The prediction of estrus synchronization in Awassi ewes by vulvar biometry and vaginal cytology

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Abstract

The characteristics of exfoliated vaginal cells and vulvar biometry following estrus synchronization via insertion of vaginal sponges impregnated with 6-Methyl- 17-Acetoxyprogesterone (MAP) were investigated with the aim to predict estrus in ten adult Awassian ewes .The animals were maintained on green and concentrated ration with water ad libitium . All measurements in the ewes were taken before insertion of intravaginal sponges and at 0hrs , 24hrs , 72hrs after removed of intravaginal sponges. Nine out of ten ewes showed estrus behavior in synchronization rate 90%.The results of vaginal cytology showed dominancy of parabasal cells 49.9% before insertion of intravaginal sponges, and dominancy of intermediate cells 62.4% at 0 hour after removed of intravaginal sponges , while at 24hrs and 72 hrs. the dominant cells were nucleated superficial cells and intermediate cells in percentage rate of 44.3% and 41.8% respectively. The results showed that the measurements of vulvar biometry of synchronized ewes during the study period increase in the vertical dimension of vulva significantly ($P \leq 0.05$) only after 24hrs of removing of intravaginal sponges .

Key words: vulvar biometry ,vaginal cytology , ewe

استخدام قياسات المهبل والخلايا المهبلية قبل وبعد توحيد الشبق بواسطة الاسفنجات المهبلية لغرض توقع الشبق في الاغنام العواسية

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المستخلص

تم الاعتماد على مواصفات خلايا المهبل المتوسفة وقياسات فتحة الفرج للتنبوء بحدوث الشبق في 10 نعاج خضعت لعملية تزامن الشبق باستخدام الاسفنجات المهبلية المحمله بهرمون 6-Methyl- 17-Acetoxyprogesterone (MAP). تم اخذ القياسات قبل وضع الاسفنجات المهبلية وفي الساعات 0 ، 24 و 72 بعد ازالتها. ضهرت علامات الشبق في 9 نعاج بنسبة 90% واضهرت نتائج فحص خلايا ضهارة المهبل ان الخلايا السائدة قبل وضع الاسفنجات هي خلايا جنب القاعدية بنسبة 49.9% امة ذات النواة والخلايا الوسطية بنسبة 44.3% و 41.8% على التوالي كما اضهرت نتائج الدراسة زيادة معنوية ($P \leq 0.05$) في البعد الشاقولي للفرج في الساعة 24 بعد ازالة الاسفنجات.

Introduction

The breeding of animal is depending on the reproductive efficacy which influences the economic values (2) . Most breeds of ewes are anoestrus for some portion of the year and the seasonality of breeding activity in sheep limits the yearly productivity

of ewes (3) therefor , Estrus synchronization is commonly used in order to induce estrus and ovulation during the anoestrus period , to synchronize the estrus and shorten the duration of lambing interval in addition to minimize labor costs during the season .

The main reproductive processes in the ewe can be altered to the advantage of the farmers, these include , Artificial insemination (AI) and embryo transfer (ET).These are examples of how grand improvements can be made in both genetics and reproductive management of sheep by using synchronization methods (11) . The present study was carried out to assess the efficiency of the vaginal cytology and vulvar biometry in detection of induced estrus and to assess the efficiency of detection of induced estrus .

Materials and Methods

The study was carried out in the animal house of the Veterinary Medicine College \ University of Kufa , its performed in March 2014 .The adult Awassi breed ewes aged 3-5 years old and weighing 70-80 kg. The ewes were housed together in separate cages and fed green grass in the morning and concentrate in the evening, fresh water were provided ad libitum .

All ewes were examined clinically before estrous synchronization by using intravaginal sponges impregnated with 40 mg of 6-Methyl-17-Acetoxyprogesterone (MAP) (Pharmacia & Upjohn, Orangeville, Canada). which left for 14 days before the injection of 600 IU PMSG (Intervet International B.V., Boxmeer, Holland) administered IM at the time of sponges removal. After removal of sponges and injection of PMSG, two adult rams that has sexual experience introduced to detect the estrus .

The vaginal smears were taken before insertion of the vaginal sponges and every 24 .48 and 72 hrs according to the method mentioned by (10).Briefly , each ewe was restrained in standing position and the swab was gently inserted with the right hand while the left thumb and forefinger were used to expose the vulva lips. At the anterior vagina the swab gently rolled against the vaginal mucosa and carefully withdrawn. The swab was immediately smeared on glass slide and stained with Geimsa's stain. The cells found in the vaginal smear were summarized as percentage epithelial cells (parabasal , intermediate, nucleated and anucleated superficial epithelial cells). The correlation between vaginal cytology and stages of the estrus was interpreted according to (10) .

For measuring the vulvar biometry the two dimensions of the vulva were used to predict the estrus in ewes by measuring the vertical dimension (the distance between superior and inferior commeasure) and horizontal dimension (distance between the broadest horizontal curvature) . These dimensions measured by using digital vernier caliper .figure 1.The vulvar biometry of each ewe was taken at the morning before sponge insertion and, at 1, 24 48 and 72 hours after removing of vaginal sponges (8). All obtained data were analyzed statically by SPSS program on computer.

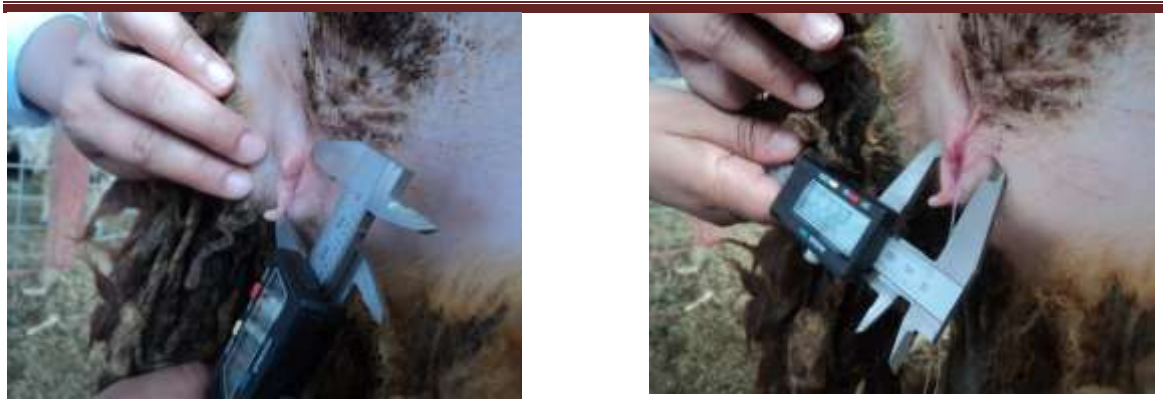


Figure (1) the measurement of horizontal and vertical dimension of ewe's vulva

Results

Nine out of 10 ewes were showing estrous after 24 hours after injection of PMSG at synchronization rate 90%, the main estrous signs were behavioral and characterized by acceptance of teaser ram.

Vaginal Cytology:

The results showed significant variation in the percentages of the vaginal epithelial in present study were presented in table (1), the presence of parabasal cells are increased before insertion of the intravaginal sponges with percentage 49.9% followed by intermediate cells , the lowest percentage were the nucleated and a nucleated superficial cells, figure(1) . After removal of intravaginal sponges ,the vaginal epithelial cells were intermediate cells, parabasal cells, nucleated and anucleated superficial cells in percentages 62.4%, 26.9%, 6.9% and 3.8% respectively ,figure 2 . After 24 hrs from removed of the sponges the results showed increase in the presence of nucleated and a nucleated superficial epithelial cells with percentages 44.3% and 30.5% respectively, figure 3 and 4.These cells returned to decrease after 72 hrs after removal of the intravaginal cells which are 26.8% and 28.6% .

Table (1) the percentages of vaginal epithelial cells before and after insertion of intravaginal sponges.

Period in estrus (hours)	Nucleated superficial cells% (range)	Anucleated superficial cells% (range)	Intermediate cells% (range)	Parabasal cells% (range)
Before insertion of sponges	4.8 (0-15)	1.6 (0-4)	43.7 (32-61)	49.9 (32-64)
0hour after removal of sponges	6.9 (3-13)	3.8 (1-8)	62.4 (49-70)	26.9 (12-36)
24 hours after removal of sponges	44.3 (34-52)	30.5 (20-40)	15.4 (10-21)	9.8 (6-16)
72 hours after removal of sponges	26.8 (21-35)	28.6 (21-33)	41.8 (37-49)	2.8 (0-7)

Vulvar Biometry

The results showed the measurements of the vulvar biometry of synchronized ewes during the study period as found in table (2) which reveals non significant increase (P≥0.05) in the vertical dimension of the vulva only after 24 hrs after remove of intravaginal sponges.

Table (2) the vulvar biometry in the synchronized ewes

Vulval biometry	Before insertion of sponges	(0)hour befor removal of sponges	(24) hours after removal of sponges	(72) hours after removal of sponges
Vertical	**2.573**	**2.581**	**2.754**	**2.595**
Horizontal	**2.375**	**2.397**	**2.419**	**2.404**

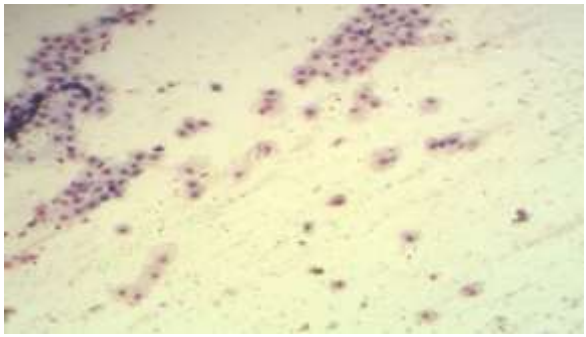


Figure (2) the dominance of parabasal cells before insertion of ntravaginal sponges

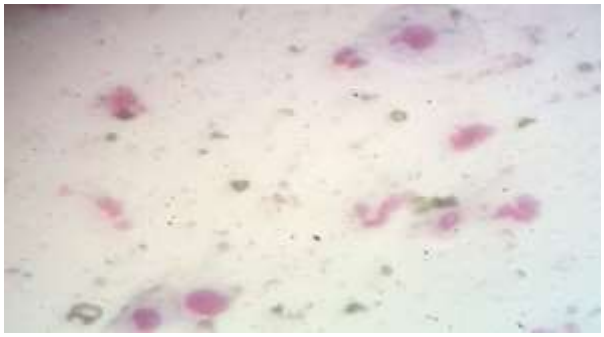


Figure (3) the dominance of intermediate cells after 0 hour from remove of intravaginal sponges

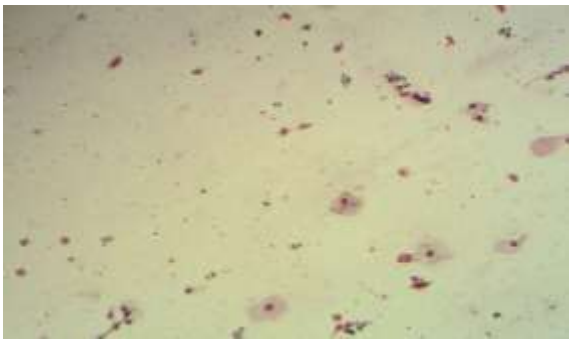


Figure (4) the dominance of nucleated superficial cells fter 24 hrs from remove of ntravaginal sponges

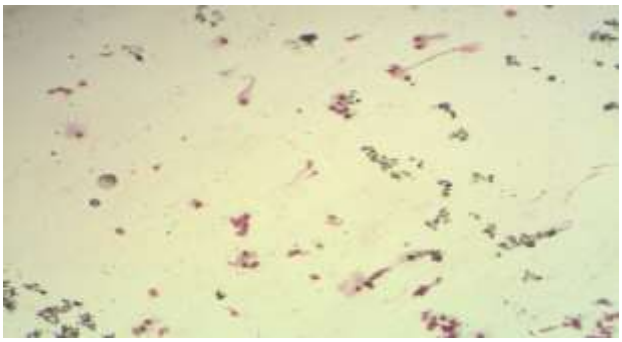
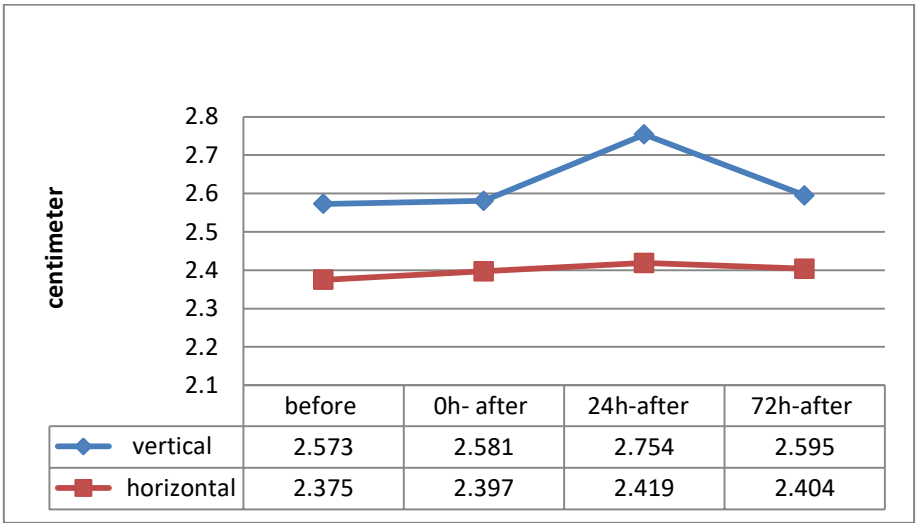


Figure (5) the presence of sperm in ewe's vagina after 24 hrs after remove of sponges



	before	0h- after	24h-after	72h-after
vertical	2.573	2.581	2.754	2.595
horizontal	2.375	2.397	2.419	2.404

Figure (6) the vulvar biometry before and after insertion of intravaginal sponges.

Discussion

Intra-vaginal sponges consider the traditional treatment of choice for estrus synchronization in small ruminants, during the breeding and anestrus seasons. Exogenous progesterone in combination with gonadotrophin can be used to induce and synchronize estrus in an ewes , in present study nine out of 10 ewes were showing estrous after 24 hours after injection of PMSG at synchronization rate 90%,this result is close to findings of other authors (1,4,5,6, 7 and 9) whose found that the estrous synchronization occur in Five(83.3%) out of the six does treated with two injections of LutalyseR (i.e. 5mg, 7 days apart) were discovered to have shown standing heat which was depicted by the presence of numerous sperm cells in their vaginal smears.

According to vaginal cytology the results showed significant variation ($P \leq 0.05$) in the percentages of different vaginal epithelial cells, table (1),our results were in agreement with (5) whose explain that the percentage of epithelial cells rose sharply between the 0-24 hours of 2nd Lutalyse R injection from 16.0±0.03% to 89.6±0.34% during the first 24 hours of standing estrus (i.e. 72-96 hours) and decreases sharply also to 13.3±1.02% about 48 hours after the first observation of standing estrus. The differences between superficial and other epithelial cells were also significant, this increase in the superficial epithelial cell occur due to the increase in the estrogen during estrous which cause exfoliation for these cell . In other hand ,the results showed that the measurements of the vulvar biometry of synchronized ewes during the study period were not increased significantly ($P \leq 0.05$) in the vertical dimension of the vulva particularly at 24 hrs after removing of intra-vaginal sponges , these results were in agreement with (5) where they found that the non-significant differences in the mean values of all measurements between the 0-72 hours and the 72- 120 hours during which mating occurred this findings may be due to that the ewes are unlike the bitches where hypertrophy of the vulva can be used to detect physiological processes leading to estrus. As found in table (2) which reveals non-significant in-

crease($P \leq 0.05$) in the vertical dimension of the vulva only at 24 hrs after remove of intravaginal .

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