

Comparison between total DNA concentration in the vaginal discharge in pregnant and non pregnant Awassi ewes

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Abstract

The study was conducted on 20 Iraqi Awassi ewes (10 pregnant and 10 non pregnant), aged between (2-5) years old. Utilized Proteinase K buffer solution (100 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl pH 8.0) for DNA extraction. The vaginal fluid were taken from the animals in order to estimate DNA concentration spectrophotometrically. The results showed that the concentration of DNA was 4.2579 gm / l in pregnant ewes, while it was 4.3601 gm/ l in non pregnant ewes, it was concluded from this study there was no significant difference in DNA concentration in the vaginal fluid between pregnant and non pregnant Awassi ewes in $p < 0.05$.

المقارنة بين التركيز الكلي للـ DNA في الإفرازات المهبلية للنعاج العواسية الحوامل وغير الحوامل

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الخلاصة

أجريت الدراسة على 20 نعجة عواسية (10 حوامل و 10 غير حوامل) تراوحت أعمارها بين (2-5) سنة. استخدم المحلول الحال (Proteinase K (100 mM NaCl, 1mM EDTA, 10 mM Tris-HCl pH, 8.0). لاستخلاص الحامض النووي (DNA) أخذت السوائل المهبلية من النعاج لتقدير التركيز الكلي للحامض النووي باستخدام المطياف الضوئي، أظهرت نتائج الفحص ان تركيز تركيز الحامض النووي 4.2579 غم/ لتر في الأغنام الحوامل بينما كان تركيزه في غير الحوامل 4.3601 غم/ لتر، نستنتج من هذه الدراسة انه لا يوجد فرق إحصائي في التراكيز للحامض النووي بمستوى $P < 0.05$.

Introduction

Deoxyribonucleic acid (DNA) profiling has rapidly become a standard and powerful tool in forensic investigations, agriculture, paternity testing, and historical investigation (1) The cytology of diestrus in control does showing numerous neutrophils, few superficial cells and few other immature epithelial cells (2). A postparturient period is characterized by low basal secretion of adenohipophysis gonadotropins with the following appropriate changes in ovarian hormones and their response to the morphology of vaginal epithelium, while the knowledge of cytological changes in vaginal epithelium and levels of ovarian hormones of ewes after parturition and of their relationships from the first several days after lambing until the 51st day of the period of observation (3). Progesterone levels remained elevated through day 28 of diestrus and pregnancy in bitch. (4) The physiological changes induced by sexual chemosignals (5). Arborization of cervical mucus appeared to be a better indicator of ovarian activity in the ewe than did arborization in mucus collected from the vagina (6). Clinical endometritis was defined as mucopurulent or purulent vulvar discharge (7). The aim of the study was designated to measure the concentrated of DNA in pregnant and non pregnant Awassi ewes in Iraq.

Materials and Methods

Twenty (vaginal discharge) samples were collected from healthy Awassi ewes aged between (2-5 years) old during the period in October to April, 10 samples from pregnant ewes (first group) and 10 samples from non pregnant (second group). The method were used for vaginal discharge collection, included the injection of (1 ml) of normal saline into vagina via long straw and waiting one minute before reabsorption, lead to normal saline mixed with the vaginal content, mucus and somatic cells (epithelial cells erosion and WBCs). The samples were collected according to (8), used cotton wool swabs were teased apart and extracted in a reaction tube (1.5 ml) for 15 minute with 338 μ l lysis buffer. In our study, the samples centrifugation (6000 per\ minute) to precipitate the cells and discarded the supernatant. Sediments cells subjected to DNA extraction in order to determination the concentration. Proteinase K buffer solution: Consist of (100 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl pH 8.0) purified by extraction with chloroform-isoamyl alcohol and precipitated with ethanol. The concentration of the DNA was measured spectrophotometrically (260 wave length). The measured in vulvar and vaginal tissues of ewes by a spectroimpedographic method at frequencies from 10 Hz to 100 kHz indicating that cell volume increased and cell density decreased in comparison with diestrus (9). The standard used Standard error, Mean, Chi square, and F. test (10).

Results

Twenty samples were subjected to DNA extraction. The concentration of DNA in the first group ranged from (4.0034 g/l - 4.5431 g/l) with the mean (4.2579 g/l) Table 1 and 10 samples from (second group) were ranged DNA from (4.1248 g/l - 4.6732 g/l) with the mean (4.3601 g/l). There was no significant difference between pregnant and non pregnant ewes in vaginal discharge DNA concentration.

Table (1) Showed the Mean, SE of (DNA g/l) concentration in the vaginal discharge in pregnant and non pregnant ewe

Pregnant ewes		Non pregnant ewes	
No of vaginal discharge	DNA g/l (M \pm SE)	No of vaginal discharge	DNA g/l (M \pm SE)
10	4.2579 \pm 0.017	10	4.3601 \pm 0.3601

Discussion

During diestrus and pregnant in ewes, the corpus luteum becomes fully developed, and the effects of its progesterone, on the vaginal discharge are marked. Vacuolar and degenerate epithelium, leucocyte infiltration, and extravasations of blood (11). Therefore, In our study, (1cc) normal saline injected into anterior vagina to collected the discharge including variable cells (WBCs, epithelial cells and debris). Cervical mucus is abundant only when the ewe is in oestrus and even then some ewes produce so little that collection of an adequate sample is impossible, because of these limitations it is worthwhile to seek other diagnostic methods and one possible area is the biochemical analysis of cervical secretions during the luteal phase of the reproductive cycle (12). In addition, the cell count in diestrus and pregnant, fewer than in estrus. The impedance angle and the tissue phase angle increased during oestrus, indicating that cell volume increased and cell density decreased in comparison with diestrus (9). In our study, (1cc) normal saline injected into anterior vagina to collected the discharge including variable cells (WBCs, epithelial cells and debris) (12) Mention the cervical secretions of clover-affected and control ewes in the luteal phase of the ovarian cycle were obtained by flushing the anterior vagina. Abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina (13). In this study the total DNA was extracted from cells by digestion with Proteinase K buffer solution: Consist of (100 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl pH 8.0) containing 0.5% SDS. While (14) write the first step of digestion, vaginal epithelial cells in the mixed stains were lysed with

Proteinase K and SDS. DNA samples revealed the cell number (Somatic cells) in the vagina. During diestrus the mucus becomes minimal, cohesive, and usually contains little cellular debris (15). In our study the observation of DNA concentration reflect the cells number into the vaginal fluid. Because DNA is present in the nucleus of cells and is the genetic information of all organisms (14). The mean of total DNA in pregnant ewes (4.2579 g/l) while the mean in non pregnant reached (4.3601 g/l). In spite of different levels of hormones between pregnant and non pregnant ewes, the results revealed no significant between total DNA when isolated from vaginal discharge in two groups. This result might be due to the effect of progesterone in the reproductive tract by histological architecture (epithelial lining), low resistant (attractive of few WBCs) and the importance of blood supply through pregnant and non pregnant periods. Furthermore the lower levels of estrogen in both groups.

References

1. Lewis, R. 2007. Human genetic. seven ed., P. 207.
2. Leigh, O. O. & Fayemi, O. E. 2011. Observations on Estrus as Monitored by Receptivity to Mating Activity and Pattern of Vaginal Exfoliates In *Trypanosoma brucei*-Infected WAD Goat does. Int. J. Morphol., 29 (3):762-768.
3. Krajnicáková, M.; Bekeová, E.; Maracek, I.; Hendrichovský, V. & Elecko, J. 1992. Dynamics of changes in the cytological picture of vaginal smears and circulating ovarian hormones during the puerperal period in ewes. Vet. Med. (Praha), 37(8): 449-458.
4. Nett, T. M.; Akbar, A. M.; Phemister, R. D. P. A.; Holst, L.; Reichert, Jr. E. & Niswender, G. D. 2012. Feedback Levels of Luteinizing Hormone, Estradiol and Progesterone in Serum During the Estrous Cycle and Pregnancy in the Beagle Bitch. Theriogenology, 74 (7): 1248-1255.
5. Gelez, H.; Archer, E.; Chesneau, D.; Campan, R. & Fabre-Nys, C. 2004. Importance of Learning in the Response of Ewes to Male Odor. fabre@tours.inra.fr.
6. Raeside, J. I. & McDonald, M. F. 1959. Arborization of cervical mucus in the ewe. Endocrinolm, 1 (18): 350-358.
7. Westermann, S. 2010. A clinical approach to determine false positive findings of clinical Endometritis by vaginoscopy by the use of uterine bacteriology and cytology in dairy cows Theriogenology, 74 (7): 1248-1255.
8. Wiegand, P.; Schurenkamp, M. & Schutte, U. 1992. DNA extraction from mixture of body fluid using preferential lysis. Int. J. Leg. Med., 104:359-360.
9. Adam, L.; Aizinbud, E.; Tadmor, A. & Schindler, H. 2007. Impedometric properties of the vulvar and vaginal tissues of ewes during the oestrous cycle. J. Anim. Sci., 85: 1908-1913.
10. Steel, R. G. & Torries, J. H. 1980. Principles and Procedure of statistics. A. Biometrical Approach. 2nd Ed. McGraw-Hill Book Co. New York. USA.
11. Salisbury, G. W.; VanDemark, N. L. & Lodge, J. R. 1978. Physiology of Reproduction and Artificial Insemination of cattle. Second Ed.
12. Tang, B. Y. & Adams, N. R. 1978. Enzyme Activities and Protein and Carbohydrate Concentrations in Cervical Secretions at Dioestrus in Normal Ewes and Ewes With Permanent Phytoestrogenic Infertility. Aust. J. Biol. Sci., 31(3): 241- 246.
13. Martin Sheldon, I.; Gregory, S.; Lewis, S. L. & Robert, O. G. 2006. Defining postpartum uterine disease in cattle. Theriogenology, 65: 1516-1530.
14. Yoshida, K.; Sekiguchi, K.; Mizuno, N.; Kasai, K.; Sakai, I.; Sato, H. & Seta, S. 1995. The modified method of two-step differential extraction of sperm and vaginal epithelial cell DNA from vaginal fluid mixed with semen. Forensic Sci. Int., 72 (1): 25-33.
15. Elmer-Dewitt, P. 1994. The genetic revolution. PP. 46-52. Time 17 Jan.