Association Among Spinnbarkeit, Electrical Conductivity, and Crystallization of Cervical Mucus and Pregnancy Rate in Egyptian Baladi Cows

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Abstract

Alteration of the biophysical and biochemical characteristics of cervical mucus (CM) due to differences in steroid hormones through the estrus cycle leads to different pregnancy rates. This investigation aims to study the possible relationship between CM properties with biochemical profiles, macro-mineral levels, and steroid hormones concentrations, and their effects on pregnancy rates of Egyptian cows. Fourteen Baladi cows were used and synchronized. The model log-likelihood ratio was highly significant (P=0.0009), and reported that the spinnbarkeit (SPK), electrical conductivity (EC), and crystallization (CRS) had significant effect on high pregnancy rate. The 3^{rd} level of SPK (>13.5 cm) and EC (>15 mS/cm) was the highest significant (P=0.0016 & 0.0517, respectively) and a clear positive of estimate marginal effect (20.2543 & 10.6192, respectively) attitude towards the pregnancy rate. However, in case of the CRS, the significant effect was in the first two levels (P=0.0321 & 0.0425, respectively) with a high pregnancy rate, reverse the last 2 levels. Total protein, cholesterol, glucose, potassium, chloride levels, and estradiol concentrations were observed higher with increasing levels of SPK and EC and appearance of typical fern patterns (first two levels of CRS), in contrast to sodium, and progesterone concentrations that decreased with elevating levels of SPK and EC and appearance of atypical fern patterns (last two levels of CRS). There was a close correlation between CM properties and steroid hormones (P_4 & E_2). So, alterations in CM properties, especially SPK, EC, and CRS, can be utilized to foresee estrus time and, as a result, insemination time.

1. Introduction

The endocervix secretory cells continuously produce cervical mucus, which varies in quality and quantity depending on the hormonal status of the estrous cycle (Lopez-Gatius et al., 1993; Tsiligianni et al., 2001). Changes in cervical mucus (CM) rheology (flow and deformation characteristics) during the estrous cycle partially reflect the cervical epithelium's response to steroid hormones (Carlstedt et al., 1989). For instance, biophysical and biochemical properties of CM can be changed with progresses the estrous cycle of cow (Carlstedt et al., 1989; Lopez-Gatius et al., 1993). Examination of fluids secreted in the female genital system to determine fertility has become a major concern and cervical fluid is also involved in sperm survivability and delivery to the uterus (Kumar et al., 2012). CM characteristics can be utilized as a sign to detect the exact moment of artificial insemination and can be utilized as an indicator of reproductive health connected to estrous behavior, also, play a major influence in pregnancy success; thus they will boost the conception rate (Tsiligianni et al., 2000; Benbia et al., 2011; Verma et al., 2014; Joshi et al., 2017; Ondho et al., 2019; Siregar et al., 2019; Abd-El-Hafeez et al., 2020).

The Spinnbarkeit (SPK) value of CM is ascribed to the presence of big molecules and is thought to be dependent on molecular chain branching and other strong intermolecular forces (Elstein, 1974). The SPK achieves its greatest value around the periovulatory stage in cows (Hamana et al., 1971). SPK levels change during the periestrus stage, becoming lower as estrus progresses (Bishnoi et al., 1982). Once the SPK value has declined, the progesterone impact is started. Hormonal interventions of estrous induction also reduce SPK compared to spontaneous ovulators (Tsiligianni et al., 2001).

Crystallization (CRS) reaches a maximum value near the time of ovulation (Wolf, 1977). This value reaches the peak in the pregnant cows (Tsiligianni et al., 2000). Similar to SPK, CRS is higher in spontaneous estrus cows than cows entering heat post hormonal interventions (Tsiligianni et al., 2001). Several studies investigated the relationship between the electrical resistance property of CM and the oestrus in cows (Hulsure et al., 1995). The electrical resistance of cervical mucus (ERCM) decreases dramatically during oestrus, and cows inseminated with low ERCM had a higher conception rate than those inseminated with high ERCM (Leidl and Stolla, 1976; Ahmed et al., 2017).

Layek et al. (2013) reported that SPK level and the arborization structure of cervical mucus strongly correlate with plasma progesterone (P_4) concentration and time of ovulation. Joshi et al. (2017) reported that the SPK level of CM ranged from 8 to 16 cm in 46% of estrus cows, and 58% of them had primary, secondary, and tertiary venation, which is typical of ferns. CM has a pH range of 7.5-8.0 and a conductivity of 13.50-15.00 mS/cm.

We suffer a big problem in the baladi cows, where the movement is large and increasing in a way that makes it difficult for him to follow the details of the occurrence of estrus features with certainty and thus determine the most appropriate time for insemination compared to the foreign breeds, with which it is easy to follow in detail the appearance of the occurrence of estrus and determine the most appropriate time for vaccination. Therefore, observing the physical characteristics may be a strong alternative to determine the most appropriate time for insemination in baladi cows. The objective of this study is to find out possible relationships among SPK, EC and CRS of cervical mucus and pregnancy rate in Egyptian Baladi cows.

2. Materials And Methods

2.1. Animals

Fourteen Egyptian Baladi cows were selected in good body condition and normal anatomy genitalia. Cows had the same parity, aged 4–5 years and weighing 400 ± 11.35 kg. which had already calved and had undergone at least two regular cycles, and then were synchronized by an intramuscular injection of 25 mg prostaglandin-F2α. Cows were fed according to NRC (2001) and were housed in a semi-open shade with ventilation and a sprinkler system to keep them cool. All cows were reared at the Sids Experimental Station belonging to the Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation, Egypt. The animal care and use committee from the Animal Production Research Institute approved our study.

2.2. Cervical mucus (CM) and blood samples

All cows were observed twice a day for 30 minutes to detect estrus. Samples of CM and blood were immediately collected prior to natural insemination. The cow's vulva was cleaned with an antiseptic solution and swilled by distilled water and properly wiped. A flexible plastic sheath was utilized to collect CM samples to avoid vaginal mucosa rupture. A sterile syringe (10 ml) was affixed to the sheath's exterior end. The samples were gently suctioned from the cervical aperture and surrounding area before being kept at -20°C for future use.

CM samples were immediately checked for spinnbarkeit (SPK), electrical conductivity (EC), and crystallization (CRS). The SPK value was measured using a simple apparatus, as described by Tsiligianni et al. (2000). Briefly, a small drop of mucus was placed on a glass slide and covered with a glass coverslip. The SPK was determined by drawing the mucus out vertically until the mucus thread broke. This procedure was repeated twice for each sample, and the mean value was recorded and categorized into three groups (<9, 9-13.5 and >13.5 cm).

Before performing the EC measurement, CM samples were first stirred in a mixture vortex (Spinix Corporation, CA, USA) until they were lysed and turned into a free-flowing liquid. Then EC was determined in the lysed CM utilizing a pH-Conductivity Benchtop (Orion 4 star, Thermo Electron Corporation, USA) in mili-Siemens/cm unit (mS/cm) and split into three groups (9-13.5, 13.5-15 and >15 mS/cm).

CRS was measured as described by Tsiligianni et al. (2000). Smearing a drop of CM on a clean slide was used to prepare a film. The film was left to dry for 30 minutes at 25°C before being inspected under 40× magnification. CRS pattern was rated on a scale of 1 to 4; 1: typical crystals formation or typical fern patterns (TFP); 2: Formation of many typical and few atypical fern-like crystals; 3: Formation of many atypical fern-like crystals and few typical ones; and 4: Formation of only atypical fern patterns (AFP). Mucus specimens were stored at -20°C until the chemical analysis of total protein (TP), total cholesterol (TC) and glucose (GLU), sodium (Na), potassium (K), chloride (Cl), progesterone (P_4) and estradiol (E_2).

Blood serum was taken from cows' jugular veins using a 10 ml disposable syringe. Blood was collected just before insemination.

Serum and mucus TP, TC, and GLU analysis were carried out by spectrophotometer using a commercial kits (Spinreact, Spain). Na and K concentration were determined by colorimetric and turbidimetric methods, respectively, using commercial kits (Biodiagnostic Company, Giza, Egypt). Cl concentration was determined using a thiocyanate method (QCA Company, Amposta (Tarragona), Spain).

The progesterone (P_4) concentrations were determined by using a commercial RIA kit (Coat-A-Count; Diagnostic Products Corporation, DPC, Los Angeles, California, USA) and the estradiol (E_2) was purchased from Spectria1; Orion Diagnostica Oy (Espoo, Finland).

2.3. Statistical analysis

The data of cervical mucus properties (spinnbarkeit, electrical conductivity, and crystallization) were transformed by the ARCSine method (Kirk, 2013) to calculate the correlation coefficient among physical (SPK, EC, & CRS) and chemical (TP, TC, GLU, Na, K & Cl) properties of cervical mucus, as well as steroid hormones by using SAS (2014).

Data were statistically analyzed using the general linear model procedure. The differences among means were tested using Duncan's Multiple-rang test.

Box-and-whisker plots (whiskers are 1.5× the interquartile range) for each group (SAS, 2014) were used to analyze physical properties data. Box plots display batches of data are becoming a widely used tool in exploratory data analysis (Mcgill et al., 1978).

A logistic regression using the GENMOD procedure of SAS (2014) was performed to assess the significance of cervical mucus properties (spinnbarkeit, conductivity, and crystallization).

The following model was used to determine cervical mucus properties affecting pregnant in Egyptian cows:

$\text{Log}\ [p_i/1\text{-}p_i] = \alpha + \beta 0 + \beta 1x1i + \beta 2x2i + \beta 3x3i + \varepsilon$

Where :

Pi = The proportion with pregnant for observation i,

α = Intercept term.

x1i = Spinnbarkeit for observation i,

x2i = Conductivity for observation i,

x3i = Crystallization for observation i,

ε = Error term

β_0, β_1, β_2 and β_3 are regression coefficient.

The previous model was applied also to study the factors in Egyptian cows.

3. Results

Out of fourteen cows presented in Table (1), three and four cows had mucus with <9.0 cm and 9-13.5 cm SPK, respectively, only one of them became pregnant, and seven cows had mucus with >13.5 cm SPK, six became pregnant. Moreover, five cows had mucus with 9.0-13.5 mS/cm EC, only one became pregnant, three cows had mucus with 13.5-15 mS/cm EC, two became pregnant, and six cows had mucus with >15 mS/cm, five of them became pregnant. However, five and three cows had mucus with TFP (1st & 2nd levels of CRS, respectively), only one of them did not get pregnant; also, five and one had mucus with AFP (3rd & 4th levels of CRS, respectively), only one of them get pregnant.

The model log likelihood ratio (χ^2 = 35.4210) was highly significant (*P* = 0.0009), indicating that the explanatory variables included were significant in explaining the effects of SPK, EC, and CRS cervical mucus properties on high pregnancy rate in Egyptian cows (Table 1). The results indicated that the estimated marginal effects for SPK levels (<9 cm = -0.6931, 9-13.5 cm = -1.0986 & >13.5 cm = 20.2543), EC (9-13.5 mS/cm = -1.3863, 13.5-15 mS/cm = -2.3653 & > 15 mS/cm = 10.6192) and CRS (1 = 15.3241, 2 = 11.4512, 3 = -0.9822 and 4 = -1.9981) had significantly effects ($P = 0.0007$, $P = 0.0028$ & $P < 0.0001$, respectively) on high pregnancy rate in Egyptian cows.

Table 1
Marginal effects of factors that influence on high pregnancy rate in Egyptian cows.

Mucus criteria	N (%)	Pregnant (%)	Estimate marginal effects	SEM	Value χ^2	*P*. value of χ^2
Spinnbarkeit						
<9.0	3 (21.43)	1 (33.33)	-0.6931	0.75	0.29	0.5901
9.0-13.5	4 (28.57)	1 (25.00)	-1.0986	0.66	5.89	0.3414
>13.5	7 (50.00)	6 (85.71)	20.2543	0.64	9.98	0.0016
P. **value of χ^2**					**27.15**	**0.0007**
Conductivity						
9.0-13.5	5 (35.73)	1 (20.00)	-1.3863	1.11	1.57	0.2099
13.5-15.0	3 (21.42)	2 (66.67)	-2.3653	1.56	3.66	0.0756
>15.0	6 (42.85)	5 (83.33)	10.6192	1.64	12.34	0.0517
P. **value of χ^2**					**48.92**	**0.0028**
Crystallization						
1	5 (35.72)	4 (80.00)	15.3241	0.73	12.46	0.0321
2	3 (21.42)	2 (66.67)	11.4512	0.17	11.78	0.0425
3	4 (35.72)	1 (25.00)	-0.9822	0.99	3.12	0.0821
4	2 (7.14)	1 (50.00)	-1.9981	2.11	1.25	0.1543
P. **value of χ^2**					**30.27**	**< 0.0001**
Value χ^2 = 35.4210, Pr-chisq = 0.0009.						

The 3rd level of SPK and EC had the only level of cervical mucus that had highly significant (*P* = 0.0016 & 0.0517, respectively) and a clear positive of estimate marginal effect (20.2543 & 10.6192, respectively) attitude towards the pregnancy rate, while 1st and 2nd levels of SPK and EC had no significant effect on pregnancy rate. These results indicate that the 3rd level of SPK and EC is preferable indicator for a high pregnancy rate. However, in the case of the CRS effect, the significant effects are the first two levels (*P* = 0.0321 & 0.0425, respectively) and positive effects (15.3241 & 11.4512, respectively) was observed with a high pregnancy rate, in the last 2 levels.

The finding of the current study showed variation in the profiles of biochemical measurements (TP, TC & GLU), macro-minerals (Na, K & Cl), and steroid hormones (P_4 & E_2) in both serum and cervical mucus of Egyptian Baladi cows, as presented in Tables 2, 3, & 4 and Figures 1, 2 & 3.

Table 2
Mucus biochemical levels in Egyptian Baladi cows at different cervical mucus properties

Mucus criteria	**Total protein (g/dl)**	**Total cholesterol (mg/dl)**	**Glucose (mg/dl)**
Spinnbarkeit			
<9.0	$7.27±0.08^{b}$	$104.67±0.77^{b}$	$60.14±0.46^{b}$
9.0-13.5	$8.09±0.13^{a}$	$109.50±0.92^{ab}$	$62.75±0.66^{ab}$
>13.5	$8.27±0.17^{a}$	$116.29±0.65^{a}$	$65.00±0.41^{a}$
P. **Value**	0.002	0.010	0.025
Conductivity			
9.0-13.5	$7.50±0.13^{b}$	$105.60±1.27^{b}$	$60.50±0.65^{b}$
13.5-15.0	$7.77±0.12^{b}$	$112.67±1.25^{ab}$	$61.33±0.62^{b}$
>15.0	$8.53±0.08^{a}$	$116.00±1.54^{a}$	$64.40±0.55^{a}$
P. **Value**	0.0001	0.016	0.001
Crystallization			
1	$8.51±0.10^{a}$	$116.20±1.82^{a}$	$66.00±0.73^{a}$
2	$8.10±0.08^{a}$	$113.00±1.78^{ab}$	$63.20±0.95^{b}$
3	$7.44±0.11^{b}$	$106.60±1.56^{b}$	$61.00±0.41^{b}$
4	$7.50±0.12^{b}$	$107.00±1.73^{b}$	$61.00±0.52^{b}$
P. **Value**	0.0001	0.054	0.008

1: Typical crystals formation or typical fern patterns; 2: Formation of many typical and few atypical fern-like crystals; 3: Formation of many atypical fern-like crystals and few typical ones; and 4: Formation of only atypical fern patterns.

Table 3
Mucus macro-minerals of Egyptian Baladi cows at different cervical mucus properties

Mucus criteria	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)
Spinnbarkeit			
<9.0	142.33±2.39[a]	7.10±0.53[c]	152.00±1.29[c]
9.0-13.5	137.25±2.73[a]	9.50±0.40[b]	165.38±1.99[b]
>13.5	113.43±2.87[b]	11.66±0.64[a]	177.29±1.43[a]
P. **Value**	0.0001	0.001	0.0001
Conductivity			
9.0-13.5	133.20±0.87[a]	8.40±0.74[b]	158.88±3.61[b]
13.5-15.0	125.33±1.43[b]	10.17±0.31[a]	169.33±2.46[a]
>15.0	123.17±1.28[b]	11.17±0.31[a]	174.58±2.69[a]
P. **Value**	0.0001	0.005	0.006
Crystallization			
1	118.80±0.95[c]	11.06±0.25[a]	173.52±1.91[a]
2	126.67±1.43[b]	10.57±0.21[a]	170.78±1.44[a]
3	134.60±0.95[a]	8.62±0.35[b]	162.12±1.60[b]
4	136.00±1.70[a]	8.77±0.29[b]	155.30±1.16[b]
P. **Value**	0.0001	0.0001	0.005

Table 4

Mucus progesterone and estradiol concentrations in Egyptian Baladi cows at different cervical mucus properties

Mucus criteria	**Progesterone (ng/ml)**	**Estradiol (pg/ml)**
Spinnbarkeit		
<9.0	0.42±0.01[a]	34.17±0.74[c]
9.0-13.5	0.36±0.02[b]	37.98±0.84[b]
>13.5	0.19±0.01[c]	52.96±0.76[a]
P. **Value**	0.0001	0.0001
Conductivity		
9.0-13.5	0.39±0.02[a]	35.42±1.24[b]
13.5-15.0	0.32±0.03[b]	47.15±1.49[a]
>15.0	0.20±0.01[c]	49.89±1.77[a]
P. **Value**	0.0001	0.0001
Crystallization		
1	0.18±0.01[c]	50.80±0.40[a]
2	0.30±0.01[b]	45.54±0.60[b]
3	0.40±0.01[a]	38.25±0.57[c]
4	0.43±0.03[a]	35.66±0.58[c]
P. **Value**	0.0001	0.0001

Table 5
Pearson correlation coefficients by ARCSine method among physical and biochemical, macro-minerals, and steroid hormones.

Mucus criteria	**Biochemical parameters**			**Macro-minerals**			**Steroid hormone**	
	TP	**TC**	**GLU**	**Na**	**K**	**Cl**	**P_4**	**E_2**
SPK	+0.712	+0.694	+0.640	-0.813	+0.811	+0.895	-0.933	+0.928
P. **Value**	0.001	0.002	0.006	0.0001	0.0001	0.0001	0.0001	0.0001
EC	+0.866	+0.659	+0.760	-0.833	+0.725	+0.707	-0.893	+0.795
P. **Value**	0.0001	0.004	0.0001	0.0001	0.001	0.001	0.0001	0.0001
CRS	-0.823	-0.588	-0.674	+0.925	-0.815	-0.740	+0.899	-0.873
P. **Value**	0.0001	0.008	0.002	0.0001	0.0001	0.0001	0.0001	0.0001

TP, TC, GLU, K, and Cl levels were increased with increasing levels of SPK and EC and appearance of typical fern patterns (TFP, first two levels of CRS). In contrast to the previous trend, sodium level was decreased with elevating SPK and EC levels and appearance of atypical fern patterns (AFP, last two levels of CRS). The 3rd level of SPK and EC, as well as the first two levels of CRS, were over the range in the other for different mucus properties. One of the most obvious findings is that most of the biochemical tests analyzed (TP, TC & GLU), as well as studied most macro-minerals (K & Cl), had higher distribution with CM properties including >13.5 SPK, >15.0 EC and TFP. An opposite trend was observed with sodium concentration.

The box plot in Figure (3 a & b) and Table (4) showed an elevation in E_2 and reduction in P_4 distribution around the 3rd level of SPK and EC and the 1st two levels of CRS of studied cows' CM compared to the other levels. E_2 concentration were significantly ($P < 0.001$) different in all studied groups, where the median values were 34.17 & 43.76 pg/ml, 37.98 & 41.16 pg/ml, and 52.96 & 53.51 pg/ml for <9, 9-13.5, & >13.5 cm of SPK in serum and CM, respectively (Table 4 & Figure 3b). Three levels of EC (9-13.5, 135.-15 & >15 mS/cm) median values were 35.42 & 42.35 pg/ml, 47.15 & 51.95 pg/ml and 49.89 & 53.75 pg/ml in serum and CM, respectively. Levels of CRS (1, 2, 3 & 4) median values were 50.80 & 51.80 pg/ml, 45.54 & 46.31 pg/ml, 38.25 & 43.21 pg/ml and 35.66 & 49.25 pg/ml, in serum and CM, respectively.

Furthermore, P_4 concentration were significantly ($P < 0.001$) different in all studied groups, median values were 0.42 & 0.30 ng/ml, 0.36 & 0.32 ng/ml, and 0.19 & 0.21 ng/ml for <9, 9-13.5, & >13.5 cm of SPK in serum and CM, respectively. Three levels of EC (9-13.5, 135.-15 & >15 mS/cm) median values were 0.39 & 0.32 ng/ml, 0.20 & 0.21 ng/ml and 0.22 & 0.30 ng/ml in serum and CM, respectively. Levels of CRS (1, 2, 3 & 4) median values were 0.18 & 0.18 ng/ml, 0.30 & 0.31 ng/ml, 0.40 & 0.34 ng/ml and 0.43 & 0.20 ng/ml, in serum and CM, respectively (Table 4 & Figure 3a).

Pearson correlation coefficient by ARCSine method between properties of cervical mucus (SPK, EC & CRS) and studied parameters (biochemical, macro-minerals & steroid hormones) are presented in Table (5). SPK and EC were positively ($P < 0.01$ & 0.001) correlated with the concentrations of TP, TC, GLU, K, CL and E_2. In contrast, the crystallization had an inverse ($P < 0.001$) relationship with the previous parameters. An opposite trend was observed with Na levels, and P_4 concentrations.

4. Discussion

It is generally known that physicochemical alterations in cervical mucus occur during erectile ovarian steroids, aiding sperm penetration. While the determination of specific parameters such as spinnbarkeit, crystallization pattern, electrical conductivity, dry matter contents, and rheological values have been utilized to predict the optimum time for cattle insemination (Joshi et al., 2017; Ondho et al., 2019; Siregar et al., 2019; Abd-El-Hafeez et al., 2020). Also, cervical mucus properties at the moment of service/insemination affect pregnancy in cows. They are influenced by alterations in periestrual hormones concentrations.

The current study's findings revealed a strong association between steroid hormones and the physical features of CM, and the probability of ovulation. The pregnancy rates in cows with <9.0, 9.0-13.5 and >13.5 cm spinnbarkeit were 33.33%, 25.00% and 85.71%, also, 9.0-13.5, 13.5-15.0 and >15.0 mS/cm electrical conductivity were 20.00%, 66.67% and 83.33%, respectively. Whereas the 1st and 2nd levels of crystallization containing typical fern patterns had 80.00 and 66.67% pregnancy rates, respectively. This result was consistent with a study by Verma et al. (2014), Joshi et al. (2017), Ondho et al. (2019) and Siregar et al. (2019).

SPK decreases with increasing progesterone, increases when estrogen concentrations are high, and peaks during ovulation (Verma et al., 2014). The 3rd level of SPK (>13.5 cm) of cervical mucus had the highest percentage of pregnancy (85.71%) compared to the other two levels (33.33% and 25.00%). These results are in accordance with Rangnekar et al. (2002) and Verma et al. (2014). Joshi et al. (2017) reported that CM of 46% of estruses ranged in 8-16 cm. The pregnancy rate in cows inseminated with CM having a high SPK value (>13.5 cm) may be attributable to the appropriate phase of estrus because SPK reaches its peak shortly before or during the ovulatory stage under the estrogen effect and then falls as progesterone concentrations rise during the luteal phase in cows (Modi et al., 2011).

Cows inseminated with a higher range (>15 mS/cm) of EC had an 83.33% pregnancy rate, which could be due to the activation motility of the uterus and spermatozoa that improve the fertilization process. Layek et al. (2013) and Verma et al. (2014) reported a positive relationship between pregnancy rate and cervical mucus EC. The small difference found in various researches could be attributable to CM lysis before conductivity measurement and methodology. Joshi et al. (2017) reported that CM had the highest number of estruses (60.00%) in the conductivity range of 13.50 - 15.00 mS/cm in cows. Under the effect of estrogen, electrolyte concentrations begin to rise during estrus, resulting in increased EC. This high amount of electrolytes in CM is essential to activate the motility of uterine and spermatozoa, and the mucus' physical state, which allows ovum-spermatozoa interaction. A positive correlation between the EC of CM and pregnancy rate in cows was observed (Bishnoi et al., 1983; Layek et al., 2013) and our findings corroborate their results. The ability of cervical mucus to be pulled into threads during estrus is attributed to the presence of big molecules in the mucus and presumably depends on the estrus to LH peak duration being delayed (Bage et al., 2002).

In the present study, 75% of pregnant cows had a TFP in CM, which could be attributable to an increase in peripheral E_2 concentrations during estrus, indicating the optimal time for insemination. Rangnekar et al. (2002) and Verma et al. (2014) found almost identical results. Higher pregnancy rates (80.00% and 66.67%) were shown in cows inseminated with TFP in their CM compared to AFP (25.00% and 50.00%). The greater pregnancy rate in TFP is related to the facilitated sperm motility compared to nil or AFP (JeŽková et al., 2008). Our findings are in agreement with Kumaresan et al. (2009) and Layek et al. (2013). Crystallization constitutes a means to reach a more stable, lower energy state from a metastable solution by reducing the solute concentration (Weber, 1991). In each stage of estrus, ferning crystallization will be varied. It is linked to E_2 secretion as a macro-mineral stimulus in the CM (Ondho et al., 2019). According to Yavari et al. (2009), ferning can be noticed when ovulation approaches due to increased hormone E_2 produced.

Mucus criteria, especially SPK, are affected by the cervical mucus acidity and function as a non-immunogenic antibacterial in the cow's reproductive tract (Ondho et al., 2019). Lu and Morresey (2006) reported that the cervical mucus release of estrogen makes the uterus resistant to infection. The hydrolysis process of bacterial cell membranes was linked to cervical mucus containing lysozyme enzyme (LYS). LYS in CM having N-Acetyl Glucosamine is aided the hydrolysis process of bacterial cells (Chimura et al., 1993). The movement of LYS in the hydrolysis process of bacterial cells damages much of the remaining bacterial cells membrane, leaving behind CM. The cervical mucus contains substances found in bacterial cell membranes. Carbohydrates, lipids, and proteins all contribute to the structure of cell membranes. During estrus, all components of cell membrane structure, including carbohydrates, lipids, and proteins, will raise the viscosity level and SPK value of the CM. (Chimura et al., 1993).

The box plot Figure (1) showed serum TP ranged from 6.1 to 7.9 g/dl with an overall median value of 7.08 g/dl. These values were in the cow's normal range, according to Mitruka and Rawnsley (1981), who stated that the normal concentration of TP for cows is 7.56 g/dl. Siregar et al. (2019) reported that serum TP levels in the fertile and the repeat breeding Aceh cows were 6.9 and 6.6 g/dl, respectively. Our findings showed that the higher TP in serum (Figure 1) and mucus (Table 2) were observed with TFP and the greater SPK and EC values. Shiraz et al. (2010) reported that low protein levels could disrupt gonadotropin production. Therefore, the elevated protein levels identified in this study were expected to boost gonadotropin production. Manas et al. (2012) claimed that amino acids and proteins were required for the biosynthesis of gonadotropin hormones (GnRH) and LH that were responsible for the onset of ovulation. Increased gonadotropin production could lead to higher steroid concentrations in the blood. The steroid concentrations are correlated to the CM physical characteristics (Rangnekar et al., 2002).

Serum and mucus total cholesterol increased with increasing SPK and EC values and typical CRS (TFP). Cholesterol is a fatty molecule produced by the liver and circulated throughout the body (Murray et al., 2003). Murray et al. (2003) stated that cholesterol is an essential component of plasma membranes and a key component of all other steroid hormones produced in the body, such as sex hormones and corticosteroids hormones, as well as bile acids and vitamin D. Arosh et al. (1998) discovered a strong correlation between low cholesterol levels and reduced steroidogenesis concentrations. Highshoe et al. (1991) revealed that under the action of LH, cholesterol served as a precursor for the synthesis of estrogen, progesterone, and androstenedione by granulose cells. Increased steroidogenesis raises the concentration of steroids in the blood, which impacts the CM properties (Lim et al., 2014).

The current results showed the highest glucose levels in serum and cervical mucus with >13.5 cm SPK, >15 mS/cm EC, and TFP (1st & 2nd levels of CRS). The findings showed that there was clear correlation between GLU levels and tested characteristics of CM. GLU level that was decreased with reduction of SPK and EC values. TP, TC and GLU levels had a greater impact on the CM properties in this investigation. Low serum GLU levels may lead to a decline in hypothalamic GnRH release, due to a shortage of ATP, which activates cAMP as an intracellular messenger (Murray et al., 2003). The reduction in GnRH release was followed by a reduction in the of FSH and LH synthesis, which led to ovarian dysfunction or the lack of ovarian follicle growth, decrease insulin and IGF-1 concentrations, and decrease releasing of E_2 by ovarian follicles (Mulligan et al., 2007).

All values of macro-minerals were in the normal range, whether in serum or mucus with all CM properties, whereas the Na concentrations were low, but K and Cl concentrations were high for the 3rd level of SPK and EC and the 1st two levels of CRS (TFP). These results were in agreement with Siregar et al. (2019). This demonstrates the specific effect of macro-minerals concentration on cervical mucus SPK, EC, and CRS. Many factors cause macro-minerals concentration to affect CM properties. Sodium chloride in the body plays an important role in reproduction, especially in the estrus stage. During estrus, sodium chloride levels are high and can cause a rise in E_2 concentrations. Cervical mucus secretion is influenced by estrogen. There was a negative relationship between Na and both of K & Cl concentrations in the current results, which agreed with the findings of Abd-El-Hafeez et al. (2020), could be clarified by Coppock et al. (1982), who reported that chloride is absorbed more efficiently than sodium in the posterior section of kidney and intestine. According to some studies, Na content was the only mineral that changed significantly during the 3 days preceding estrus (Cowan and Larson, 1979). Lack of body sodium will cause ovarian dysfunction, which leads to repeated breeding (Roberts, 2004). Furthermore, high K administration in cows can cause delayed puberty and ovulation. Such high administration reduces corpora lutea development and a higher likelihood of anestrus (Velladurai et al., 2016). Ondho et al. (2019) reported that the sodium chloride percentage of CM has 2 peaks at the end of the estrus cycle. Dairy cows at younger ages (three years) reach their peak earlier than older cows while having a smaller NaCl % of CM at the peak. It was found to be related to the E_2 and mineralocorticoid hormone (MCTH) secretion. NaCl % in CM from each estrus stage and age was influenced by E_2 induced by LH hormone (Makmun et al., 2017; Samsudewa et al., 2019). Otherwise, high E_2 secretion causes hypofyse to produce more adrenocorticotropic hormone (ACTH). Cortisol production in the liver is stimulated by increased ACTH secretion, accompanied by elevated estrogen circulation in the liver. Cortisol induces secretory MCTH as controller of electrolyte fluid in the adrenal cortex, whereas E_2 can be eliminated via urine by conjugating water-soluble E_2. MCTH promotes the kidneys to retain more Na^+, K^+, Cl^-, and H^+. The minerals created can be reabsorbed by the entero-hepatic together with free E_2. The entero-hepatic system absorbs the minerals into each specific organ, including the cervix as one of the constituent organs of CM (Widiyono et al., 2013).

Regarding steroid hormone concentrations, there was a negative correlation between P_4 and E_2 in both serum and mucus. The lowest values for P_4 and highest values for E_2 were in cervical mucus characterized by >13.5 SPK, >15.0 EC, and typical fern pattern. P_4 and E_2 can influence CM physical properties (Rangnekar et al., 2002). A reduction in circulating P_4 would be predicted to increase LH pulse frequency (Lopez et al., 2005). Pulse frequency on LH is raised, and LH influx is restrained when P_4 maintains low concentrations causing prolonged follicular dominance (Revah and Butler, 1996). P_4 takes precedence over E_2 in controlling estrus behavior because P_4 can impede estradiol's estrus-inducing effects (Allrich, 1994). Lopez et al. (2005) suggested that P_4 concentrations in superovulated cows are greater throughout estrus than in single ovulating animals. Abnormalities in hormonal balance in the pre- and periovulatory periods have been associated with aberrant follicular/oocyte maturation (Callesen et al., 1986). Therefore, high P_4 concentrations in other tested groups led to changes in CM properties.

Concerning elevation of estradiol concentrations, Lopez et al. (2005) showed a premature rise in E_2 was noticead in cows with triple dominant follicles. Pre-ovulatory rise in E_2 concentrations triggers the onset of LH influx (Hansel and Convey, 1983). In cattle, E_2 formation from the pre-ovulatory follicle is mirrored in the serum E_2 concentration, which rises gradually until the pre-ovulatory LH increase (Bevers and Dieleman, 1987). The presence of E_2 has a significant impact on animal physiology throughout the estrus period and allowing the animal to copulate (Siregar et al., 2019). Tsiligianni et al. (2011) reported that in the follicular phase, superovulated cows possess the lowest and highest E_2 concentration of 30.95 and 54.77 pg/ ml, respectively. The elevation in E_2 concentrations in the bloodstream reaches the anterior pituitary, which increases the LH secretion. Furthermore, estrogen has an effect on the neurological system, making animals restless and causing them to mount other cows. E_2 also induces a gentle contraction of the uterus, which enables spermatozoa to be transferred to the female reproductive genitals post-natural insemination. Other impacts of high E_2 concentrations include blood vessels dilation in the genitals and the release of mucus by the vagina and cervical glands (Ramli et al., 2016). Properties of the mucus rely upon the hormones secreted during estrus (Benbia et al., 2011). Spermatozoa motility is aided by having mucus ducts that can easily penetrate and direct the sperm forward. This situation is related to estrogen's impact during estrus, which regulates glycoprotein macromolecules to reduce the distance among mucus molecules to 2-5 μm. Thus, a duct is formed and sperm can penetrate it (Hafez and Hafez, 2000).

5. Conclusion

According to the findings of this study, alterations in cervical mucus properties, especially SPK, EC, and CRS, can be utilized to foresee estrus time and, as a result, insemination time. Cows with > 13.5 cm SPK and > 15 mS/cm EC of their CM showed the highest pregnancy rate and higher percentage cows. The typical fern pattern was seen in a higher percentage of cows' CM, mainly composed of primary and secondary venation. Further studies should be conducted to explain the heterogeneity of mucus secretion in cows upon estrus synchronization. These findings may also help to update or change insemination practices to get a higher pregnancy rate by inseminating at an accurate periovulatory time in some cow breeds raised in similar climate condition, or similar reproductive characteristics.

Declarations

Author contribution

Mahmoud Yassin Mohamed: Developed the theory, Performed the computations, Software, Writing Original Draft, Writing Review and Editing, Visualization, Supervision. **Ahmed M. Abd El-Hafeez**: Developed the theory, Carried out the experiment, Data curation, Methodology, Writing draft preparation**.** **Mohamed El-said Ibrahim:** Software, Validation Investigation, Formal analysis. **Mohamed H. Ramadan**: Methodology, Writing-Original draft preparation, Investigation**. Amin M.S. Amin:** Designed the model and the computational framework, Conceptualization, Resources, Formal analysis, Visualization. **Ahmed Helal**: Conceived of the presented idea, Planned the experiments, Writing Review and Editing. All authors discussed the results, provided critical feedback and helped shape the research and contributed to the final manuscript.

Data availability

All data generated or analyzed during this study are included in this published article.

Code availability

Not applicable

Declaration of interest

All authors declare that there is no conflict of interest in this study.

Ethical statement

All research procedures were carried out in compliance with the standards set Forth Guidelines for The Care and Use of Experimental Animals by the Animal Ethics Committee of APRI, ARC, Egypt.

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Figures

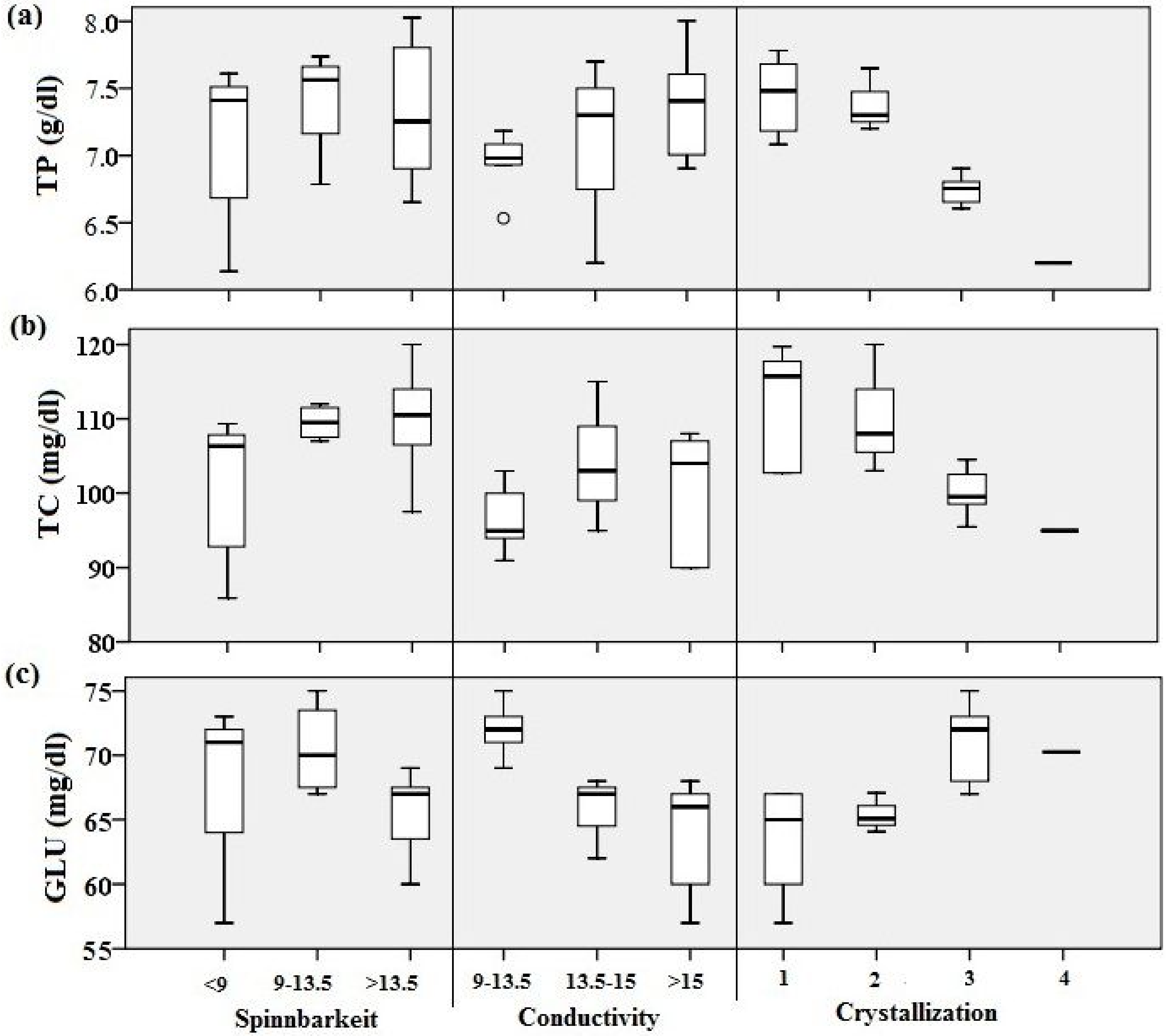

Figure 1

Boxplot analysis of cervical mucus distribution of serum metabolites of Egyptian Baladi cow. The plots show the median (line within box), 25th and 75th percentiles (box), 10th and 90th percentiles (whiskers), and outliers (circles).

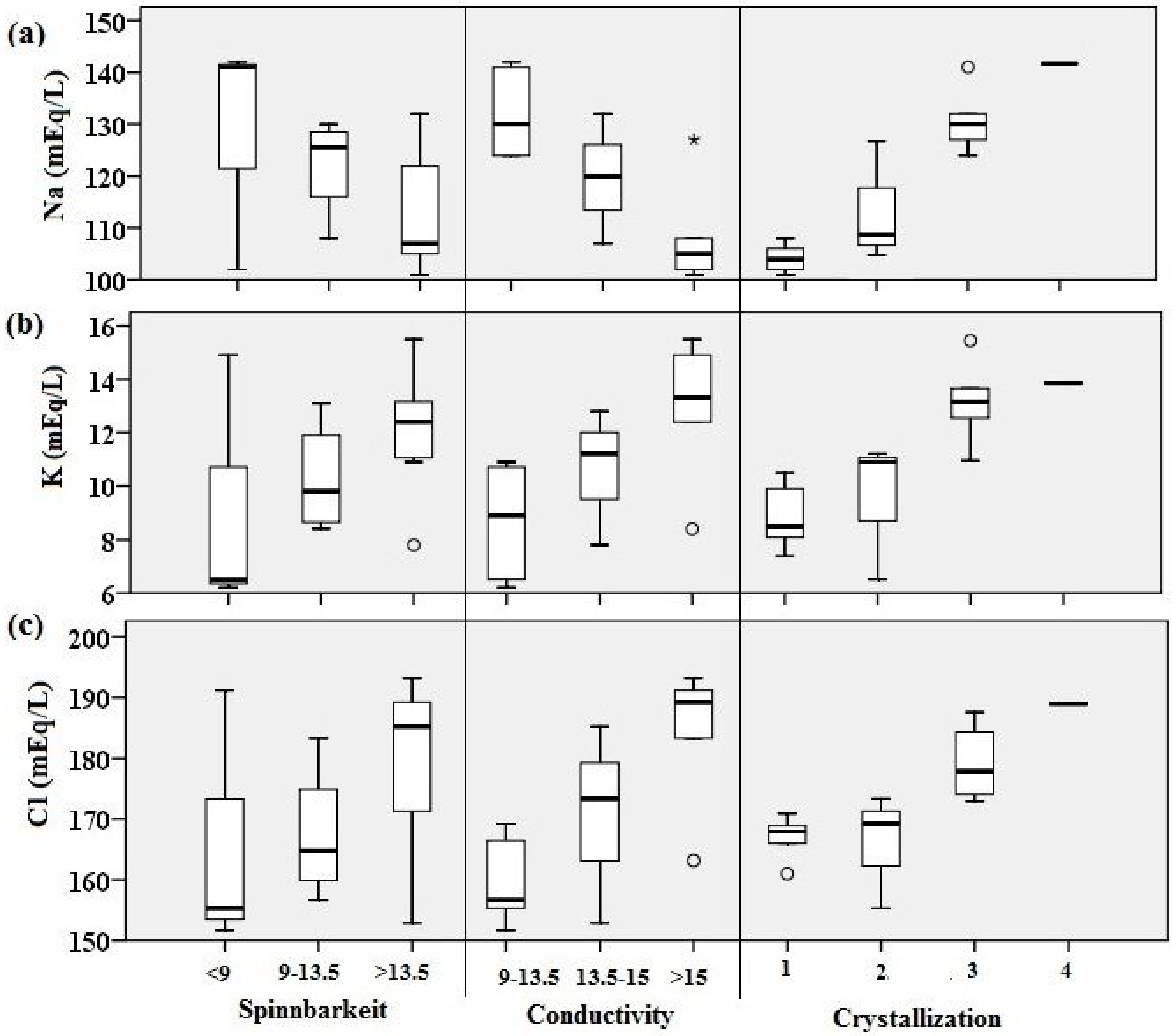

Figure 2

Boxplot analysis of cervical mucus distribution of serum macro-minerals of Egyptian Baladi cow. The plots show the median (line within box), 25th and 75th percentiles (box), 10th and 90th percentiles (whiskers), and outliers (circles).

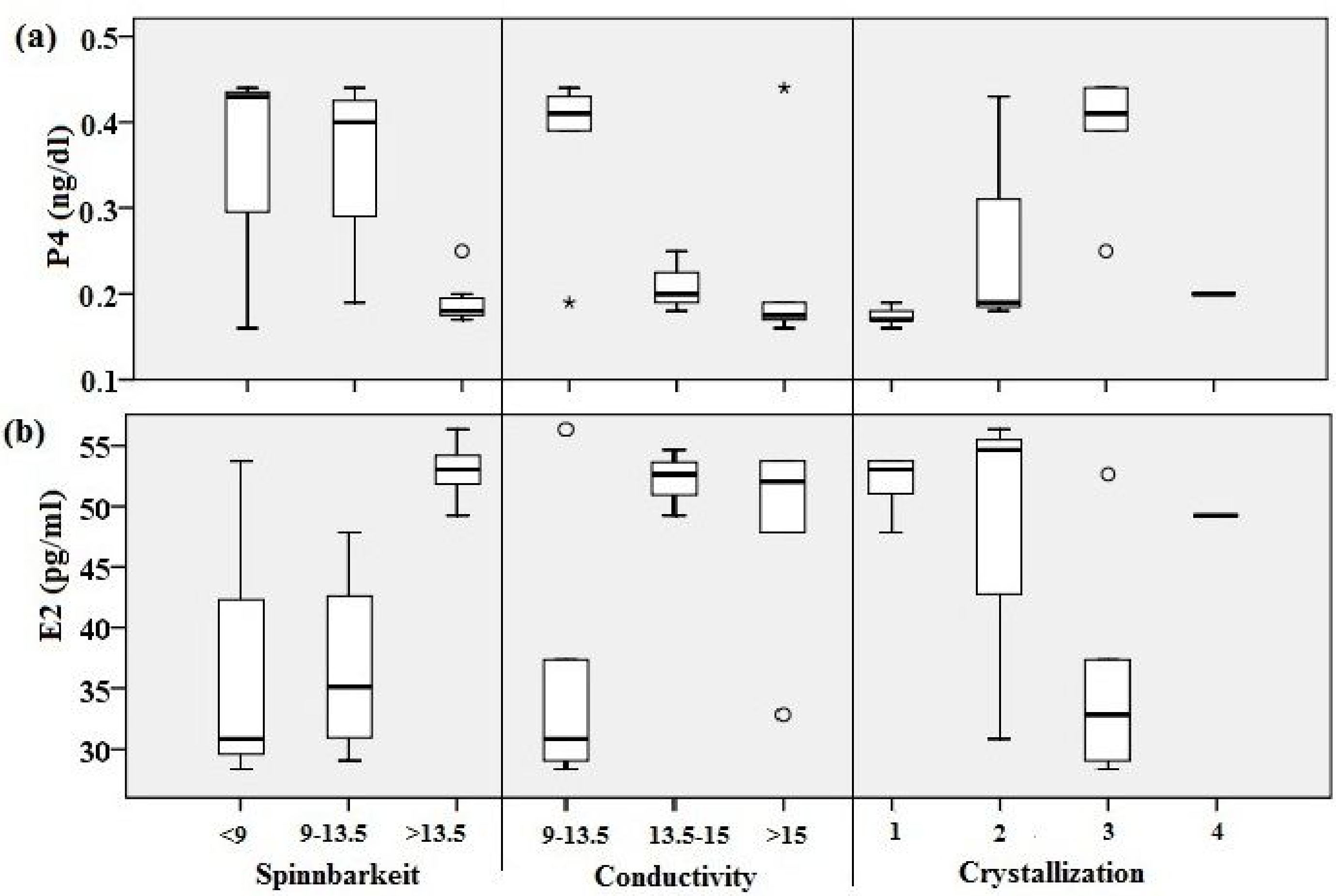

Figure 3

Boxplot analysis of cervical mucus distribution of serum progesterone (P_4, a) and estradiol (E_2, b) hormones of Egyptian Baladi cow. The plots show the median (line within box), 25^{th} and 75^{th} percentiles (box), 10^{th} and 90^{th} percentiles (whiskers), and outliers (circles).