Int. J. Morphol.,
30(4):1461-1465, 2012.

Evidence of Fractality in a Pattern of Crystallization of Bovine Cervical Mucus Obtained at Oestrus

Evidencia de Fractalidad en un Patrón de Cristalización de Moco Cervical Bovino Obtenido en Estro

[*,**]**Manuel E. Cortés;** [**,***]**Roberto Hauyón;** [*]**Fernando González &** [**,****]**Pilar Vigil**

CORTES, M. E.; HAUYÓN, R.; GONZÁLEZ, F. & VIGIL, P. Evidence of fractality in a pattern of crystallization of bovine cervical mucus obtained at oestrus. ***Int. J. Morphol., 30(4)***:1461-1465, 2012.

SUMMARY: Fractality has emerged as a feature of the organization of some complex natural systems. Several biological secretions show fractal-like patterns for their crystallization phenomena but their presence in crystallizations of bovine cervical mucus (BCM) is yet unknown. In order to assess the fractality of BCM crystallization, samples of this fluid were taken from heifers at oestrus, their crystalline patterns photographed and its morphology analyzed. Among the many images obtained for BCM crystallizations, one of them had a highly symmetrical geometric arrangement, possessing three zones characterized by pine-like, arboriform structures, evidencing a remarkable similarity between them. Moreover, fractal dimensions obtained for these zones were statistically equal when analyzed by using specialized software. In summary, this brief communication shows, for the first time, that a pattern of crystallization of BCM at oestrus possesses a fractal-like organization.

KEY WORDS: Bovine cervical mucus; Cervical mucus crystallization; Fractal dimension; Fractality; Oestrus.

INTRODUCTION

Bovine cervical mucus (BCM) is produced by mucus-secreting epithelial cells located in the cervix and comprises two phases, aqueous and gel (Rutllant *et al*., 2005). The aqueous phase contains 92-95 % of water and several dissolved low-molecular-mass compounds, e.g., ions and metabolites (Tsiligianni *et al*., 2001); and the gel phase is formed mainly by glycoproteins called mucins (Rutllant *et al*., 2005; Pluta *et al*., 2011). Biophysical and biochemical properties of BCM are controlled by changes in sex steroid levels during the oestrous cycle (Rutllant *et al*., 2002). BCM is secreted during the whole cycle, yet its volume increases at oestrus under oestrogenic influence, which also induces its crystallization in branched arborescent patterns resembling fern fronds or palm leaves (Bone, 1954; Abusineina, 1962; Noonan *et al*., 1975), among other geometric arrangements (Cortés, 2012). Such crystalline patterns are more frequent at oestrus than in any other phase of the cycle (Alliston *et al*., 1958; Abusineina; Noonan *et al*.).

Crystallizations in arboriform patterns are not exclusive to BCM, but can also be observed in human cervical mucus (Papanicolaou, 1946), ocular fluids (Battaglia Parodi & Giusto, 1993), gall precipitates and human saliva (Shen *et al.*, 2005), among others. Fractal-like crystallization patterns have been reported for all of these secretions (Battaglia Parodi & Giusto; Shen *et al*.; Yang *et al*., 2011). A fractal is a structure comprised of smaller parts that resemble the whole in a smaller scale (Mandelbrot, 1993) and possess attributes such as roughness, irregular shape at every level, high degree of organization, self-similarity, lacunarity, and a characteristic fractal dimension (FD) (Losa, 2009).

Considering the current importance that the study of fractal geometry has for biomedicine, especially in the field of anatomy and microscopy (Losa; Landini, 2011), and the aforementioned fractality observed in other biological secretions, our objective was to assess whether among the crystallizations of BCM at oestrus it is possible to find fractal-like geometric patterns.

[*] Departamento de Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile.
[**] Programa Teen STAR de Educación Afectivo-Sexual, Pontificia Universidad Católica de Chile, Santiago, Chile.
[***] Programa de Formación Pedagógica, Facultad de Educación, Pontificia Universidad Católica de Chile, Santiago, Chile.
[****]Fundación Médica San Cristóbal, Santiago, Chile.

MATERIAL AND METHOD

Animals studied. The study involved ten Holstein Friesian heifers (15 months old) from the herd at the Experimental Station of the Faculty of Agronomy and Forestry Engineering, Pontificia Universidad Católica de Chile at Pirque, Chile.

Collection of cervical mucus samples. BCM samples were obtained from heifers at oestrus. Oestrus was confirmed by palpation of the genital organs per rectum as reported by other researchers (López-Gatius & Camón-Urgel, 1991). Mucus samples were collected by using a 50-mL sterile plastic tube and stored at 4ºC for later analysis (the interval between sampling and analysis was less than 24 h). The samples were subsequently analyzed in the laboratory, where those showing opacity, blood content or contamination were discarded.

This study considered all aspects concerning animal welfare and obtained the approval of the corresponding bioethical committee. In addition, the process of sample collection was under the permanent supervision of an experienced veterinary surgeon.

Analysis of crystallizations of cervical mucus. The crystallizations were analyzed by smearing the mucus samples on clean glass slides in all directions using a needle according to the 'spread-out' technique (Odeblad, 1995). Then, the samples on the slides were air-dried at room temperature (20-25ºC) for at least 15 min before being observed using a light microscope (Olympus CK X41). These relevant images were subsequently photographed.

Analysis of the characteristics of fractality. Most of the images obtained presented crystallizations including a combination of shapes resembling fern fronds or palm leaves (not shown), which have been previously reported by other authors (Bone; Alliston *et al.*; Abusineina; Noonan *et al*.). However, for one heifer, a particularly remarkable crystalline pattern was found whose geometric arrangement presented a high level of symmetry (Fig. 1A), an was the only one sample found with such characteristics. Due to the highly symmetrical organization present in this crystallization and the evident resemblance between some areas of its structure, the image was converted to black and white (Fig. 1B) and analysed with Fractalyse v. 2.4 (Lab. ThéMA, Université de Franche-Comté, FR) using the box-counting method. This methodology consists in overlapping a grid on the whole scene to be dimensioned, and then counting how many boxes within the grid possess at least one element of the set to be dimensioned. The box-counting method has been reported as a useful way to estimate FD in other biological specimens (Shen *et al*.; Losa). The mathematical expression underlying this methodology is:

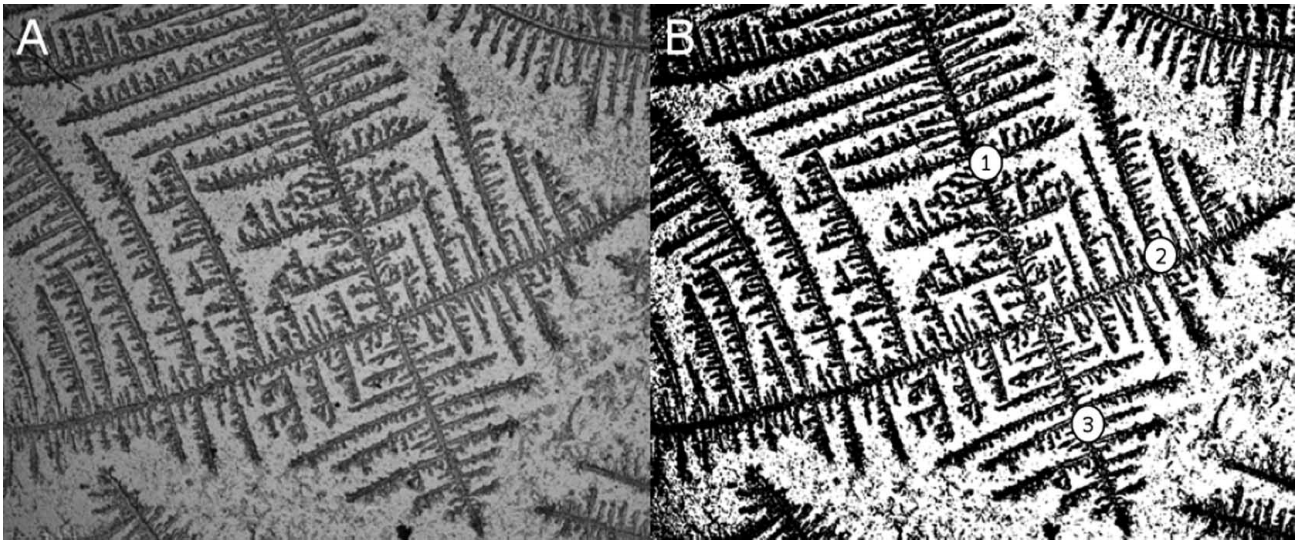

Fig. 1. Highly symmetrical crystallization pattern observed for bovine cervical mucus obtained at oestrus. A. This crystallization is remarkable due to its high level of symmetry and comprises four well-defined axes (stems) projecting from the same central point. The axes are arranged perpendicularly, forming angles of approximately 90°, and are delimiting four well-defined quadrants. From each axis, branchings (venations) of variable length originate, forming pine-like, arboriform structures within the whole of the image. This crystalline pattern is classified as crystallization Type A according to the classification proposed by Abusineina (1962). B. Black and white image for the described crystallization. The centre of each area to be studied is indicated by the numbers 1, 2 and 3, corresponding to Zone 1, Zone 2, and Zone 3 respectively. These zones possess a remarkable morphological similarity between them.

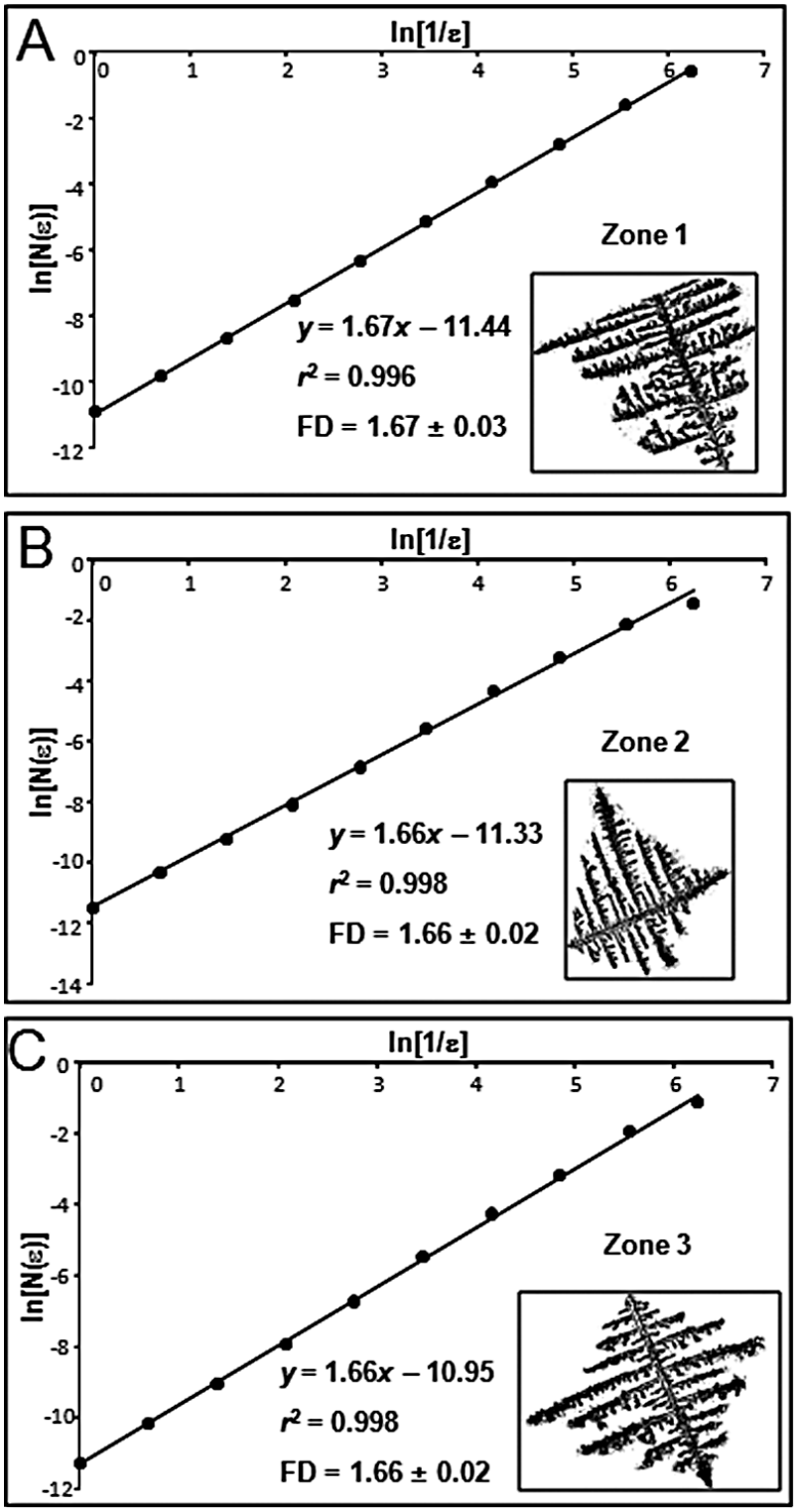

Fig. 2. ln[N(ε)] versus ln[1/ε] plots for each one of the studied zones in the crystallization of bovine cervical mucus obtained at oestrus. The analyzed zone is located in the lower right corner of each graph (A, B and C). A. Zone 1, FD (± SD) = 1.67 ± 0.03; B. Zone 2, FD = 1.66 ± 0.02; and C. Zone 3, FD = 1.66 ± 0.02. N(ε) = number of non-empty boxes in the grid; e = varying length of the box sides; FD = fractal dimension; SD = standard deviation.

$$FD = \lim_{\varepsilon \to 0} \frac{\ln[N(\varepsilon)]}{\ln[1/\varepsilon]}$$

where N(ε) represents the number of non-empty boxes in the grid, and e is the varying length of the box sides (Losa).

When performing the analysis, FD values were determined for areas within the whole image. Specifically, three zones (hereafter referred to as Zones 1, 2 and 3) showing the biggest resemblance in the studied scene were analyzed (Fig. 1B). Fractalyse rendered ln[N(ε)] versus ln[1/ε] plots for these zones and the regression line for each graph was obtained by using Excel based on the Fractalyse output.

RESULTS

The ln-ln plots and their corresponding analyzed zones (in the lower right corner) are shown in Fig. 2. Fig. 2A shows the plot for Zone 1, with FD (± standard deviation, SD) of 1.67 ± 0.03. Zone 2 had a FD of 1.66 ± 0.02 (Fig. 2B). Finally, Zone 3 had a FD of 1.66 ± 0.02 (Fig. 2C). In general terms, FD obtained for the three zones was statistically the same.

The selected crystallization areas shown a very similar structure, but their original orientation (Fig. 1B) does not enable a more detailed observation of such similarity. Fig. 3A shows the original spatial arrangement of the three zones of crystallization. The high resemblance in the geometric pattern of these zones could be observed in more detail after rotating and aligning them parallel to the main axis (Fig. 3B). This resemblance is confirmed by the aforementioned FD values, which are similar to each other.

DISCUSSION

To the best of our knowledge, this brief report is the first to show that the geometric arrangement of the arboriform

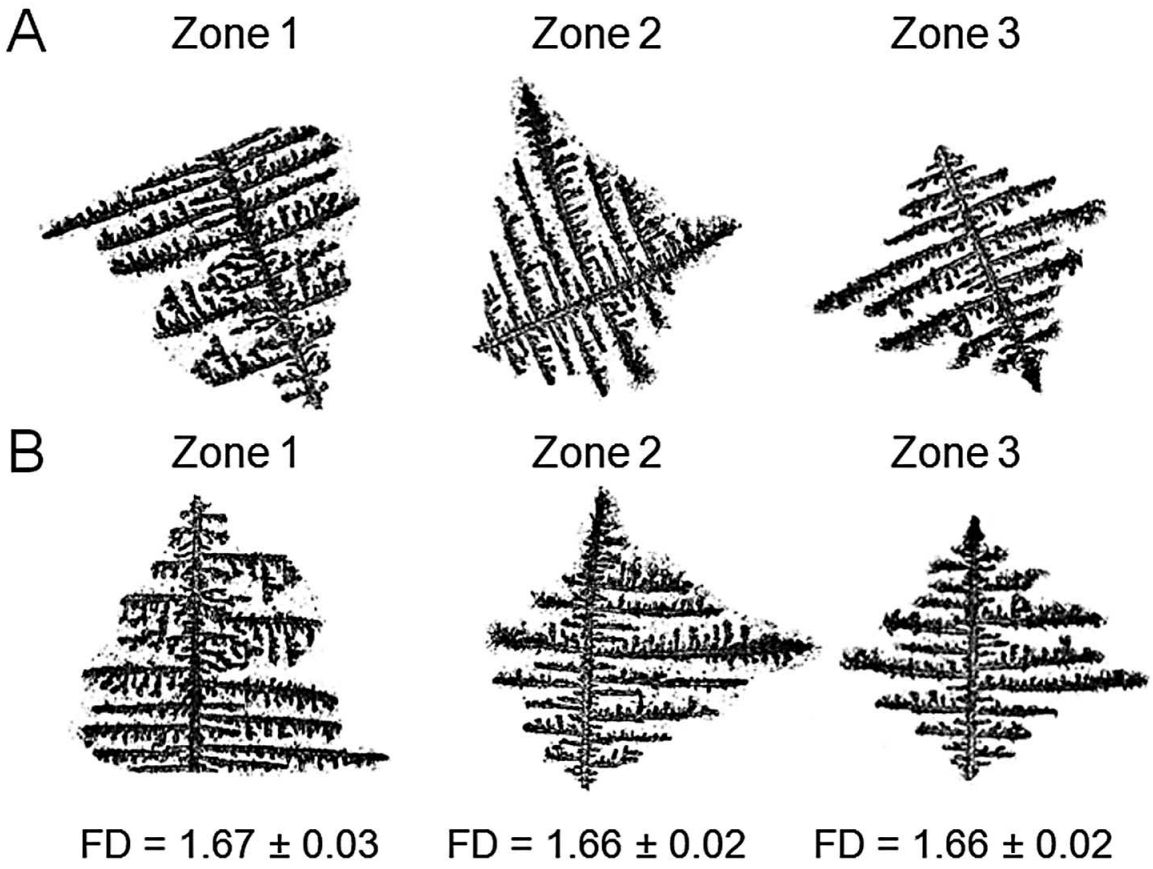

Fig. 3. Resemblance between the studied zones in the crystallization of bovine cervical mucus obtained at oestrus. A. From left to right, it is possible to observe the original arrangement of the three studied zones. B. When aligning the three areas according to the main axis, the high degree of geometric and structural similarity can be observed, a resemblance which is also evident in its FD values, approximately the same. FD = fractal dimension.

crystallizations of BCM at oestrus would be characterized by a fractal-like organization. The fractality is evidenced when observing that the FD values determined for the three different studied areas within the whole image are statistically the same (Fig. 2). Moreover, when conveniently ordering the analyzed zones, these revealed great geometric and structural similarity (Fig. 3B). The presence of fractality in BCM is in agreement with preliminary reports on the existence of a fractal-like pattern in the arborizations of human cervical mucus (Yang *et al.*). In the present study, FD values determined for the three crystallization zones are, interestingly, very close to 1.7, the characteristic FD value for processes of diffusion-limited growth (Losa), such as ice crystallization, which also possesses a fractal-like geometry.

Even though this short communication reports on just one case of crystallization presenting fractality, further studies ought to be carried out in order to determine the frequency of this phenomenon, as well as to find out more about its scope and usefulness, opening a new line of research in this area. Regarding the latter, the development of a method to assess the features of BCM fractality (and its alterations) at the different stages of the cycle, mainly oestrus, would yield some indicators of the endocrine and reproductive status of the animal. This could be of relevance to clinical veterinary practice considering that it is known that BCM characteristics not only change according to the fluctuating levels of ovarian sex steroids during the cycle (Bone; Noonan *et al*.), but also that the characteristics of mucus can change and present alterations due to fertility problems (Rutllant *et al.*, 2002).

Conflict of interest statement. None of the authors of this brief report has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the manuscript.

ACKNOWLEDGEMENTS. M.E. Cortés thanks CONICYT - Chile for the scholarship (# 21080589) granted for PhD studies. The authors also wish to thank Dr. Jorge L. Alvarado (Pontificia Universidad Católica de Chile), Prof. Jaime Rössler (Universidad de Chile) and Prof. Dr. Leonard F. Blackwell (Massey University) for their critical comments about this manuscript.

CORTES, M. E.; HAUYÓN, R.; GONZÁLEZ, F. & VIGIL, P. Evidencia de fractalidad en un patrón de cristalización de moco cervical bovino obtenido en estro. ***Int. J. Morphol., 30(4)***:1461-1465, 2012.

RESUMEN: La fractalidad ha surgido como una característica propia de algunos sistemas biológicos complejos. Varias secreciones biológicas presentan patrones de tipo fractal al cristalizar; no obstante, su presencia en las cristalizaciones de moco cervical bovino aún se desconoce. Con el objetivo de investigar la presencia de fractalidad en las cristalizaciones de moco cervical bovino, se obtuvieron muestras de este fluido de vaquillas en estro, fotografiándose los patrones cristalinos observados. Entre las típicas disposiciones similares a frondas de helechos observadas para la cristalización del moco cervical, se encontró una imagen con un arreglo geométrico altamente simétrico, constando de tres zonas caracterizadas por estructuras arborescentes similares a pinos, muy semejantes entre sí. Más aún, las dimensiones fractales obtenidas para esas zonas fueron estadísticamente iguales al ser dichas áreas analizadas con un programa computacional apropiado. En resumen, esta comunicación breve demuestra por primera vez que uno de los patrones de cristalización de moco cervical bovino en estro está caracterizado por una organización de tipo fractal.

PALABRAS CLAVE: Cristalización del moco cervical; Dimensión fractal; Estro; Fractalidad; Moco cervical bovino.

REFERENCES

Abusineina, M. E. A study of the fern-like crystalline patterns of the cervical and vaginal mucus of cattle. *Vet. Rec., 74(22)*:619-21, 1962

Alliston, C. W.; Patterson, T. B. & Ulberg, L. C. Crystallization patterns of cervical mucus as related to estrus in beef cattle. *J. Anim. Sci., 17(2)*:322-5, 1958.

Bone, J. F. Crystallization patterns in vaginal and cervical mucus smears as related to bovine ovarian activity and pregnancy. *Am. J. Vet. Res., 15(57)*:542-7, 1954.

Cortés, M. E. *Morphological and ultrastructural characterization of different types of bovine cervical mucus using light and scanning electron microscopy.* PhD Thesis, Santiago, Pontificia Universidad Católica de Chile, 2012.

Landini, G. Fractals in microscopy. *J. Microsc., 241(1)*:1-8, 2011.

López-Gatius, F. & Camón-Urgel, J. Confirmation of oestrus rates by palpation per rectum of genital organs in normal repeat dairy cows. *Zentralbl. Veterinarmed. A, 38(7)*:553-6, 1991.

Losa, G. A. The fractal geometry of life. *Riv. Biol., 102(1)*:29-59, 2009.

Mandelbrot, B. B. *Los objetos fractales: Forma, azar y dimensión.* 3ª ed. Barcelona, Tusquets, 1993.

Noonan, J. J.; Schultze, A. B. & Ellington, E. F. Changes in bovine cervical and vaginal mucus during the estrous cycle and early pregnancy. *J. Anim. Sci., 41(4)*:1084-9, 1975.

Odeblad, E. *The spread out technique. Advantages, pitfalls and biological interpretation.* Barcelona, Actas IV Simposium Internacional sobre Regulación Natural de la Fertilidad, 1995. pp.295-303.

Papanicolaou, G. N. A general survey of the vaginal smear and its use in research and diagnosis. *Am. J. Obstet. Gynecol., 51*:316-28, 1946.

Battaglia Parodi, M. & Giusto, D. D. Ocular fluid ferning test and fractals. *Ophthalmic Res., 25(5)*:307-13, 1993.

Pluta, K.; Irwin, J. A.; Dolphin, C.; Richardson, L.; Fitzpatrick, E.; Gallagher, M. E.; Reid, C. J.; Crowe, M. A.; Roche, J. F.; Lonergan, P.; Carrington, S. D. & Evans. A. C. Glycoproteins and glycosidases of the cervix during the periestrous period in cattle. *J. Anim. Sci., 89(12)*:4032-42, 2011.

Rutllant, J.; López-Béjar, M.; Santolaria, P.; Yániz, J. & López-Gatius, F. Rheological and ultrastructural properties of bovine vaginal fluid obtained at oestrus. *J. Anat., 201(1)*:53-60, 2002.

Rutllant, J.; López-Béjar, M. & López-Gatius, F. Ultrastructural and rheological properties of bovine vaginal fluid and its relation to sperm motility and fertilization: a review. *Reprod. Domest. Anim., 40(2)*:79-86, 2005.

Shen, Y.; Xie, A.; Zhang, j.; Cui, F. & Zhu, H. Fractal structures precipitated from A gall of patient. *Phys. B Condens. Matter, 363(1-4)*:61-8, 2005.

Tsiligianni, T. H.; Karagiannidis, A.; Brikas, P. & Saratsis, P. H. Chemical properties of bovine cervical mucus during normal estrus and estrus induced by progesterone and/or PGF2a. *Theriogenology, 56(1)*:41-50, 2001.

Yang, G.; Yang, Z.; Zhou, H. & Huang, T. Fractal analysis of the fern-leaf crystallization of cervical mucus. *J. Yunnan Univ. Natly., 20(1)*:75-8, 2011.

Correspondence to:
Prof. Manuel E. Cortés, PhD
Investigador Asociado, Programa Teen STAR
Pontificia Universidad Católica de Chile
Alameda B. 0'Higgins 340
Santiago
CHILE

Email: manuelcortes@uc.cl

Received: 06-04-2012
Accepted: 03-07-2012