

## ALGERIAN SHEEP ARE NONSEASONAL BREEDERS: "CLINICAL, CYTOLOGICAL AND HISTOLOGICAL STUDIES"

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### Abstract

4377 pregnant ewes have been used in this study to realise a curve of lambing. This curve prove that the three most important breeds of Algerian sheep can breed all year round.

To prove also that Algerian sheep are no seasonal breeder, a cytological and histological study of ewes vaginal mucosa have been done. The exfoliative cytology over all the phases of the oestrous cycle and pregnancy consistent in appearance and appear to be influenced directly by the changes in endogenous ovarian steroid hormones.

The histological features of the anterior vaginal epithelium of the ewe sampled all year round, before, during, after oestrus and at pregnancy are described. In the present study, we have never found an arrest of the cyclical genital activity of ewes at any moment of the year. This finding agree with results of the curve of lambing. We can conclude that Algerian ewes are nonseasonal breeders.

**Key words:** Ewes, Curve of lambing, seasonal and non seasonal breeding, vaginal exfoliative cytology, vaginal histology.

### Résumé

Un total de 4377 brebis gestantes ont été utilisées dans le cadre de cette étude, dans le but de réaliser une courbe d'agnelage caractéristique de nos différentes races ovines Algériennes. Cette courbe a pu mettre en évidence que nos brebis sont capables de se reproduire tout au long de l'année.

Pour le besoin de confirmer nos résultats cliniques, nous avons fait le suivi cytologique et histologique de la muqueuse vaginale sur un échantillon de 36 brebis. La cytologie exfoliative vaginale de toutes les phases du cycle sexuel et de la gestation, a été consistante en apparence, et paraît être influencée directement par les changements endogènes en hormones stéroïdiens ovariens.

Les caractéristiques histologiques de l'épithélium de la muqueuse vaginale antérieure montrent qu'il n'y a aucun arrêt de l'activité sexuelle chez la brebis, et ce, tout au long de l'année. Ces résultats s'accordent parfaitement avec ceux de la courbe d'agnelage et nous permettent de conclure que la cyclicité des brebis de nos trois principales races ovines, n'est pas saisonnière.

**Mots clés :** Brebis, courbe d'agnelage, cyclicité sexuelle, saisonnalité, cytologie et histologie vaginale.

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### ملخص

تم استخدام 4377 نعجة حاملية في هذه الدراسة لرسم المنحنى البياني للولادات عند أهم سلالات الأغنام الجزائرية، ولقد تبين من هذا المنحنى أن هذه السلالات يمكنها أن تضع مواليدها طول أيام السنة.

للكشف عن عدم وجود تناسل فصلي عند أهم سلالات الأغنام الجزائرية، قمنا بإجراء دراسة نسيجية و خلوية لعينات من مخاطية المهبل لهاته النعاج. أثبتت نتائج هذا الفحص عن وجود استمرارية في عمل هذه المخاطية، كونها مرتبطة بالإفرازات الهرمونية الستيرويدية للمبيض في جميع مراحل دورة الشبق عند هاته النعاج.

خلال هذه الدراسة النسيجية والخلوية، لم نستطع ملاحظة أي توقف لنشاط مخاطية المهبل عند النعاج في أي وقت من أوقات السنة، وقد كان نشاطها دوري ومستمر.

نتائج الدراسة النسيجية و الخلوية كانت موافقة للمنحنى البياني للولادات لهاته النعاج، وهذا يمكننا من استنتاج عدم وجود تناسل فصلي عند نعاج أهم سلالات الأغنام الجزائرية.

**الكلمات المفتاحية:** نعاج، منحنى الولادات، الدورة التناسلية. فصيلة التناسل، دراسة نسيجية و خلوية للنسيج المهبلية عند الأغنام.

The most domestic breed of sheep developed in colder climates, such as the RAMBOUILLET, TEXEL, SOUTH-DOWN, and consequently, as seasonally polyoestrous. The availability of feed and the climatic conditions are such that the new born will not survive unless they are born at the most optimal time this favoured a fall breeding season and delivery in the spring.

The other group includes the nonseasonal breeders who had their origin around the Mediterranean Sea where climatic conditions are not as severe and the new-born can survive all year round. This group includes the MERINOS, KARAKUL and PERSIAN BLACKHEAD. This is may be the case of ALGERIAN breeds such as OULED DJELLAL, HAMRA and RUMBI.

The exfoliative cytology and histology of the vagina is a sensitive indicator of the stage of the oestrous cycle in many species, presumably reflecting the balance between the influence of estrogens and progesterones [1,2]. In some species such as dog, mouse, Rabbit, sow, ewe, such changes are fairly consistent and can be used as a reliable diagnostic aid in determining the stage of the oestrous cycle or early pregnancy diagnosis [3,4,5].

With the aim of establishing the nature of breeding in the different breeds of Algerian ewes by the mean of the cyclical changes that do occur in the vaginal epithelium, the present study began first by a clinical survey of lambing among the twelve months of the year.

## MATERIALS AND METHODS

**PART ONE:** to study the nature of breeding in our three breeds of ewes, we have established a curve of lambing over more than 4300 lambing ewes. We have raised the lambing ewes at every month of the year as a manner to demonstrate the seasonality or the nonseasonality breeding in our Algerian ewes.

**PART TWO:** Thirty six cytological and histological examinations of biopsies from the anterior vagina have been done from an abattoir over a period of 12 months (3 samples per month; each sample represents one of the three breeds of Algerian sheep: (OULED DJELLAL; HAMRA, and RUMBI).

After each sample, the genital tract of the sampled ewes is examined carefully and classified into one of six groups according to the gross appearance of the ovaries and the uterus (proestrus, oestrus, métoestrus, dioestrus, anoestrus and pregnancy).

**1) Collection of cells:** the samples were done carefully from the anterior vagina with AYRE spatula. The diluted mucus was then spread onto a labelled clean microscope slide using another slide as for the normal preparation of blood smears. The slides were immediately fixed in 10% Formol saline, before staining with haematoxylin and Eosin (H.E) [6].

The epithelial cells present in 20 fields were counted on each slide under light microscopy at x 400 magnification, and a mean value calculated per field for each smear. The cell types were classified as follows:

**Parabasal cells:** round, oval or polyhedral basophilic cells with a large round or centrally located ovoid vesicular nucleus.

**Intermediate cells:** larger than Parabasal cells, round, oval or polygonal in shape with large vesicular nucleus showing a distinct chromatin network.

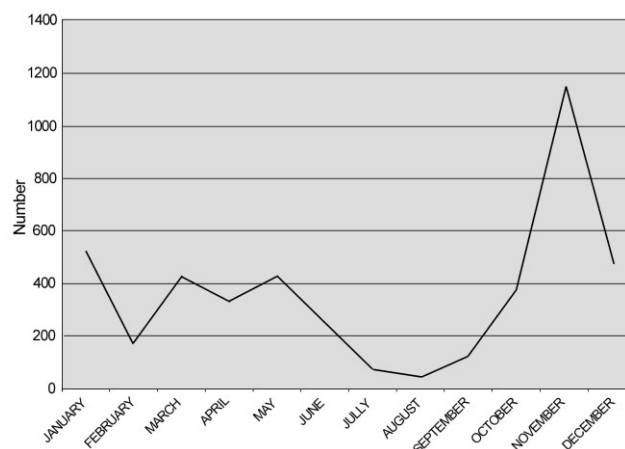
**Superficial cells:** large flat, polyhedral – shaped cells with a transparent basophilic cytoplasm and dense centrally located pyknotic nucleus.

**Cornified cells:** large or small in size round, oval or polygonal in shape, with eosinophilic pink, orange or red cytoplasm. The nucleus is usually pyknotic.

**2) Collection of tissues:** with a special instrument called “NOVAC canula” or “CUZZI curette”, we removed from the upper vagina a small piece of the superficial mucous membrane about 4 x 2 mm. The biopsy is transferred immediately to labelled tubes containing 3 to 5ml of an alcoholic BOUIN-type fixative. After an overnight fixation, the biopsy is transferred to 80 percent ethanol. After that, the biopsy is trimmed under the dissecting microscope using a single-edged razor blade. The trimmed tissue is embedded in paraffin wax. From each biopsy three or more sections, 5 to 7 (thick are mounted on one slide, stained with haematoxylin and eosin (H.E)).

## RESULTS

### A. Curve of lambing (Fig1)



**Figure 1:** Repartition of lambing within the twelvemonths of the year of Algerian ewes.

We can see among results in table 1 that the three breeds of Algerian ewes can produce a lamb across the twelve months. Repartition of lambing is not homogeneous, but the highest percentage of lambing occurs in autumn, which corresponds to the breeding period of the spring, corresponding with increasing length of daylight.

Month	Number of lambing ewes	%
January	521	11,90%
February	167	3,81%
March	424	9,68%
April	331	7,56%
May	426	9,73%
June	244	5,57%
July	76	1,73%
August	48	1,09%
September	124	2,83%
October	380	8,68%
November	1154	26,36%
December	482	11,01%
Total	4377	100%

**Table 1:** Lambing frequencies within the twelvemonths of the year of Algerian ewes.

### B. Vaginal cytology

\* During pro-oestrus and oestrus (Fig. 2 and 3): pro-oestrus and oestrus smears were very clear and characterised by presence of great number of superficial cells and Cornified cells. We can also find a great number of intermediate cells and the scarcity of Parabasal cells. Rare leukocytes may appear in the smears.

\* During métoestrus (Fig. 4): smears of this period showed an increase in the number of leukocytes and persistently large number of intermediate cells, and small number of superficial and Cornified cells.

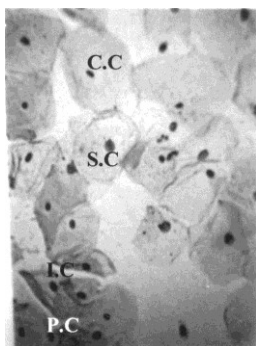


Fig.2

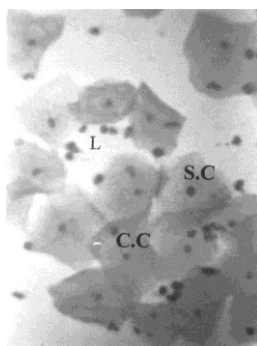


Fig.3

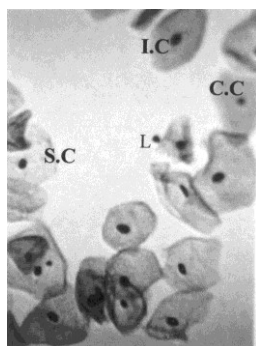


Fig.4

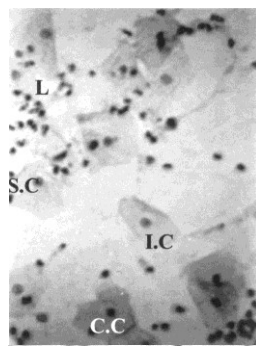


Fig.5

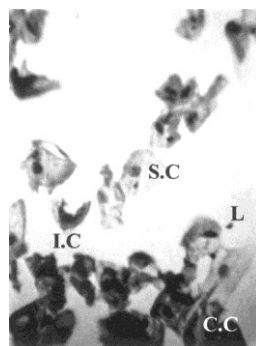


Fig.6

**Figure 2:** Photomicrograph of ovine vaginal smear, taken in pro-oestrus, showing the presence of the four types cells, of which cornified cells (C.C) and intermediate cells (I.C) predominate (H.E  $\times$  160).

**Figure 3:** Photomicrograph of ovine vaginal smear, taken at oestrus, showing, the predominance of cornified cells (C.C) (H.E  $\times$  160 )

**Figure 4:** Photomicrograph of ovine vaginal smear, taken at metoestrus showing intermediate cells (I.C) and leucocytes (L) adherent to mucus strand (H.E  $\times$  160).

**Figure 5:** Photomicrograph of ovine vaginal smear, taken at dioestrus showing large number of neutrophils and intermediate cells (H.E  $\times$  160).

**Figure 6:** Photomicrograph of ovine vaginal smear, taken at pregnancy showing the predominance of intermediate cells (I.C) or shipped cells (H.E  $\times$  160).

\* During dioestrus (Fig.5): at this stage of cycle, the predominant type of epithelial cell was the intermediate cell. Other types of epithelial cells were also represented in few numbers and leukocytes were present in fairly large number.

\* During pregnancy (Fig.6): pregnancy smears approaches those of the métoestrus period, with only a difference characterised by the presence of a great number of a new kind of cells called « shipped cells ».

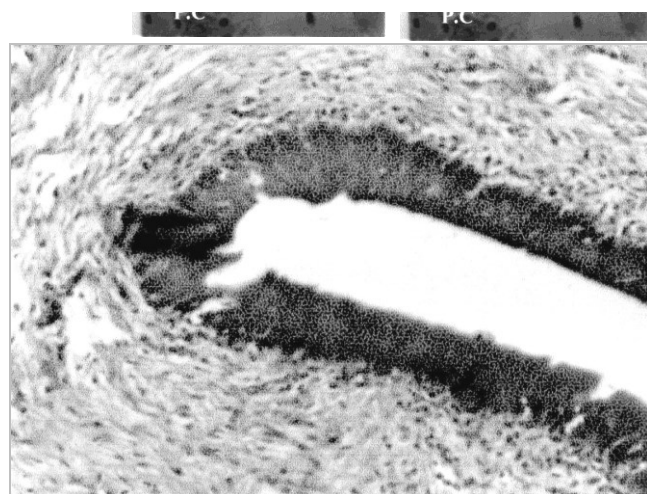
### C. Histology of the vaginal mucosa

The vaginal of the ewe is lined by a stratified epithelium. This epithelium normally undergoes regular cyclical changes related to the oestrous cycle and during pregnancy.

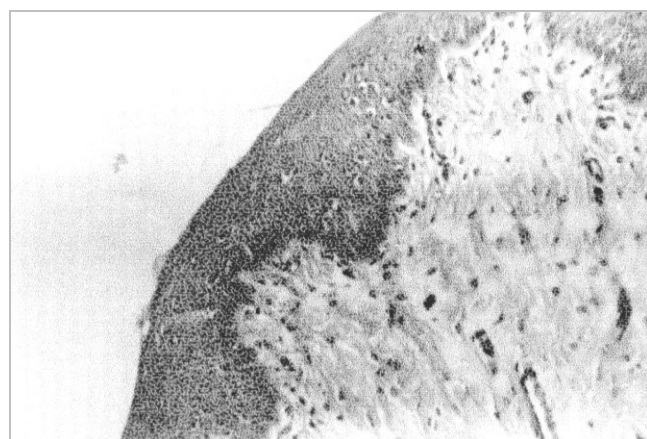
\* **During pro-oestrus and oestrus:** the epithelium proliferate rapidly reaching a depth of 16 to 20 cells during late oestrus. The luminal cells were flattened squames with deep staining nuclei, and the deeper cells were polygonal with bright staining spherical nuclei. Small numbers of leukocytes were seen in the sub-epithelial tissue and keratinisation was observed at oestrus (Fig. 7 and 8).

\* **During métoestrus and dioestrus:** during 15 period, the depth of the epithelium decreases sharply at first and then more slowly to 3 to 5 cells. Crypts seen at the oestrus period tend to become shallower or disappear. The stratum germinativum becomes cuboidal and the more superficial cells though still irregularly arranged, stain fairly uniformly (Fig. 9 and 10).

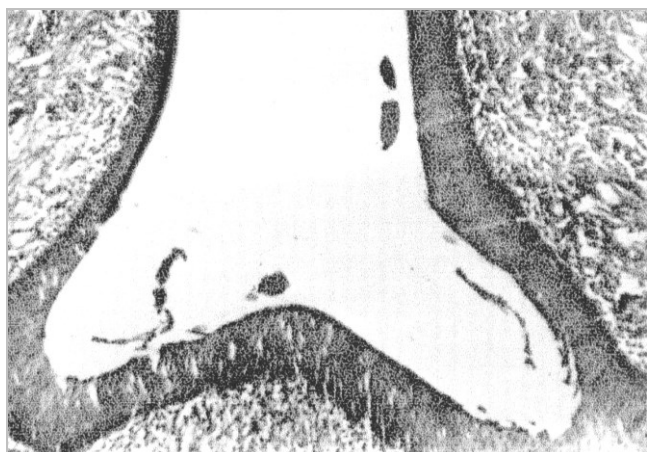
**During pregnancy:** in the pregnant animal the simplification process continues still further. The epithelium eventually comprises two or at the most three layers of cells arranged regularly. The luminal cells were cubiform and prismoidal with densely staining nuclei (Fig11).



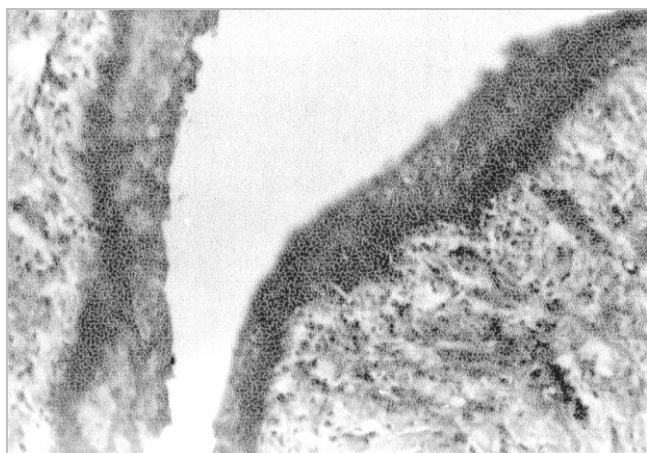
**Figure 7:** Vaginal epithelium of cyclic ewes during pro-oestrus. The cell depth is between six to seven cells (H.E  $\times$  220).



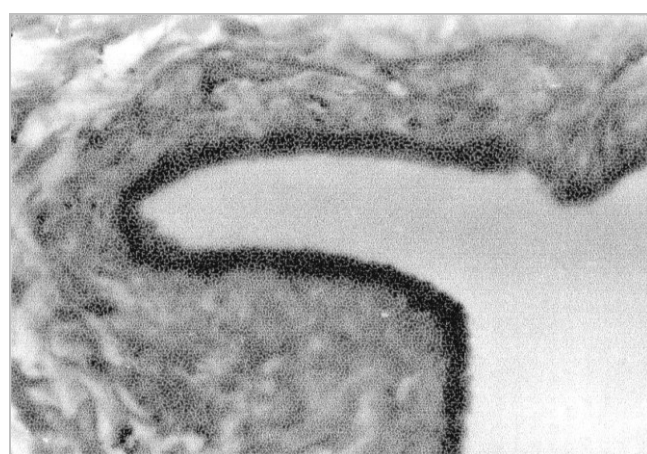
**Figure 8:** Vaginal epithelium of cyclic ewes during oestrus. The cell depth is between 16 to 20 cells, and showing deep epithelial ridges (H.E  $\times$  210).



**Figure 9:** Vaginal epithelium of cyclic ewes during metoestrus. The superficial cells are being sloughed into the lumen (H.E  $\times$  450).



**Figure 10:** Vaginal epithelium of cyclic ewes during dioestrus. The epithelial depth of 3 to 5 cells gives an irregular appearance to the rows of nuclei (H.E  $\times$  450).



**Figure 11:** Vaginal epithelium of pregnant ewes. The depth of epithelium is 2 to 3 cells only with a typical regularity in arrangement of the cells (H.E  $\times$  115).

## DISCUSSION AND CONCLUSION

Across these results we can say that Algerian breeds of ewes can produce a lamb all over the twelve month. The theory according to what the stimulus for the annual onset of sexual activity declining length of daylight is wrong. In Algeria, the best results are obtained when breeding occurs in spring and summer; this results in a high percentage of lambing in autumn and early winter.

Bonnes *et al.* [7] bring back the same finding in MERINOS, Ile de France Preaple and ROMANOV breeds in France. Goot [8] and Arthur *et al.* [9] bring back the same results in Great Britain and Spain.

Neutrophils, round squamous epithelial cells and polygonal epithelial cells with or without keratinisation were recorded as being present in vaginal smears of ewes sampled during the oestrous cycle and pregnancy. Similar results have been reported by Cole and Miller [10], Robinson and Moor [11].

In our experience the vaginal biopsy method offers a reasonably simple yet accurate means of detecting the stage of the oestrous cycle, or pregnancy diagnosis, or anoestrus diagnosis. In all our samples, we have always found in cytological or histological samples, only cycling ewes or ewes in pregnancy and at any moment of the year. We have a biopsy corresponding to an arrest of cyclicity in the three breeds of ewes in Algeria.

In this context, we can say that cytological, histological and clinical results agree with our theory that our ewes are nonseasonally breeders.

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## REFERENCES

- [1]- Papanicolaou G.N., *Amer. J. of obst. and Gyne.*, (1946), pp. 51-317.
- [2]- Papanicolaou G.N., *Atlas of exfoliative cytology*, Cambridge M.A., Harvard University Press, (1954).
- [3]- Miroud K. and Noakes D.E., *Br. Vet.*, (1990), pp. 146-387.
- [4]- Done T.T. and Heard T.W., *Veterinary record*, (1965), pp. 88-64.
- [5]- Richardson C., *Vet. Rec.*, (1972), pp. 90-264.
- [6]- Martoja R. et Martoja M., "Initiation aux techniques histologiques", Masson Editions, (1967).
- [7]- Bonnes G. Desclaude J. et Gadoud R., "Reproduction des mammifères d'élevage", INRA Collections, (1988).
- [8]- Goot H., *J. Agr. Sci.*, (1969), pp. 73-77.
- [9]- Arthur G.H., Noakes D.E. and Pearson H., "Veterinary reproduction and obstetrics", 6<sup>th</sup> edition, (1992).
- [10]- Cole H.H. and Miller R.F., *Am. J. Anat.*, (1935), pp. 57-39.
- [11]- Robinson T.J. and Moore N.W., *J. Endocr.*, (1956), pp. 19-288. □