04[a]	37.5 ± 2.4[a]
Non-pregnant cows	7-7.5	0.27±0.01[a]	12.1 ± 2.3[b]
	7.5-8	0.21±0.02[a]	18.1 ± 1.3[b]
	>8	0.24±0.05[a]	16.1 ± 2.1[b]

a, b: values within the same column having different superscripts are significantly at (P <0.05).

Estradiol-17β concentration and estrus intensity expression in Egyptian Baladi cows

Table (7) showed that concentration of estradiol-17β at estrus was significantly (P <0.05) higher in cows which had high estrus intensity expression compared with those cows which have weak estrus intensity expression. The present result clarified that there was a positive relationship between estrus intensity expression and concentrations of estradiol-17β during estrus. The current finding corresponded with that reported by Lyimo *et al*. (2000) who found higher correlation between the visual sings of estrus and estradiol-17β concentrations. Similar trend was observed by SáFilho *et al*. (2009) and Atkins *et al*. (2010) who suggested that a significant positive correlation between estradiol concentration and high incidence of estrus expression. In addition, Jinks *et al*. (2013) and Perry *et al*. (2014) found a positive relationship between peak concentrations of estradiol, and exhibited intensity of estrus in cows. Rolfs *et al*. (2010) reported that positive association between the conception rate, higher concentrations of estradiol-17β and estrus expression in cows. Mogheiseh *et al*. (2019) suggested that low concentration of estradiol-17β at mating were the main causes for the occurrence of repeat breeding syndrome in dairy cows. Ozturk and Demir (2010) reported that estradiol-17β at artificial insemination plays a vital role in establishing the timing of uterine receptivity. Pre-ovulatory concentrations of estradiol-17β influenced sperm transport, embryo survival and the uterine environment (Miller, 1976, Hawk, 1983 and Perry and Perry, 2008).

Table 7. Estradiol-17β (mean ± SE) (pg/ml) concentration and estrus intensity expression in thirty Egyptian Baladi cows

Estrus intensity	No. of cows	Estradiol-17β (pg/ml)
Weak estrus intensity expression[1]	15	15.4±1.3[a]
High estrus intensity expression[2]	15	32.7±1.1[b]

a, b: values within the same column having different superscripts are significantly at (P <0.05), 1-Weak estrus intensity expression: These cows, which appeared one sign only during the estrus. 2-High estrus intensity expression: These cows, which appeared ≥3 signs at least during the estrus.

CONCLUSION

The results clarified the cows, which had vaginal mucus discharge (VMD**)**: transparent, thin, and pH value at estrus >8 recorded higher conception rate. Estradiol-17β concentration was significantly (*P <0.05)* higher during estrus period in cows, which displayed high estrus intensity.

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الخصائص الطبيعية للمخاط المهبلى وعلاقتها بمعدل الحمل فى الأبقار البلدية المصرية

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أُجريت الدراسة الحالية لتقدير تأثير الخصائص الطبيعية للمخاط المهبلى على معدل الحمل فى الأبقار البلدية المصرية. استخدم فى هذه التجربة عدد ٤٠ بقرة بلدى. تم متابعة الأبقار بعد مرور اسبوعين من الولادة. تم تقسيم الأبقار بناء على شفافية المخاط المفرز من المهبل الى الى ثلاثة فئات (شفاف، معتم وأكثر عتامة)، اللزوجة الى (خفيف، ثقيل) و الاس الهيدروجينى الى (٧-٧.٥ ، ٧.٥ – ٨ ، >٨). أوضحت النتائج ان نسبة الأبقار التى حملت كانت أعلى (٧٣.٣%) معنويا ($P < 0.05$) فى الأبقار ذات المخاط المهبلى الشفاف بالمقارنة بالأبقار ذات المخاط المهبلى المعتم والاكثر عتامة (٤٠ و ٢٠%) على التوالى. كانت نسبة الأبقار التى حملت أعلى (٨٧.٥%) معنويا ($P < 0.05$) فى الأبقار ذات المخاط المهبلى الخفيف بالمقارنة بالأبقار ذات المخاط المهبلى الثقيل ٢٥%. كان معدل الحمل أعلى (٧٨.٦%) معنويا ($P < 0.05$) فى الأبقار التى كان مستوى الاس الهيدروجينى فيها أكبر من (٨) بالمقارنة بتلك الأبقار التى كان الاس الهيدروجينى يتراوح بين (٧- ٨). لايوجد اى اختلاف معنوى فى تركيز هرمون البروجستيرون عند الشياع فى الأبقار التى حملت والأبقار التى لم تحمل ذات المخاط المهبلى مختلف الاس الهيدروجينى. بينما تركيز هرمون الاستراديول عند الشياع كان أعلى معنويا ($P < 0.05$) فى الأبقار التى حملت عنه فى الأبقار التى لم تحمل. كان تركيز هرمون الاستراديول أعلى معنويا ($P < 0.05$) فى الأبقار التى أظهرت قوة فى مظاهر سلوك شياع عنه فى الأبقار التى أظهرت ضعف فى مظاهر سلوك الشياع. أوضحت النتائج أن معدل الحمل كان أعلى فى الأبقار التى كانت تفرز مخاط مهبلى شفاف ، خفيف و الاس الهيدروجينى له أعلى من(٨).كان تركيز هرمون الاستراديول أعلى عند الشياع فى الأبقار التى أظهرت قوة فى مظاهر سلوك شياع.