Investigation of the availability of vaginal electrical resistance during estrus synchronization in ewes

KOLDAŞ ÜRER ECE[1]*, KÖSE AYŞE M.[1], GÖZER AHMET[1], BAHAN ONUR[1]

[1] Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Mustafa Kemal University, Hatay, Turkey

SUMMARY

Vaginal electrical resistance (VER) value measurement, which is a noninvasive method, is used to determine the estrus, appropriate insemination or mating time in sheep. The aim of this study was to investigate the availability of vaginal resistance values to evaluate the success of estrus synchronization program and to estimate early pregnant and returning ewes. Besides, it was also aimed to reveal the pregnant and non-pregnant ewes during the first trimester of pregnancy. Thirty-four healthy Awassi ewes were used in the study. Intravaginal sponges were inserted to ewes for 11 days and 0.075 mg d-cloprostenol was injected intramuscularly at the day of sponge withdrawn. Ewes were mated with fertile rams in estrus. Key times of the study were the day sponge insertion (D_0), the day of estrus (D_E), sixty hours after sponge removal (h_{60}), eighteen days after estrus ($D18_{pe}$), thirty-five days ($D35_{pe}$) and fifty days after estrus ($D50_{pe}$). VER value was measured on D_0, D_E, h_{60}, $D18_{pe}$, $D35_{pe}$ and $D50_{pe}$. Progesterone concentration was measured from blood samples of D_0, D_E and $D18_{pe}$. The change in VER value and progesterone concentration on different days (D_0, D_E, $D18_{pe}$) of the estrus synchronization protocol was significant ($P<0.001$). While VER measurement was not significant in the early diagnosis of pregnancy ($P>0.05$) on $D18_{pe}$, it was found to be significant ($P<0.05$) in identification of returning ewes. VER cut-off value was found to be <255 ohm for determination of returning ewes. There was no significant ($P>0.05$) difference in VER values of pregnant and non-pregnant ewes at $D35_{pe}$ or $D50_{pe}$. As a result, VER value measurement in the first trimester of pregnancy does not seem to be useful in determining both of the presence and the day of pregnancy. On the other hand, VER measurement can be useful in evaluating synchronization success and determining the first spontaneous estrus after induced estrus in the absence of teaser rams under field conditions.

KEY WORDS

Vaginal impedance, pregnancy, returning ewe.

INTRODUCTION

Sheep are seasonal polyestrous animals that cycling every 16-18 days during the breeding season. Awassi sheep may not be considered a prolific breed due to low litter size, approximately 1.08 [1]. Therefore, it can be said that there is a high demand for fertility programs, whether professional or conventional breeding models. The average duration of estrus is 35-45 hours[2], and it's difficult to detect as in other sheep breeds. The importance of estrus detection increases with the intensive applies of estrus synchronization and artificial insemination in sheep. The only accurate indication on the field that a sheep stands estrus is acceptance of a ram in mounting. Techniques have been developed by using this indicator such as vasectomized teaser males[3], marker crayon on the male[4], electronic mount detectors[5] and video tracking of behavioral patterns[6]. However, there are difficulties in applying each method in field conditions associated with increased herd size[5]. It has been suggested that the general condition of the rams, the ratio of rams to ewes in the herd, the competition between rams for estrus ewes may change the reliability of the use of teaser rams in detecting estrus[7].

The most informative method to evaluate female's fertility is to measure steroidal hormones in the bloodstream[8]. However, it's rarely applied due to the individual handling of each animal, the extra cost of analysis, and the need for professional staff. Therefore, it seems more advantageous to use noninvasive methods following the reflection of steroidal hormones in the detection of estrus. During estrus, the NaCl level of the vaginal mucus increases with the effect of estrogen, adrenocorticotropic hormone and aldosterone, which increases the vaginal electrical resistance (VER)[9]. VER value was frequently used to detect estrus or ovulation and proper time of insemination or mating in many species[10-13].

We hypnotized that the VER measurement, which is an available method in the field conditions, can be used in many stages of the estrus synchronization program. Therefore, three consecutive phases of a single synchronization program were evaluated for the purposes of the study: i) to determine the mean VER values and serum progesterone (P4) concentrations of ewes on the key times of the estrus synchronization protocol, ii) to investigate the use of VER values in the detection of pregnant,

Corresponding Author:
Ece Koldaş Ürer (ecekoldas@gmail.com, ecekoldas@mku.edu.tr).

non-pregnant and estrus ewes before the ultrasound imaging, and iii) to compare the VER values of ewes in first trimester of pregnancy with non-pregnant ewes. In other words, it was aimed to demonstrate the effectiveness of VER measurements in evaluating the success of estrus synchronization, in the early diagnosis of ewes that conceive or not after synchronization, and in revealing pregnant ewes on the first trimester and non-pregnant ewes.

MATERIALS AND METHODS

This study was conducted in Belen, Hatay province in eastern Turkey, in the breeding season (late June to July). The study was approved by the local animal research ethics committee of Hatay Mustafa Kemal University, with the approval number of 2021/06-07.

Animals and Management

Thirty-four healthy Awassi ewes with a mean body weight of 42-47 kg, aged 3-5 years were used. Ewes were housed in a semi-enclosed shelter with open air ventilation system, away from rams and allowed 8-12 hours of natural grazing. Ewes were received regular antiparasitic treatments (Okzan®, Ceva, Turkey; Blotic %7®, Topkim, Turkey) and vaccinations (Biocan-R®, Interhas, Turkey; Supervac-9®, Vetal, Turkey; Brudoll-M®, Dollvet, Turkey; Poxvac®, Vetal, Turkey).

Study Design

Estrus was synchronized with Medroxyprogesterone acetate (MAP) impregnated intravaginal sponges in 34 ewes. After the sponge withdrawal, rams joined the flock for 3 days. Blood samples were collected from all ewes for serum P4 measurement at the time of sponge insertion (D_0) and on the day of estrus (D_E). VER values of ewes were measured and noted at D_0, D_E and 60 hours after sponge removal (h_{60}). 18th days following the estrus ($D18_{pe}$), blood samples were collected from all ewes and VER values were also measured and noted on the same day. Subsequently, at 35 days after estrus ($D35_{pe}$) and 50 days after estrus ($D50_{pe}$), ultrasound imaging for pregnancy diagnosis was performed and VER values were measured. Estrus synchronization protocol and whole study design are given in Fig. 1. To illuminate the points where VER measurement can be used in the synchronization protocol, the experiment was designed as follows;

Evaluation of the success of estrus synchronization: Key times were considered D_0, D_E, and h_{60}. The variation of mean serum P4 concentrations (on D_0, D_E, and D18pe) and VER values (on D_0, D_E, h_{60}, and $D18_{pe}$) of ewes were evaluated.

Identification of returning ewes after synchronization: The usability of the VER value in determining the returning ewes was investigated. Key time was considered $D18_{pe}$. VER and P4 concentrations were evaluated. Ewes were divided into two groups according to serum P4 levels; Group E (n=17) with serum concentrations below 1ng/ml and Group L (n=17) with serum concentrations above 1ng/ml. Group E was assumed to be in estrus, while Group L was assumed to have a luteal structure in the ovary. A cut-off value was calculated using the estrus variable. VER values measured below the cut-off value was indicated ewes stood estrus, while values above the cut-off value was indicated ewes did not stand estrus. False positive and false negative estrus detection rates were calculated. Accordingly VER>cut-off value and P4<1 ng/ml were considered false negative, VER<cut-off value and P4>1 ng/ml were considered false positive.

Early identification of pregnant ewes: The usability of the VER value in determining the early pregnancy diagnosis was investigated. $D18_{pe}$, $D35_{pe}$, and $D50_{pe}$ were considered key times. Pregnancy diagnosis results at $D35_{pe}$ and $D50_{pe}$ and VER value in $D18_{pe}$ were examined. A cut-off value was calculated using the pregnancy variable. Values below the VER cut-off were used to identify ewes that were not pregnant, and values above the cut-off were used to identify ewes that were pregnant.

Monitoring the VER value in the first trimester of pregnancy and comparing it with non-pregnant ewes: Key times were $D35_{pe}$ and $D50_{pe.}$ Ewes were divided into two groups according to pregnancy results regardless of serum progesterone concentration in $D18_{pe}$; Group NP (n=17) non-pregnant ewes in both examinations and Group P (n=17) pregnant ewes in both examinations. VER values of Group NP and Group P were measured and the relationship between the groups and within groups was evaluated.

Estrus Synchronization

Ewes were treated with intravaginal sponges containing 60 mg of MAP (Esponjavet, Hipra, Spain) for 11 days. At the time of sponge removal, intramuscular 0.075 mg d-cloprostenol (Senkrodin®, Veta , Turkey) was administered. Ewes in estrus after sponge removal were mated to five fertile rams naturally. The rams were joined to the flock twice daily for two hours (early morning and late evening), for three days, starting from 24 hour after the sponge's removal. All ewes were exposed to

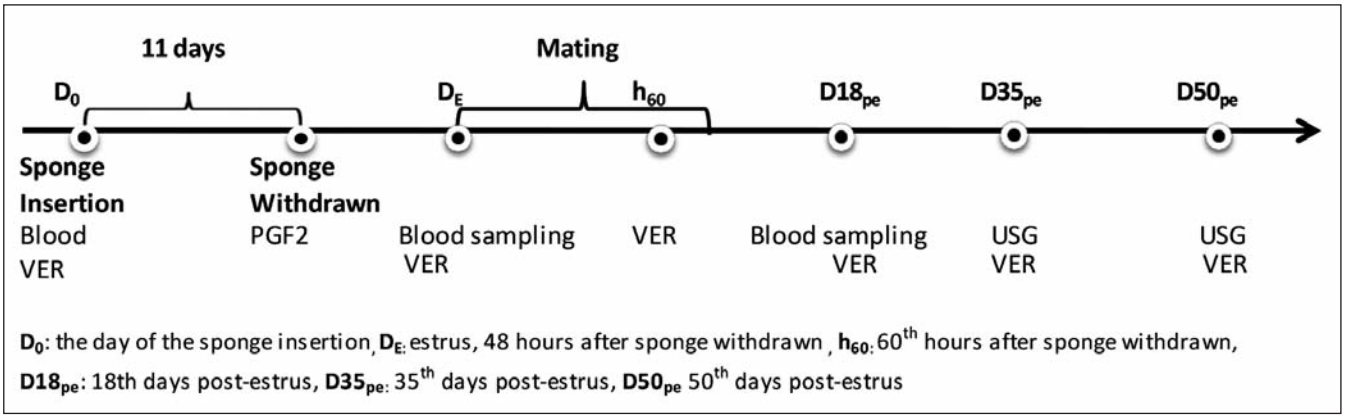

Figure 1 - Estrus synchronization protocol and parameters monitored during and after the protocol.

Table 1 - VER values and P4 concentrations of ewes during estrus synchronization protocol. Different superscripts in same line show significant differences (P<0.05).

	D_0	D_E	h_{60}	$D18_{pe}$	P
VER (ohm, Ω)	485,45±21,12[a]	295,15±10,65[b]	322,73±16,86[b]	337,58±21,38[b]	<0,001
Progesteron (ng/ml)	0,68±0,18[b]	0,28±0,02[b]		2,42±0,47[a]	<0,001

D_0: the day of the sponge insertion, D_E: estrus, 48 hours after sponge withdrawn, h_{60}: 60th hours after sponge withdrawn, $D18_{pe}$: 18th days post-estrus

rams. The day when at least half of the ewes stood still during mating with ram was considered the estrus day of the flock (D_E). 3 days (72 hours) after sponge removal, rams left the flock and did not come into contact with the ewes again.

Ultrasound imaging

Transabdominal ultrasonography was performed in the ewes by real-time ultrasound device with 6-8 MHz probe (Falco 100, Pie Medical, Netherlands). All examinations were performed by the same veterinarian. In the diagnosis of pregnancy, visualizing of vesicle, viable embryo with heartbeat at $D35_{pe}$ and placentomes, fetal heartbeat and ossification, vesicles of embryo at $D50_{pe}$ were accepted pregnancy criteria.

Determination of vaginal electrical resistance

Values of VER were determined with a heat detector (Draminski Electronics in Agriculture, Poland) according to method previously descripted [14]. Before the determination of VER, vulva of ewes was cleaned by a mild antiseptic solution containing 1% potassium permanganate. Then heat detector put in place with vagina, and the average value of three consecutive measurements was recorded.

Blood sampling and analyzes

Blood samples were taken from v. jugularis into tubes without coating anticoagulant. The blood samples were centrifuged at 3000 rpm for 5 minutes; their serum was removed and stored at -20 ºC until the analysis of P4 by Direct Chemiluminescence method (ADVIA Centaur® XP Immunoassay System Ready-Pack, Progesterone (PRGE), REF 01586287, Siemens, USA).

Statistical analysis

All statistical analyses were performed using the SPSS software package (SPSS 23.1.0.). The variation of P4 and VER values during estrus synchronization analyzed with repeated measures ANOVA. Similarly, relationship between the VER values of pregnant and non-pregnant ewes analyzed with repeated measures ANOVA. Greenhouse Geisser's test was used to adjust the degrees of freedom in case of the violation of the normality assumption. ROC (Receiver Operating Characteristic) analyzes was done to determine the cut-off value of VER for estrus detection and early pregnancy diagnosis.

RESULTS

The change of VER value and P4 concentrations during estrus synchronization is given in Table 1. The cut-off for VER value was found to be <255 ohm (AUC: 0,721, 95% CI: 0.532-0.909) on the determination of returning ewes (P=0,031) with 54.8% sensitivity and 93.8% specificity (Fig. 2). In detection

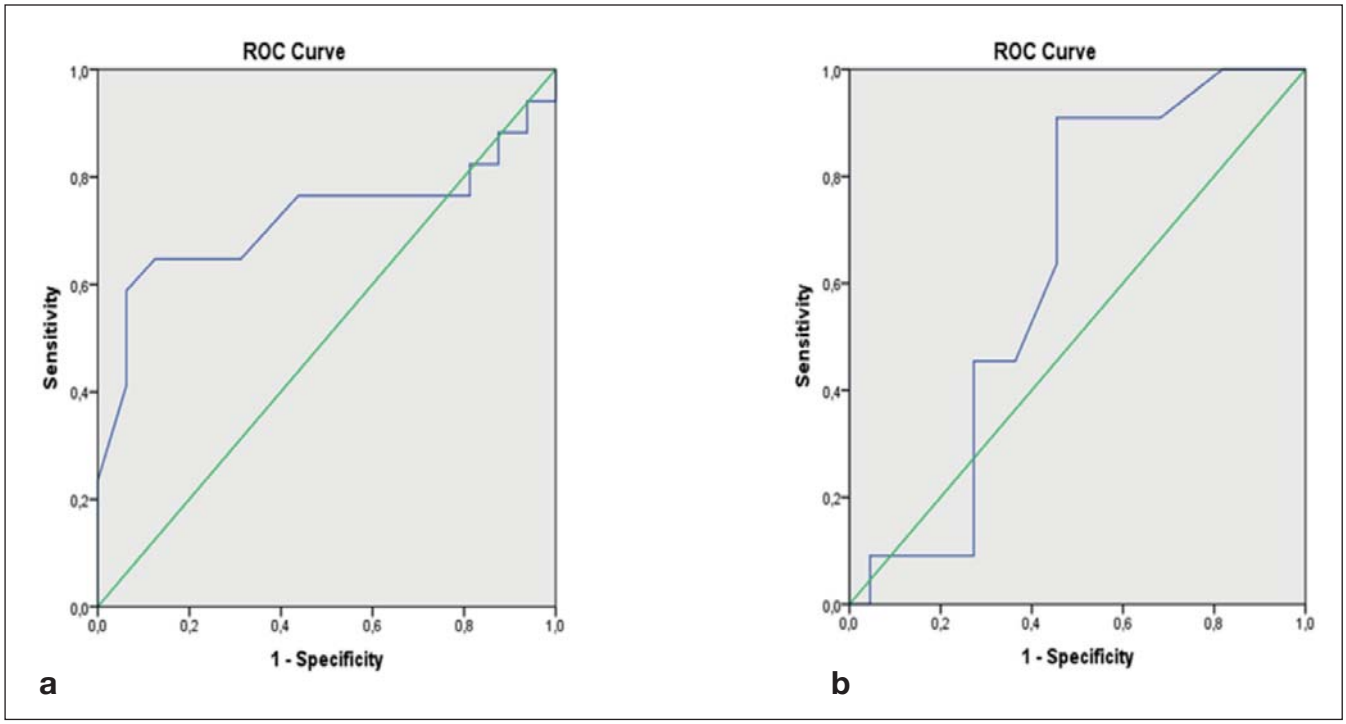

Figure 2 - The ROC curve at D18pe in a) estrus detection and b) early pregnancy.

Table 2 - VER value change at $D35_{pe}$ and $D50_{pe}$ in pregnant and non-pregnant ewes.

		VER		P		
	Groups	$D35_{pe}$	$D50_{pe}$	VER	VER x Pregnancy	Pregnancy
Pregnancy	Group NP	440,59±28,84	331,88±24,05	0,002	0,861	0,528
	Group P	451±50,27	361,25±18,71			

$D35_{pe}$: 35 days after estrus $D50_{pe:}$ 50 days after estrus.

of estrus, 20.58% (7/34) false negative (VER>255 and P4<1 ng/ml) and 2.94% (1/34) false positive (VER<320 and P4>1 ng/ml) diagnosis were obtained according to VER measurement on $D18_{pe}$.

A separate cut-off value was calculated for early pregnancy diagnosis, considering that there may be ewes who could not maintain their pregnancy until ultrasound imaging, even if they were on luteal phase (serum P4 value>1ng/ml) at $D18_{pe}$. Early pregnancy diagnosis related cut-off value of VER was found insignificant (P=0.229) (with 90.9 % sensitivity; 95% CI, 23.5 - 83.1 and 54.5% specificity; 95% CI, 28.8 - 96.7), (<270 ohm for non-pregnant ewes; AUC: 0.630, 95% CI: 0.440-0.821), (Fig. 2). When the retrospective records of the pregnant ewes were examined after the ultrasound examination, it was determined that 70.6% (12/17) of the ewes in Group L and 29.4% (5/17) of the ewes in Group E were pregnant.

Values of VER were found to be similar between pregnant and non-pregnant ewes in $D35_{pe}$ and $D50_{pe}$ (P>0.05), while the change of VER values was statistically significant in both Group P and Group NP (P<0.05), (Table 2).

DISCUSSION

Although the breeding season of Awassi sheep varies according to the environmental factors such as region or climate, it starts in April and continues until September [15]. In regions closer to the equator, such as Jordan, the breeding season may not begin until mid-July [16]. In the Mediterranean region, where we also conducted this study, June-July is considered the breeding season [17]. It was reported that mean P4 concentrations before mating were 0.19±0.13 ng/ml (0.62±0.44 nmol/L) and during the luteal phase in non-pregnant ewes was 3.4±1.03 ng/ml (11.0±3.3 nmol/L)[18]. On the other hand, it is known that during the first estrus cycle of transition to the breeding season, the maximal serum P4 concentrations are lower compared to the mid-breeding season in sheep[19]. The mean serum P4 concentration of ewes was 0.68±0.18 ng/ml at the time of sponge insertion (D_0) in this study, indicating that some ewes may still be in the transition period considering previous studies [16]. The P4 concentration of ewes decreased during estrus (0.28±0.02 ng/ml) and increased sharply after 18 days of estrus (2.42±0.47 ng/ml). This fact indicates that estrus was successfully induced by estrus synchronization program.

A positive correlation was notified between VER values and blood serum P4 concentrations [20]. Although the P4 concentration was low, the highest VER value of the study (485.45±21.12 ohm) was measured (P<0.001) on D_0, probably due to the absence of estrogen. VER values decreased to the lowest value (295.15±10.65 ohm) during estrus. Since the earlier studies examining the efficacy of VER measurement in sheep [21], it is known that lower vaginal electrical impedance values were observed in the follicular phase compared to the other phases. However, a single VER value that would indicate estrus in sheep seems unfeasible. Some variations of VER values are possible at the same stage of different cycles of the same sheep [14]. In a recent study, the VER value was measured just before the artificial insemination and it was reported that the VER value of the ewes that would become pregnant was lower than that of the ewes that would not become pregnant, and the VER value of the ewes that would become pregnant was <300 ohm[22]. Since the VER increased at the 60th hour of sponge withdrawal (322.73±16.86) compared to estrus, it can be thought that most ewe's estrus stage was already ends. The mean serum P4 value (2.42±0.47 ng/ml) of the ewes on $D18_{pe}$ indicates that some ewes have a luteal structure on their ovaries. Similarly, VER value on the same day (337.58±21.38) may indicate that ewes are in the luteal phase rather than the follicular phase. As a result, it was concluded that the VER value can be used to reveal the success of estrus synchronization, even if it is measured in key days of synchronization. However, measurement of VER in the transition period is not recommending since there will be individual differences among the herd.

The common practice in breeding herds of sheep is to mate under controlled conditions after estrus has been detected during the breeding season[23]. It is known that the main purpose of synchronization is to ensure the effective planning of the lambing season and to ensure homogeneous lambing in terms of matching the supply and demand in the market[24]. However, pregnancy rate obtained with the breeding program may vary depending on management factors and individual differences such as age, feeding and housing[25]. Ewes that do not conceive after an estrus synchronization protocol mate with rams in the subsequent estrus. However, if the number of non-pregnant sheep is higher than admissible, the basic homogeneity target may be impaired in farms where the sheep/ram ratio may be insufficient or where the rams are unavailable. Accordingly, it is necessary to identify non-conceived females as early as possible and to implement a new fertility program. Earliest studies reveal that the P4 concentration in non-pregnant animals is below 1 ng/ml at 18-22 days after mating in sheep[26]. Considering that the mean estrus cycle duration in Awassi ewes is 16-18 days[15], we hypothesized that ewes without luteal structure in their ovaries should be in estrus again simultaneously 18 days after induced estrus. Serum P4 concentration was used to reflect the presence of luteal structure. It was assumed that if the serum P4 concentration is above 1ng/ml, luteal structure is present, if it is below 1ng/ml, there is no luteal structure, and the ewe was stand estrus. Ewes at re-estrus on $D18_{pe}$ had a VER cut-off value <255 ohm. However, it should be considered that cut-off success is approximately 59% in identifying estrus, and 94% in identifying non-estrus ewes. Therefore, it is not surprising that there were 20.58% (7/34) false negative estrus (VER>255 and P4<1 ng/ml) and 2.94% (1/34) false positive

estrus (VER<255 and P4>1ng). It is thought that false negative diagnosis may be due to the presence of acyclic animals. When the individual records of these animals were examined, it was seen that 6 out of 7 ewes were exhibited a similar profile at D_0 and D_E, and did not conceive. On the other hand, false positive diagnoses may be caused by measurement error. It is thought that the rather low rate of false positive estrus diagnosis can be ignored if mating used as key method for conception on post-synchronization re-estrus. However, measuring only the VER value to decide on artificial insemination may be very erroneous practice.

The cut-off value which may indicate early pregnancy at $D18_{pe}$ was not significant (P>0.05). Measuring progesterone concentration of sheep 16-18 days after mating is recommended as an early pregnancy indicator [27]. However, it was reported that the test had sensitivity as low as 60%[28]. In this study, it was determined that 29.4% (5/17) of ewes with P4 concentration <1ng/ml in $D18_{pe}$ were pregnant on ultrasound examination. While 4 of these 5 ewes were diagnosed estrus (p4<1ng/ml and VER<255 ohm) in both VER and progesterone measurements at $D18_{pe}$, one of them (1/5) was diagnosed with false negative estrus (p4<1ng and VER>255 ohm). A possible explanation of this hormonal profile could be the measurement error. However, in this study, where we were aimed to reveal usability of VER measurement in field conditions, we considered measuring serum progesterone values as the gold method and approve the VER values reflect steroid concentration in bloodstream. On the other hand, 29.4% of non-pregnant animals were found in pregnancy examination although serum progesterone concentration is >1ng/ml on $D18_{pe}$. We think that there may be early embryonic death which would expect in any flocks. The cut-off value of VER was significant in identifying the returning ewes (<255 ohm) while was not significant in identifying early pregnancies (<270 ohm). It is difficult to say that the statistical significance and numerical difference between the cut-off values can only be caused by early embryonic death. We think that this result may be caused by variables such as the presence of acyclic sheep, early embryonic losses, measurement error and many other possible failures that were not identified in this study. As a result, VER value (<255 ohm) measurement 18 days after mating can be used in detecting re-estrus but is not as successful as the use of teaser rams and does not indicate early pregnancy. However, we consider it usable when rams are not available as it can provide a preliminary information and estimate of the average number of ewes returning in a synchronized flock. It is also recommended that similar studies be carried out in larger flocks in the middle of the breeding season. It is thought that specificity and sensitivity rates may be higher in these herds as individual variations can be minimized.

There are few studies investigate the variation of VER value after pregnancy diagnosis. In a human study, it was revealed that cervical stromal impedance was higher in pregnant women than in non-pregnant women, but this difference was reported to be particularly remarkable in the last trimester of pregnancy[29]. In goats, it was reported that the VER value was the lowest in estrus and the highest in the luteal phase, but there was no difference between diestrus and pregnancy[30]. We hypothesized that non-pregnant ewes would stand estrus again on the days corresponding to the 3th and 4th cycles after induced estrus; therefore, VER values in nonpregnant ewes would be lower than pregnant ewes on luteal phase. However, the difference in VER value between Group P and Group NP was not significant (P>0.05). It can be thought that some ewes had a luteal structure in their ovaries although they were not pregnant or may be acyclic ewes in Group NP. The estrus cyclicality after synchronization may also have changed due to individual differences. However, since the presence of CL and serum P4 levels were not revealed in this study, it would be speculation to assume that this is the case. Nevertheless, our result corresponds well with that previous reports[29,30]. The VER value in the first trimester of pregnancy in ewes does not indicate a pregnancy-related point.

In this study, the availability of VER measurement in the estrus synchronization program was investigated and it was concluded that it could be useful in determining the first estrus after induced estrus in field conditions. It can also be used to evaluate synchronization success in the herd but failed to identify first trimester pregnancies. It is recommended to measure the VER value 18 days after estrus, to detect ewes that were likely not conceived, if rams are not available. This practice can significantly increase the rate of homogeneous lambing in synchronized herds.

ACKNOWLEDGEMENT

This study is dedicated to our colleagues and friends Dr. Pınar Ambarcığlu Kısaçam, Dr. Mehmet Ali Kısaçam, Dr. Erhan Tek, and Assoc Prof İbrahim Ozan Tekeli, who passed away on February 6, 2023 in Turkey in a greater earthquake, and we wish for them to find eternal peace in paradise. Particularly, we wish to thank Dr. Pınar Ambacıoğlu Kısacam for her valuable contributions on statistical analysis in this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

References

1. Kridli R.T., Abdullah A.Y., Al-Smadi N.M. (2007). Reproductive performance and milk yield in Awassi ewes following crossbreeding. Small Rumin Res, 71(1-3): 103-108. Doi: 10.1016/j.smallrumres.2006.05.007
2. Alkass J.E., Hermiz H.N., Baper M.I. (2021). Some aspects of reproductive efficiency in Awassi ewes: A review. Iraqi J Agric Sci, 52(1): 20-27. doi: 10.36103/ijas.v52i1.1232
3. Chenoweth P.J., Landaeta-Hernández A.J., Flöercke C. (2014). Reproductive and Maternal Behavior of Livestock. Genet Behav Domest Anim, 5: 159-194. doi: 10.1016/B978-0-12-394586-0.00005-6
4. Radford H.M., Watson R.H., Wood G.F. (1960). A crayon and associated harness for the detection of mating under field conditions. Aust Vet J, 36: 57-66. doi: 10.1111/j.1751-0813.1960.tb03764.x
5. Alhamada M., Debus N., Lurette A., Bocquier F. (2016). Validation of automated electronic oestrus detection in sheep as an alternative to visual observation. Small Rumin Res, 134: 97-104. doi: 10.1016/j.smallrumres.2015.12.032
6. Díaz A., Orihuela A., Aguirre V. Clemente, N., Pedernera, M., Flores-pérez, I., Vázqueza R., Ungerfeld R. (2021). Ewes prefer subordinate rather than dominant rams as sexual partners. Appl Anim Behav Sci, 238: 105306. doi: 10.1016/j.applanim.2021.105306
7. Kohno H., Okamoto C., Iida K., Takeda T., Kaneko E., Kawashima C., Miyamoto A., Fukui Y. (2005). Comparison of estrus induction and subsequent fertility with two different intravaginal devices in ewes during the non-breeding season. J Reprod Dev, 51(6): 805-812. doi: 10.1262/jrd.17037
8. Hodges K., Brown J., Heistermann M. (2010): Endocrine monitoring of reproduction and stress. In: Wild mammals in captivity: principles and techniques for zoo management, Eds. Kleiman D.G., Thompson K.V., Baer C.K., 447-468, University of Chicago Press, Chicago, IL.
9. Fehring R.J. (1996). A comparison of the ovulation method with the CUE ovulation predictor in determining the fertile period. J Am Acad Nurse

Pract, 8:461-466. doi: 10.1111/j.1745-7599.1996.tb00604.x
10. Theodosiadou E., Amiridis G.S., Tsiligianni T. (2014). Relationship between electrical resistance of cervical mucus and ovarian steroid concentration at the time of artificial insemination in ewes. Reprod Biol, 14(3): 234-237. doi: 10.1016/j.repbio.2014.03.001
11. Murtaza A., Khan M.I.U.R., Abbas M., Hameed N., Ahmad W., Mohsin I., Tahir M. Z. (2020). Optimal timing of artificial insemination and changes in vaginal mucous characteristics relative to the onset of standing estrus in Beetal goats. Anim Reprod Sci, 213:106249. doi: 10.1016/j.anireprosci.2019.106249
12. Zuluaga J.F., Saldarriaga J.P., Cooper D.A., Cartmill J.A., Williams G.L. (2008). Evaluation of vaginal electrical resistance as an indicator of follicular maturity and suitability for timed artificial insemination in beef cows subjected to a synchronization of ovulation protocol. Anim Reprod Sci, 109(1-4): 17-26. doi: 10.1016/j.anireprosci.2007.10.002
13. Gürler H., Koldaş E., Binli Önyay F., Akçay A. (2018). Efficiency of vaginal electrical impedance to determine the stage of the reproductive cycle in bitches. Med Weter, 74(3):179-181. doi: 10.21521/mw.6080
14. Talukder M.R.I., Hasan M., Rosy T.A., Bari F.Y., Juyena N.S. (2018). Monitoring vaginal electrical resistance, follicular waves, and hormonal profile during oestrous cycle in the transition period in Bangladeshi sheep. J Vet Res, 62(4): 571-579. doi: 10.2478/jvetres-2018-0080
15. Talafha A.Q., Ababneh M.M. (2011). Awassi sheep reproduction and milk production: Review. Trop Anim Health Prod, 43(7): 1319-1326. doi: 10.1007/s11250-011-9858-5
16. Husein M.Q., Kridli R.T. (2003). Effect of progesterone prior to GnRH-PGF2α treatment on induction of oestrus and pregnancy in anoestrous Awassi ewes. Reprod Domest Anim, 38(3): 228-232. doi: 10.1046/j.1439-0531.2003.00411.x
17. Canooğlu E., Saribay M.K. (2015). Morphology of the reproductive tract and reproductive physiology [Turkish]. In: Obstetrics and infertility in farm animals, Ed. Semacan A., Kaymaz M., Fındık M., Rişvanlı A., Köker A., 467-490, Medipres, Malatya, Turkey.
18. Zarkawi M. (1997). Monitoring the reproductive performance in Awassi ewes using progesterone radioimmunoassay. Small Rumin Res, 26(3): 291-294. doi: 10.1016/S0921-4488(97)00011-4
19. Bartlewski P.M., Beard A.P., Rawlings N.C. (1996b). Ovarian function in ewes at the onset of the breeding season. Anim Reprod Sci, 57(1-2): 67-88. doi: 10.1016/s0378-4320(99)00060-3
20. Theodosiadou E., Tsiligianni T. (2015). Determination of the proper time for mating after oestrous synchronisation during anoestrous or oestrous by measuring electrical resistance of cervical mucus in ewes. Vet Med, 60(2): 87-93. doi: 10.17221/7982-VETMED
21. Bartlewski P.M., Beard A.P., Rawlings N.C. (1999a). The relationship between vaginal mucous impedance and serum concentrations of estradiol and progesterone throughout the sheep estrous cycle. Theriogenology, 51(4): 813-827. doi: 10.1016/s0093-691x(99)00029-1
22. Rahman M.M., Naher N., Isam M.M., Hasan M., Naznin F., Bhuiyan M M.U., Bari F.Y., Juyena N.S. (2020). Natural vs synchronized estrus: determinants of successful pregnancy in ewes using frozen-thawed Suffolk semen. J Anim Reprod Biotechnol, 35(2): 183-189. doi: 10.12750/JARB.35.2.183
23. Dursun Ş. (2019). Effect of different short term synchronization protocols on estrus and fertility in non-pregnant ewes during the breeding season. J Hell Vet Med, 70(2): 1461-1466. doi:10.12681/jhvms.20813
24. Nozieres-Petit M.O., Moulin C.H. (2021). The management of lamb heterogeneity is a tool for farmers' marketing strategies. Animals, 11(2): 551. doi: 10.3390/ani11020551
25. Özyurtlu N., Bademkıran S.(2010). Estrus synchronization and ınduction of estrus methods in sheep [Turkish]. Dicle Üniv Vet Fak Derg, 1(1):17-22.
26. Shemesh M, Ayalon N, Mazor T.(1976). Early pregnancy diagnosis in the ewe, based on milk progesterone levels. Reproduction, 56(1): 301-304. doi: 10.1530/jrf.0.0560301
27. Karen A., Beckers J.F., Sulon J., de Sousa N.M., Szabados K., Reczigel J., Szenci O. (2003). Early pregnancy diagnosis in sheep by progesterone and pregnancy-associated glycoprotein tests. Theriogenology, 59(9): 1941-1948. doi: 10.1016/S0093-691X(02)01289-X
28. Gvozdic D., Ivkov V. (1994). Early pregnancy diagnosis in ewes. Acta Vet Belg, 44(1994): 215-219.
29. Gandhi S.V., Walker D., Milnes P., Mukherjee S., Brown B.H., Anumba D.O. (2006). Electrical impedance spectroscopy of the cervix in non-pregnant and pregnant women. Eur J Obstet Gynecol Reprod Biol, 129(2): 145-149. doi: 10.1016/j.ejogrb.2005.12.029
30. Nain S., Kumar D., Prakash B., Purohit G. (2020). Vaginal electrical resistance (VER) measurements in goats in different reproductive states. Int J Livest Res, 10(6): 122-126. doi: 10.5455/ijlr.20200329032847