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A Trial For Prediction of Pregnancy, Fetal Viability and Number and Parturition Status Using Estrone Sulphate Profiles In Blood, Saliva and Faeces of Goats

G. Shawki; Abou El-Roos^{*} M.E.A. and S.I. Selim^{**}

^{*}Department of Theriogenology, Fac. Vet. Med., Zagazig Univ., Zagazig

^{*}Department of Theriogenology, Fac. Vet. Med., (Moshtohor) Zagazig Univ., Benha

^{**}Department of Physiology, Cent. Lab., Fac. Vet. Med., Zagazig Univ., Zagazig

ABSTRACT

The objective of this study is to clarify the profiles of estrone sulphate (ES) in maternal blood, saliva and fecal samples of she-goats during pregnancy and trend parturition. The hormonal levels were used as a monitor to assess the advancement of gestation, fetal number and viability. Eighteen she-goats (16 - 24 months) were used in 2 groups. Five animals (non-pregnant) were used as control, and 13 females (naturally mated at estrus) were used as pregnant group. Pregnancy was detected using transabdominal ultrasonography and confirmed by abdominal palpation at 2.5-3 months, and were follow-up till parturition. Peripheral plasma was obtained from all females, also in parallel saliva and faecal samples were collected for ES extraction and measured by RIA assayed.

The estrone sulphate levels in all samples of pregnant goats were greater than 1ng/ml (plasma and saliva) and 1ng/gm (faeces) higher than those of non-pregnant ones. Considering the monthly changes, ES level in pregnant goats were non-significantly increased within at 1st month when compared to those of the non-pregnant ones, thereafter were gradually increased significantly for pregnant during the 2nd (2-3.5 months) and the 3rd (> 3.5 months) trimesters of gestation. The maximum mean values were detected during the 3rd trimester while during the 1st trimester (< 2 month) were significantly low when compared to the other trimesters and non-significant difference with non-pregnant goats. Concerning fetal viability, ES concentration in all samples significantly declined steadily within 4th month of gestation few days before abortion of dead feti when compared than that observed at same stage of females bearing alive kids, the variation between two groups was highly significant. So, it is suggested that a concentration of ES > 3ng/ml plasma, > 2ng/ml saliva and > 2ng/gm faeces is highly indicative of fetal viability at late gestation. Regarding fetal number, maternal ES levels in all samples from the 2nd month were increased progressively to term in singleton (n=2), twin bearing (n=4) and triplet bearing (n=4) goats. There was no difference in plasma, saliva and faecal ES concentrations between singleton and twin bearing goats up to 2nd trimester but was significantly higher in triplet bearing goats than singleton ones. While mean ES levels at 3rd trimester were significantly higher in goats carrying twin and three fetuses than that of singleton ones. Overall, values of this hormone during the 1st trimester useless to predict the number of fetuses, while during 2nd and 3rd trimesters were significantly available to predict of a large litter in goats. The ES profiles in all samples were drastically increased during the early prepartum period (days 7 to 1) reaching a plateau. Furthermore prepartum ES concentrations were significantly lower in dystocial than eutocial goats especially at last 3 days before parturition. Subsequently, ES concentrations increased gradually and linearly as gestation progresses and were thereafter drastically elevated at last week prepartum. It is concluded that the determination of ES in blood, saliva and faeces of goats may be useful for pregnancy diagnosis, predictive value of alive fetal number (= 3 mon) and viability, as well as may be used for clarify the nature of parturition in goats.

INTRODUCTION

Estrogens are a prerequisite for maintenance of pregnancy and initiation of parturition in cattle (1). It is well established that steroid hormones influence the development and maturation of fetus during intrauterine fetal life (2). Most estrogens are produced by the placenta and can be measured in blood (3), urine (4), milk (5), faeces (6) and saliva (7), in

which fetal placental tissue was able to convert both androstendione and testosterone into estrogen in vitro (8). Estrone and estradiol are the major steroids synthesized by the fetoplacental unit in the cow, and most of estrone is immediately conjugated in the placentomes (9,4) among these conjugated steroids, ES is the predominant throughout gestation.

The estrone sulphate is an estrogen metabolite originating mainly in the conceptus

during gestation (10). Meanwhile, it is important to determine the profile of maternal peripheral ES concentrations during early pregnancy in order to confirm normal placental formation, because early embryonic loss during implantation is a serious problem in cattle (11). Recently, the relationship between maternal plasma ES concentration and the condition of parturition, such as dystocia and retention of placenta, and the status of the newborn calf has been analyzed (12,13). In goats, pregnancy testing can be performed by measurement of ES in a milk sample taken within the first half of pregnancy; this test was extremely accurate for distinguished between pregnancy (true or pseudo) and non pregnancy (14).

The detection of saliva and fecal estrogen as a method for pregnancy confirmation would be more preferable than both progesterone and estrone determination in milk or blood because it is much easier method for sampling and it is especially important in primipara where no milk is produced by the udder (15,16,6,7). The fecal ES levels were significantly lower in non-cycling goats than those of cycling ones (6). Meanwhile, salivary ES concentration in pregnant mares was more accurately reflected the reproductive status of the mare (17). While, a few number literatures on the salivary ES are available in goats. The study reported herein was designed to clarify the relationship between ES concentrations in maternal plasma, saliva and faeces to the nature of pregnancy and parturition in she goat.

MATERIALS AND METHODS

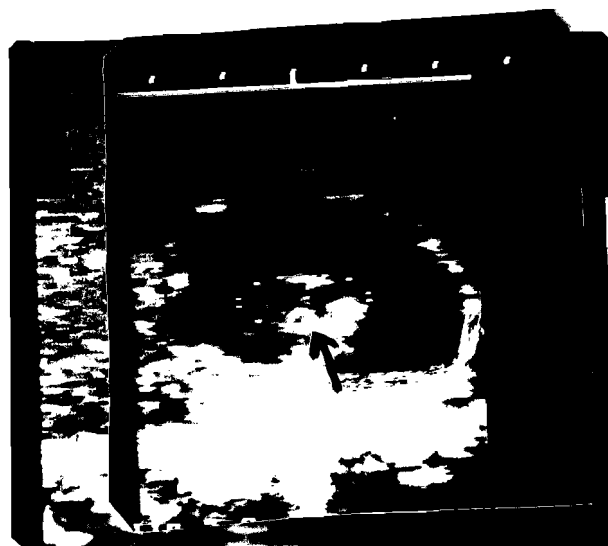
Animals:

A total of 18 balady she goats (aged 16 - 24 months), belonging to a Privet farm at Meniet El-Kamh Center, Sharkia Province, were used in the present work. All animals were good healthy condition, free from venereal diseases and both internal and external parasitic infestation. They were fed on balanced ration in addition to Barseem during winter and hay during summer and all females were naturally mated at estrus. Pregnancy was detected using trans-abdominal ultrasonography (β -mode, real time portable ultrasound machine of Pie-Medical scanner, Eq B V

Head quarters, Maastricht, Holland, Model 200-V) with a 5MHz and 3.5 MHz linear-array transducer was applied 3 and 5 weeks postmating (Fig. 1) as well as the abdominal palpation at 75-90 days postmating was applied. The non-pregnant animals were used as control. While, the pregnant ones, were observed monthly, and the fetal viability, fetal number and nature of parturition (normal or abnormal) were recorded.



(a)



(b)

Figure 1. Ultrasonography of pregnant uterus at 25 days (a) and at 35 days (b) in goat. Note the hyperechoic image (white) which represents the fetus and the surrounding amnion, or its (black) representing the fetal fluid.

Sampling:

Blood plasma, saliva and fecal samples were collected at two weeks interval during the 1st month post-mating from all animals. In pregnant animals, the samples were collected once at the 2nd, 3rd and 4th month of gestation; while during the last month samples were collected at two weeks interval until the expected last week before parturition, then the samples were obtained daily until parturition.

Peripheral blood (10 ml) was collected via jugular vein puncture into heparinized tubes were placed immediately on ice, then centrifuged (1700 xg/10 minutes), the harvested plasma was stored at -20 °C until hormonal analysis. Saliva samples were collected using a wooden chopstick (15-20 cm long) tipped with 2 gm absorbent cotton inserted into the mouth, when the cotton was soaked with sufficient amounts of saliva it removed from the stick and compressed in a 10-20 ml disposable syringe to squeeze the saliva from the cotton. Saliva samples obtained were mixed with sodium azide (5 mg/ml) and kept frozen at -20 °C until hormonal extraction and assay (7). Fecal samples (5-10 gm) were collected into plastic pages and immediately placed on ice, then were stored at -20 °C until hormonal extraction. 0.5 gm faeces mixed with 0.5 ml water and 4 ml methanol were vortexed for 3 minutes; then 3 ml petroleum ether was added and vortexed for 10 seconds. After centrifugation at 1500 xg/10 minutes, 0.2 ml of methanol extract was transferred into a new vial then diluted with 0.6 ml distilled water and 5 ml of petroleum ether/ diethyl ether (v/v 9:1). The mixture was vortexed for 3 minutes then ether layer was evaporated at 40 °C, later the residue was diluted with 1 ml buffer and stored at -20 °C until hormonal assay (18,19).

Hormonal assay

Plasma, fecal and salivary ES concentrations were measured using RIA (20,7). Estrone sulphate was measured by RIA with [6-7-³H (N)]-estrone sulphate (Du Pont

New England Nuclear, Research Products, Boston, USA) and a specific antibody (anti-estrone-3-sulphate-6-carboxymethyloxime-BS rabbit serum; Sigma Chemicals C./ St. Louis UAS) (20,21). Antibody solution was prepared at a dilution of 1:40000 with phosphate buffered saline (PBS) containing 0.25% of normal rabbit serum and 50 mM of ethylene diamine tetracetic acid (Dojin Co.), pH was adjusted at 7.1. Intra- and interassay coefficient of variance (CV) of estrone sulphate were 10% and 14% respectively, and the sensitivity of the assay was 1.0 pg/ml⁻¹.

Data analysis:

Differences between comparable groups were demonstrated with student "t" test. All computations were done using personal computer, using statistical programmed SPSS/PC 3.1 of SPSS Inc.

RESULTS**The ES during pregnancy**

The results obtained were presented in Tables 1 and 2. There was no difference in plasma, saliva and fecal ES levels between non pregnant and pregnant goats within the 1st month of gestation, then showed progressive increments of ES concentrations were noticed in all samples with advancement of pregnancy. Overall, there was a significant ($P < 0.05$) elevated ES levels in pregnant goats when compared with non-pregnant ones. The concentration of ES started to increase around the 2nd month of gestation and then elevated at the 3rd month in blood plasma (Table 1 and Figure 2); while in saliva and fecal samples it was increased at the 3rd month. On the other hand, ES levels were gradually increased from the 3rd to 5th months of gestation. The ES concentrations in all samples during the 1st, 2nd and 3rd trimesters of gestation are shown in Table (2) and Figure (3). The ES levels were significantly increases at the 2nd and 3rd trimesters when compared with those during the 1st trimester. While, the hormonal profiles

during the 1st trimester showed no change than those for non pregnant ones except for fecal samples ($P < 0.05$).

The ES profiles in relation to fetal viability:

The relationship between ES concentrations and fetal viability are shown in Table (3) and Figure (3). Estrone sulphate concentrations in all samples were gradually increased during the first 3 months of gestation for dams bearing alive and dead feti. There was no variation between them up to the 3rd month. Subsequently, ES levels in samples at 4th month of gestation were significantly ($P < 0.05$) higher for dams carrying alive feti than those in goats aborted dead feti. The mean values of ES were 1.05 ng/ml plasma, 0.82 ng/ml saliva and 0.90 ng/gm faeces for dams aborted dead feti within 4th month (few days before abortion), while the data for dams bearing alive feti at the same period were 3.73 ng/ml plasma, 2.14 ng/ml saliva and 2.38 ng/gm faeces.

The ES levels in relation to fetal number:

Table (4) showed no variation in ES concentrations in all samples between singleton, twin and triplet bearing goat within

the 1st trimester and non pregnant ones. Moreover, plasma level of ES were significantly higher ($P < 0.01$) for triplet than twin and singleton bearing dams within the 2nd trimester of gestation, but no difference between twin and singleton pregnancies for ES levels. There were significant variation in ES level between singleton, twin and triplet pregnancies during the 3rd trimester of gestation Table (4).

Prepartum ES profile and its relation to nature of parturition

From the present data showed in Tables (5 and 6) and Figure (5). There were drastic elevation for ES in all samples during early prepartum period (days 7 to 1). In respect to the nature of parturition, prepartum ES levels were significantly ($P < 0.05$) lower in dystocial (that animals need interference during parturition from traction till caesarian section) than eutocial (that animal delivered without help) goats within the last 3 days before parturition, and there were no changes between dystocial and eutocial animals observed between day -7 and -4 prepartum Table (6).

Table 1: Monthly changes in estrone sulphate concentrations in plasma (ng/ml), saliva (ng/ml) and faeces (ng/gm) in pregnant goats (mean \pm SEM).

Animals	Plasma	Saliva	Faeces
Non-pregnant	0.36 \pm 0.05	0.17 \pm 0.02	0.19 \pm 0.08
Pregnant	2.79 \pm 0.30*	1.54 \pm 0.21*	2.11 \pm 0.22*
1 st month	0.61 \pm 0.24	0.28 \pm 0.09	0.35 \pm 0.08
2 nd month	1.26 \pm 0.13 ^a	0.56 \pm 0.17*	0.66 \pm 0.15*
3 rd month	2.73 \pm 0.30 ^a	1.63 \pm 0.28 ^a	2.15 \pm 0.18 ^a
4 th month	3.68 \pm 0.48 ^a	2.03 \pm 0.33 ^a	2.64 \pm 0.40 ^a
5 th month	5.66 \pm 0.54 ^a	3.20 \pm 0.34 ^a	4.75 \pm 0.47 ^a

• :Excluding the last week before parturition and aborted animals (3).

* :Significant from non-pregnant at the same column at $P < 0.05$.

a :Significant from the 1st month in the same column at $P < 0.05$.

Table 2: Estrone sulphate concentrations in plasma (ng/ml), saliva (ng/ml) and faeces (ng/gm) in relation to the trimesters of pregnant goats (mean \pm SEM).

Animals	Plasma	Saliva	Faeces
Non-pregnant	0.36 \pm 0.05	0.17 \pm 0.02	0.19 \pm 0.08
Pregnant:			
1 st trimester (< 2 months)	0.66 \pm 0.24	0.28 \pm 0.09	0.54 \pm 0.08*
2 nd trimester (2-3 months)	2.00 \pm 0.22 ^a	1.10 \pm 0.18 ^a	1.39 \pm 0.15 ^a
3 rd trimester (> 3.5 months)	4.67 \pm 0.51 ^a	2.62 \pm 0.34 ^a	3.05 \pm 0.45 ^a

• :Excluding the last week before parturition and aborted animals (3).

* :Significantly from non-pregnant at the same column at P < 0.05.

a :Significant from the 1st trimester in the same column at P < 0.05.

Table 3: Estrone sulphate concentrations in plasma (ng/ml), saliva (ng/ml) and faeces (ng/gm) in relation to fetal viability in goats (mean \pm SEM).

Samples	Alive feti					Dead feti			
	Months of gestation					Months of gestation			
	1 st	2 nd	3 rd	4 th	5 th •	1 st	2 nd	3 rd	4 th ••
Plasma	0.51 \pm 0.05	1.09 \pm 0.14	2.69 \pm 0.33	3.73 \pm 0.55	6.02 \pm 0.44	0.56 \pm 0.09	1.13 \pm 0.35	1.60 \pm 0.47	1.05 \pm 0.6*
Saliva	0.31 \pm 0.0*	0.54 \pm 0. 0*	1.53 \pm 0.25	2.14 \pm 0.32	3.33 \pm 0.31	0.26 \pm 0.02	0.61 \pm 0.06	0.92 \pm 0. 29	0.82 \pm 0.23*
Faeces	0.36 \pm 0.0 ^a	0.64 \pm 0.0*	1.78 \pm 0.24	2.38 \pm 0.41	3.83 \pm 0.02	0.40 \pm 0.02	0.59 \pm 0.19	1.20 \pm 0.35	0.90 \pm 0.22*

• :Excluding the expected last week before parturition.

••: Dead foeti occurred at 4th month.

* :Significant from the same month in the same row at P < 0.05.

Table 4: Estrone sulphate concentrations in plasma (ng/ml), saliva (ng/ml) and faeces (ng/gm) in relation to the number of alive feti in goats (mean \pm SEM).

Samples	Trimesters of pregnancy and number of alive foeti.									Non pregnant
	1 st trimester (< 2months)			2 nd trimester (2-3.52months)			3 rd trimester (> 3.5 months)•			
	Single-tone	Twin	Triplet	Single-tone	Twin	Triplet	Single-tone	Twin	Triplet	
Plasma	0.51 \pm 0.20**	0.69 \pm 0.12	0.78 \pm 0.17	1.63 \pm 0.21	2.20 \pm 0.29	3.15 \pm 0.33**	3.15 \pm 0.17	4.10 \pm 0.31*	5.08 \pm 0.47**	0.36 \pm 0.05
Saliva	0.29 \pm 0.08	0.38 \pm 0.06	0.41 \pm 0.05	0.87 \pm 0.14	1.30 \pm 0.20	1.57 \pm 0.16**	1.66 \pm 0.13	1.98 \pm 0.2	2.63 \pm 0.22**	0.17 \pm 0.02
Faeces	0.23 \pm 0.06	0.46 \pm 0.11	0.66 \pm 0.12	1.10 \pm 0.15	2.18 \pm 0.48	2.13 \pm 0.21**	2.05 \pm 0.20	3.86 \pm 0.56*	4.50 \pm 0.68**	0.19 \pm 0.08

• :Excluding the last week before parturition.

* :Significant from single in the same trimester in the same row at P < 0.05.

** :Significant from single in their trimester in the same row at P < 0.01.

Table 5: Estrone sulphate concentrations in plasma (ng/ml), saliva (ng/ml) and faeces (ng/gm) during the expected last week before parturition in goats (mean \pm SEM).

Days before parturition	Plasma	Saliva	Faeces
-7 days	6.69 \pm 0.52	3.55 \pm 0.20	4.66 \pm 0.33
-6 days	7.01 \pm 0.63	3.22 \pm 0.21	5.10 \pm 0.24
-5 days	7.94 \pm 0.48	4.30 \pm 0.25	5.24 \pm 0.44
-4 days	7.72 \pm 0.56	4.21 \pm 0.38	5.71 \pm 0.32
-3 days	8.49 \pm 0.42*	4.79 \pm 0.34	6.86 \pm 0.44
-2 days	8.85 \pm 0.43*	4.86 \pm 0.27	6.49 \pm 0.41
-1 or 0	9.49 \pm 0.29*	5.89 \pm 0.09	7.71 \pm 0.23

0 : Day of parturition.

* : Significant from the 7th day in the same column at $P < 0.05$.

Table 6: Estrone sulphate concentrations in plasma (ng/ml), saliva (ng/ml) and faeces (ng/gm) in normal and abnormal parturition in goats (mean \pm SEM).

days prepartum	Plasma		Saliva		Faeces	
	Eut.	Dyst.	Eut.	Dyst.	Eut.	Dyst.
-7	6.90 \pm 0.69	4.68 \pm 0.52*	3.53 \pm 0.28	2.08 \pm 0.21*	4.47 \pm 0.41	3.15 \pm 0.44*
-6	6.58 \pm 0.93	5.03 \pm 0.82	3.22 \pm 0.30	2.23 \pm 0.27*	4.62 \pm 0.43	2.52 \pm 0.29*
-5	7.98 \pm 0.89	5.05 \pm 0.42*	4.30 \pm 0.35	3.65 \pm 0.22 ^a	4.87 \pm 0.57	4.10 \pm 0.23 ^a
-4	7.57 \pm 0.84	6.05 \pm 0.47	4.40 \pm 0.46	2.95 \pm 0.10 ^{a*}	5.70 \pm 0.46	3.65 \pm 0.54*
-3	8.9 \pm 0.64 ^a	5.98 \pm 0.37*	4.52 \pm 0.49	3.13 \pm 0.34 ^{a*}	6.25 \pm 0.57 ^a	4.55 \pm 0.39 ^{a*}
-2	9.10 \pm 0.62 ^a	4.88 \pm 0.36*	4.8 \pm 0.42 ^a	2.98 \pm 0.51	6.33 \pm 0.52 ^a	3.28 \pm 0.41*
-1 to 0	9.67 \pm 0.42 ^a	4.68 \pm 0.20	5.88 \pm 0.12 ^a	2.80 \pm 0.43*	7.37 \pm 0.33 ^{a-}	3.18 \pm 0.38*

Eut.: Eutocial cases (normal parturition).

Dyst.: Dystocial cases (abnormal parturition).

* Significant change from normal in the same sample and row at $P < 0.05$.

a: Significant change from -7 in the same column at $P < 0.05$.

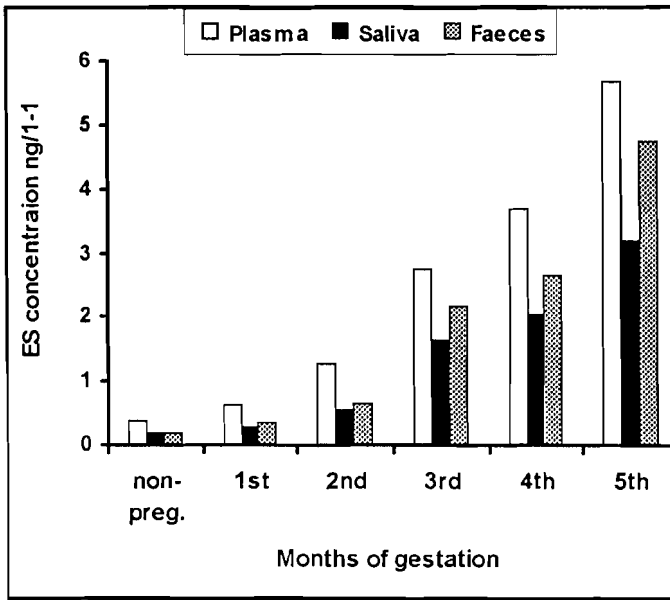


Figure. 2. Monthly changes of ES concentrations in plasma (ng/ml), saliva (ng/ml) and faeces (ng/gm) in pregnant goats

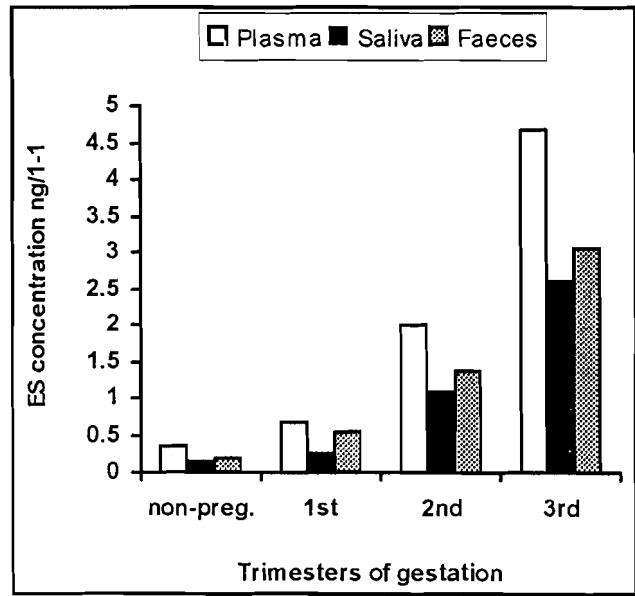


Figure. 3. Levels of ES in plasma (ng/ml), saliva (ng/ml) and faeces (ng/gm) in relation to trimesters of gestation in goats.

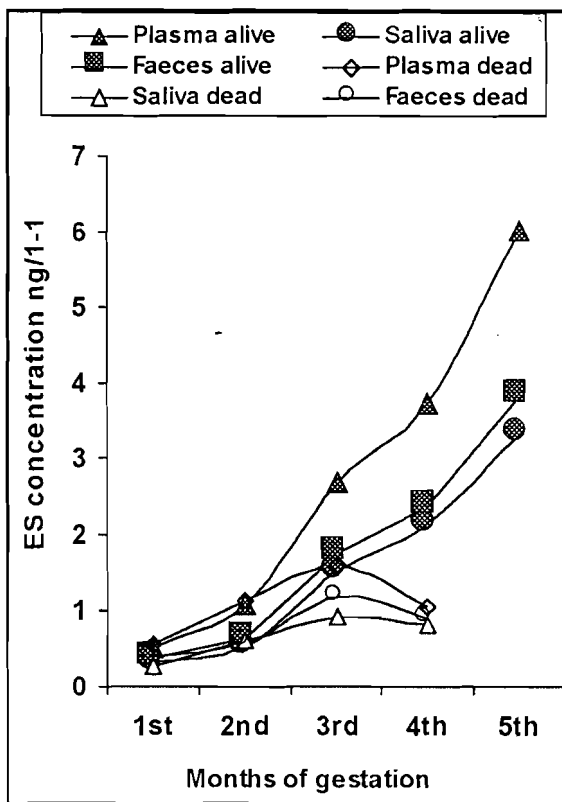


Figure 4. The profiles of ES in plasma (ng/ml), saliva (ng/ml) and faeces (ng/gm) in relation to fetal viability in goats

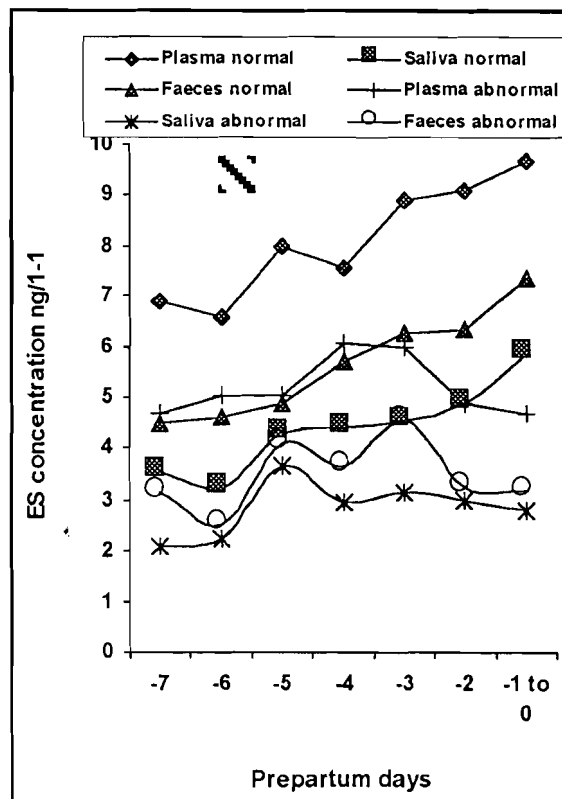


Figure. 5. The levels of ES in plasma (ng/ml), saliva (ng/ml) and faeces (ng/gm) early prepartum in relation to the nature of parturition.

DISCUSSION

The identification of substances produced by the conceptus and its placenta would have particular value for early detection of pregnancy and fetal status in domestic animals and in woman. The accuracy of progesterone profile was 80% in the pregnant cows because the progesterone is derived from CL not from the placenta or conceptus (3,11,22,23,24,25). So it may lead to false diagnosis of pregnancy, while, estrone sulphate (ES) is related to the development of the endocrine properties of the placenta and presumably of a viable conceptus, so, the levels of ES may have a higher diagnostic and predictive value for pregnancy status (22). Over 90% of ES is aqueous solubility, so that ES can be estimated in most maternal body fluids (22,14,15,17,7)

This investigation was carried out to detect ES concentrations in maternal plasma, saliva and faeces in goats post-mating till term to clarify the pregnancy, fetal number and viability also the nature of parturition. With regard to pregnancy, Tables (1 and 2) shows no change in ES level within 1st month and 1st trimester of pregnant goats compared with non-pregnant ones, these were concomitantly of (14,26,27,28), they revealed that ES in pregnant goat were increased from days 30-50, 36, 45 and 60 respectively. Our findings were good in agreement with reported by Refsal, *et al.* (29) they found serum ES level was no change during 1st trimester of pregnant goats when compared to non-pregnants. Also, the fecal ES level were significantly higher in pregnant goats than those in non-pregnant ones during 5th to 6th week of gestation (6). Likewise, it was reported that the bovine plasma ES level was detected around first 50 days of gestation with the sensitive assay system (11,21), while in sow the plasma ES levels were in measurable amounts around day 25 of gestation allowing early confirmation of pregnancy (30). Also plasma ES levels could be a useful indicator of pregnancy within the 1st trimester of gestation in cows (11). In this study ES levels were significantly elevated

compared with non-pregnant goats beginning from the 2nd month to the 5th month of gestation and/ or from the 2nd and 3rd trimester of gestation, similar findings were recorded previously by various investigators (2,31,32). It was reported that the accuracy of gestation based upon ES levels was 100% in the milk samples of pregnant goats (33) and in serum samples (6) at the same period of pregnant animals, the level of ES increased from 2-6 ng/ml (2nd month) to 12.9 ng/ml (5th month), these data were slightly higher than those of our results. Meanwhile, the present results were lower than those of plasma ES at the 2nd and 5th month of gestation in goats (28), while were higher than those in ewes at the same period of gestation (36). The salivary levels of ES in pregnant mares (17) were higher than those our results, and similar findings for fecal ES levels from the 2nd trimester of gestation in mares were recorded also (34,35). While, our findings were higher than that reported previously (36) for plasma ES levels in ewes at same period of gestation.

In respect of fetal viability, the results showed in Table (3) revealed a no change for ES levels in all samples between animal bearing alive or dead feti within the first 3 months of gestation, while at the 4th month, in which the dead feti were aborted, ES levels were significantly decreases than those in goats delivered alive healthy kids. Similarly, it was showed that the plasma ES levels decreased slightly a few days before abortion and were similar to that recorded in non-pregnant goats when estimated at and after abortion (37,38). Whether placental dysfunction or embryonic death is reflected in reduced concentration of ES in bovine milk, so, the levels of ES may had a diagnostic and predictive value for bovine fetal viability (22); also (25) urinary estriol is used in women to evaluate the endocrine function of the feto-placental unit (22). The ES levels in bovine milk could provide strong evidence for the presence of a viable conceptus (5), and plasma ES in sheep (36).

Concerning, fetal numbers, our results present in Table (4), showed no difference in plasma, saliva and fecal ES concentrations between singleton, twin and triplet bearing goats within the 1st trimester, thereafter, the levels of ES were significantly ($P < 0.01$) higher for 3 fetuses carrying dams than twin and singleton within the 2nd trimester but no difference in ES levels between singleton and twin bearing goat at the same period. Subsequently, our results added a significant variations for ES levels in all samples between singleton, twin and triplet pregnancies within the 3rd trimester and the variations were highly significant ($P < 0.01$) and significant ($P < 0.05$) between triplet and singleton, twin and singleton respectively. Our results were similar with others as (32,39) who indicated goat's serum level of ES was up to 2 ng/ml in females carrying (1-2 fetuses) and up to 4.6 ng/ml in females carrying (3-more fetuses) within the 2nd trimester, and was 6.9 ng/ml for triplet bearing goats at the end of the 3rd trimester. Also, it was reported that fecal ES levels were significantly higher in goats carrying twin than those carrying single kid (6). Similarly the plasma levels of ES was significantly higher in twin than singletons in goats (40), however they suggested that plasma ES determination isn't reliable for the prediction of number of fetuses due to the high variation between animals. It shown no difference for plasma ES level between ewes with single and multiple fetuses during mid pregnancy, but during 3rd trimester there were significant difference between ewes carrying single, twin and triplet fetuses, so they used ES level in plasma as a possible indicator of the number of viable fetuses in ewes (36). These findings were similar to that obtained previously (24) the level of bovine plasma ES was significantly higher for twin bearing cows than singleton bearing ones. This suggested that most of unconjugated estrogens synthesized by the fetoplacental unit are immediately conjugated to sulphate in the placentomes; this could be a precautionary measure so as to ameliorate any deleterious effect of excessive unconjugated estrogen on maternal tissues during gestation (24). In early stage of gestation, the conceptus is assumed to be the main source of

unconjugated estrogen for the endometrial sulfotransferase enzyme (41), consequently, since the conceptus is the source for the enzyme's substrate estimation of plasma ES levels allows the prediction of the litter size in the sow (42). In agreement, (9) showed twin bearing cows were had higher ES levels during late gestation when compared to singleton cows, this could be directly attributed to the presence of twin placenti and acquisition of enhanced steroidogenic activity with placental growth and development.

From the present data showed in Tables (5, 6) the levels of ES in all samples were drastically increased during the early prepartum period (days 7 to 1). Furthermore, prepartum ES concentrations were significantly lower in dystocia than eutocia goats especially at the last 3 days before parturition. Results almost similar were observed during the last 10 days preceding parturition in goats (26), and were up to 12 ng/ml plasma before parturition (27). As well the levels of ES in maternal body fluids of cows increase progressively throughout gestation to peak at parturition (18,43). Likewise, as observed in the present study (12) plasma ES concentrations 3 to 6 days prepartum was higher in eutocia cattle than in dystocia cattle especially during last 3 days prepartum. Estrogens stimulate $\text{PGF}_{2\alpha}$ synthesis from placental membranes and myometrium and correlated positively with relaxation of the pelvic ligaments prepartum (44,45). Therefore, these results suggest that a decrease in secretion rate of ES from the fetoplacental unit before kid delivery, may result in falling maternal ES below the level necessary for normal estrogen control of parturition, e.g. of myometrial activity, cervical dilatation, fetal behavior, righting reflexes and late correctional movements, resulting in difficult parturition.

In the present study the measurement of estrone sulphate concentration in plasma, saliva and faeces of goats provide a good tool for pregnancy detection, in addition to prediction of fetal viability and number. Also, the insufficient production of ES prepartum in our results might be used for expectation of dystocia in goats.

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الملخص العربي

محاولة للتنبؤ عن الحمل وعدد الأجنة وحيويتهم وكذا طبيعة الولادة على أساس مستوى سلفات الإسترون في بلازما ولعاب وروث إناث الماعز

جمال شوقي - محمود السيد عابد أبو الروس* - صلاح إبراهيم سليم**

قسم التوليد والتناسل-كلية الطب البيطري بالزقازيق ومشتهر فرع بنها*

المعمل المركزي-قسم الفسيولوجيا- كلية الطب البيطري بالزقازيق**

تهدف هذه الدراسة إلى توضيح مستويات سلفات الاسترون في بلازما إناث الماعز وكذا في اللعاب والروث وقياسه بطريقة المناعة الإشعاعية أثناء فترات الحمل حتى الولادة وإيجاد العلاقة بين مستوياته بالحمل وتقدمه وعدد وحيوية الأجنة وكذا طبيعة الولادة (طبيعية أو عسرة). لذلك استخدم عدد ١٨ من إناث الماعز تتراوح أعمارهم من ١٦ إلى ٢٤ شهراً، منهم ٥ حيوانات غير عشار و١٣ حيوان عشار بعد توثيهم طبيعياً أثناء فترة الشبق ومعرفة الحمل باستخدام الموجات فوق صوتية في البداية ثم الجس عن طريق البطن بعد ذلك (٢،٥-٣ شهور) بعد التوثيب، ثم لوحظت الحيوانات من التوثيب حتى الولادة. أخذت عينات البلازما واللعاب والروث بمعدل (عينة واحدة/أسبوعاً أثناء الشهر الأول ثم عينة شهريا حتى الشهر الخامس ثم عينة يومياً أثناء الأسبوع الأخير قبل الولادة).

وأظهرت النتائج زيادة معنوية في مستوى الأسترون سلفات في كل العينات للحيوانات العشار عنه في الحيوانات الغير عشار. وبالنسبة للتغيرات الشهرية في مستويات الهرمون لوحظ أن الفرق غير معنويًا أثناء الشهر الأول بين الحيوانات العشار والغير عشار، ثم ارتفع مستوياته تدريجياً ومعنويًا من الشهر الثاني حتى الخامس وكانت أعلى مستوياته في الثلث الأخير من الحمل (٤-٥ شهراً) وكان ٤,٦٧ نجم/ملل بلازما، ٢,٦٢ نجم/ملل لعاب و ٣,٠٥ نجم/جم روث. وبالنسبة لحيوية الأجنة أظهرت النتائج انخفاض معنويًا في مستوى الهرمون في جميع العينات التي أخذت من أمهات تحمل أجنة ميتة وقد أجهضت بعد ذلك ولكن في حالة الأجنة الحية لم ينخفض الهرمون بل ارتفع مستوياته. لذلك إذا كان مستوى سلفات الإسترون أكثر من (٣ نجم/ملل بلازما، ٢ نجم/ملل لعاب أو ٢ نجم/جم روث) أثناء الثلث الأخير من الحمل قد يكون دليل على حيوية الأجنة. وبالنسبة لعدد الأجنة أثناء الحمل وجد زيادة معنوية في مستوى الهرمون في بداية النصف الثاني من الحمل في حالة حمل ٣ أجنة عنه في الجنين الواحد وكانت الزيادة غير معنوية مع التوأم وأيضاً غير معنوية بين التوأم والجنين الواحد في هذه الفترة من الحمل. ولكن أثناء الثلث الأخير من الحمل (٤-٥ شهور) كانت الزيادة معنوية في ٣ أجنة عنه في التوأم، وأيضاً في حمل التوأم عن الجنين الواحد. لذلك أهمية هذا الهرمون لمعرفة عدد الأجنة أثناء الثلث الأخير من الحمل عند معرفة مستوياته في البلازما أو اللعاب أو الروث. لوحظ أثناء الأسبوع الأخير قبل الولادة ارتفاع شديد تدريجياً في مستوى الهرمون وكان أعلى مستوياته ٣ أيام قبل الولادة في حالات الولادات الطبيعية عنه في حالات الولادات الغير طبيعية حيث انخفض مستوى الهرمون حوالي ٣ أيام قبل الولادة وكان الفرق معنوياً.

نستخلص من هذه الدراسة أن معرفة مستويات سلفات الأسترون في بلازما أو لعاب أو روث إناث الماعز يستخدم كوسيلة جيدة في تشخيص الحمل وكذا حيوية وعدد الأجنة (أثناء النصف الثاني من الحمل)، وأيضاً أن معرفة نقص مستويات سلفات الأسترون خلال الأسبوع الأخير من الحمل قد تشير إلى توقع حدوث عسر الولادة.