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Analysis of HSP-90, Annexin A1 and Annexin A2 biomarkers in healthy mares and mares suffering from endometritis

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Abstract
Endometritis, which affects around 60% of barren mares, is one of the most common causes of subfertility and infertility in breeding mares. The diagnosis of endometritis can be done by various methods. Heat shock protein (HSP-90) are significantly produced by heat shock and other kinds of chemical and physical stress. In some studies, shot gun proteomics was also used to identify heat shock proteins in mares suffering from endometritis. Annexins 1 to 5 are expressed locally in the horse's endometrium, with ANXA1 and ANXA2 being the most prevalent. The present study was designed to analyse the presence of HSP90, Annexin A1 and A2 biomarkers in low volume uterine lavage as well as serum samples from healthy as well as mares suffering from endometritis. The results indicated that above mentioned biomarkers were found in the low volume uterine lavage samples as well as in the serum samples but the concentration was found to be very low, and no significant difference was found between the values of optical density obtained by the ELISA reader. The reason for the results obtained could be the actual low concentration of these biomarkers present in the uterine lavage samples as well as in serum samples of the mares. It can be concluded that the analysis of HSP-90, Annexin A1 and A2 biomarkers by using ELISA is not remarkable for the diagnosis of endometritis in mares.

Keywords: Equine, Endometritis, HSP90, Annexin A1, Annexin A2

Introduction
Endometritis, which affects around 60% of barren mares, is one of the most common causes of subfertility and infertility in breeding mares [1, 2, 3]. The diagnosis of this condition is based on the mare's medical history, external exams, vaginal examinations, rectal palpation, and reproductive tract ultrasonography. Laboratory diagnostic procedures, such as uterine cytology, uterine culture, endometrial biopsy, and endoscopic examination of the mare's vaginal organs, are also employed [4, 5, 6]. Endometritis detection can be difficult at times, and this might be owing to insufficient diagnostic procedures and misunderstanding of the findings [7, 8, 2, 9, 4, 5].

Heat shock proteins (HSPs) are significantly produced by heat shock and other kinds of chemical and physical stress [10]. In both prokaryotic and eukaryotic species, their expression fluctuates with normal physiological processes and is also influenced by pathological circumstances [11]. Shot gun proteomics was also used to identify heat shock proteins in mares suffering from endometritis [12]. The most common reason of mare infertility is endometritis, which causes embryonic death or early abortion, lowering conception chances [13]. As a result, it would make sense to look at HSPs in equine endometrium during oestrus to see if they have a role in changing the endometrial environment. Morphometric changes in the endometrium are a normal feature of the oestrus but, to date, they have not been associated with patterns of HSP90 immuno-expression.

The calcium-regulated phospholipids and membrane-binding proteins known as annexins (ANXA) are a multigenic family of proteins [14]. They are phospholipid-binding proteins that are calcium-dependent. They also participate in endocytosis and exocytosis [15]. Annexin A2 was previously found in mare endometrium and the yolk sacs of a 20-day old conceptus of horses [16]. Annexins 1 to 5 are expressed locally in the horse's endometrium, with ANXA1 and ANXA2 being the most prevalent [16]. By controlling the activity of phospholipase A2, annexin A1 is capable of influencing the release of arachidonic acid [17]; an important fatty

acid present in membrane phospholipids. Annexins were formerly considered to be intracellular proteins. However, it has recently been proven that annexins (annexins A1 and A2) may be carried out of the cell via unusual secretory routes [18, 19, 20]. Annexin1 is a 37 kD endogenous anti-inflammatory peptide that is controlled by glucocorticoids and is prevalent in neutrophils and other blood cells [21]. Although annexin1 generally inhibits neutrophil activity, cleavage in the N-terminal region of protein which leads in the production of anti-inflammatory peptides that can potentially promotes inflammatory consequences [22, 23]. Annexin A2 on the cell surface and in the cell, matrix regulates cell adhesion and migration via interacting with proteases and the cell matrix [24, 25]. Annexin A2 was identified in vivo in syncytiotrophoblasts and has since been shown to be necessary for cancer invasion and metastasis, indicating a probable role in conceptus invasion [26].

Keeping in view the above findings, the present study was designed to study the presence of biomarkers in uterine lavage sample and serum sample from the healthy mares and mares with endometritis.

Materials and Methods

Place of work

The present study was conducted at various Stud Farms in and around Pune and the Department of Animal Reproduction, Gynaecology and Obstetrics, Mumbai Veterinary College, Mumbai – 400012. The mares in study were available at the Stud Farms. All mares were reared under standard feeding and managemental practice.

Selection of Animals

The mares with or without history of abortion were screened by uterine swab cytology and trans – rectal sonography for endometritis. Afterwards, the screened mares were divided into two groups *i.e.,* mares suffering from endometritis (Group-I) and mares with healthy uterus (Group II), in each group 15 mares were taken for the study.

All the selected mares were in an ideal body condition score and having age between 4 to 6 years were selected for the study.

Collection of samples

The mares were restrained in a travis or stock for the trans rectal examination and collection of swabs as well as lavage samples. Uncooperative mares were controlled by a twitch applied to the ear pinna or the upper lip. No chemical restraint was used. No special preparation was required for per-rectal and ultrasonographic examination.

The blood samples and low volume uterine lavage samples were also collected from all 30 selected mares. The samples were then analysed for Heat shock protein (HSP 90) analysis (Biomarker) and Annexin A1 and A2 analysis (Biomarker).

Analysis of Biomarkers

The analysis of biomarkers i.e., HSP-90 (Heat shock protein – 90), Annexin A-1 and Annexin A-2 was done using commercial equine specific ELISA kits. The analysis was done from serum samples as well as low volume uterine lavage samples both were preserved at -80°C. The results of the ELISA were read in a standard ELISA reading machine and recorded.

Results and Discussion

Heat shock protein (HSP 90) analysis

Commercial ELISA kits were used for detection of Heat shock protein – 90 in uterine lavage samples and in blood serum samples. Results showed no significant difference and the HSP 90 biomarker was not detected in any of the samples from group I as well as group II, the standard curve was plotted for the same (Figure – 01 and Table - 01).

HSP90 is found to be localized in the supranuclear region of the glandular epithelial cells, particularly during oestrus and dioestrus [27]. Heat shock proteins (HSPs) are preserved chaperones that assume significant parts in protein breakdown, development, and refolding. HSP10, HSP40, HSP70, HSP90, HSPB1, HSPD, and HSPH1 families are among the adenosine triphosphate-subordinate chaperones ordered in view of their atomic mass, which ranges between 10-100 kD. HSPs are required for cellular responses, protein homeostasis, and survival under stressful situations [28].

In present study the HSP90 were found in the uterine lavage samples as well as in the serum samples but the concentration was found to be very low, and no significant difference was found between the values of optical density obtained by the ELISA reader. The reason for the results obtained could be the actual low concentration of these biomarkers present in the uterine lavage samples as well as in serum samples of the mares. No exact reference could be traced as per the ELISA for the HSP90 biomarker in equine.

Annexin A1 and Annexin A2 analysis

Commercial ELISA kits were used for detection of Annexin A1 and Annexin A2 in uterine lavage samples and in blood serum samples. Results showed no significant difference and the Annexin A1 and Annexin A2 biomarkers were not detected in any of the samples from group I as well as group II, the standard curve was plotted for the same (Figure – 02, 03 and Table – 02, 03).

Recent research using annexin removal or elimination models has revealed fresh information on the biological activities of numerous annexin proteins. The temporary depletion of annexin caused by RNA interference and the production of dominant negative mutant proteins demonstrated that proteins play a role in membrane activities ranging from membrane structural regulation to specific membrane transport events. Although such capabilities correlate well with annexins' capacity to connect with cell membranes in a reversible and controlled manner, certain activities are membrane independent, most likely because annexins may also engage particular protein-protein interactions. This might be shown in annexin A1 and A2 knockout mice, which display strange control of neutrophil extravasation or lacks in plasmin age, in addition to other things [14]. The equine endometrium communicates annexins 1-5, with ANXA1 and ANXA2 being the most successive [29]. Pickles *et al*., 2010 expressed that assuming the enactment of pony leukocytes in vivo outcomes in the delivery and resulting cleavage of annexin1, the N-terminal peptides framed could tie to the neutrophils and lessen the development of free extremists in light of molecule boosts. Although, annexins have long been known to be a multigenic family of Ca2 + -regulated membrane-binding proteins, only in recent years have direct inactivation and inactivation approaches discovered functional properties of various annexin proteins [14].

In present study the Annexin A1 and Annexin A2 were found in the uterine lavage samples as well as in the serum samples but the concentration was found to be very low, and no significant difference was found between the values of optical density obtained by the ELISA reader. The reason for the results obtained could be the actual low concentration of these biomarkers present in the uterine lavage samples as well as in serum samples of the mares. No exact reference could be traced as per the ELISA for the Annexin A1 and A2 biomarkers in equine.

Table 1: Elisa readings of HSP90 biomarker

S.no.	1	2	3	4	5	6	7	8	9	10	11	12
A	1.68	0.019(a^U)	0.010(i^U)	0.014(a^S)	0.017(i^S)	0.016(A^U)	0.017(I^U)	0.019(A^S)	0.012(I^S)	NV	NV	NV
B	1.12	0.011(b^U)	0.013(j^U)	0.018(b^S)	0.019(j^S)	0.019(B^U)	0.017(J^U)	0.016(B^S)	0.011(J^S)	NV	NV	NV
C	0.65	0.012(c^U)	0.012(k^U)	0.012(c^S)	0.016(k^S)	0.011(C^U)	0.016(K^U)	0.010(C^S)	0.019(K^S)	NV	NV	NV
D	0.38	0.012(d^U)	0.010(l^U)	0.012(d^S)	0.018(l^S)	0.015(D^U)	0.012(L^U)	0.013(D^S)	0.019(L^S)	NV	NV	NV
E	0.21	0.016(e^U)	0.018(m^U)	0.011(e^S)	0.011(m^S)	0.014(E^U)	0.019(M^U)	0.011(E^S)	0.019(M^S)	NV	NV	NV
F	0.16	0.012(f^U)	0.016(n^U)	0.013(f^S)	0.015(n^S)	0.012(F^U)	0.018(N^U)	0.016(F^S)	0.011(N^S)	NV	NV	NV
G	0.09	0.014(g^U)	0.013(o^U)	0.011(g^S)	0.011(o^S)	0.011(G^U)	0.016(O^U)	0.016(G^S)	0.013(O^S)	NV	NV	NV
H	B	0.014(h^U)	NV	0.014(h^S)	NV	0.016(H^U)	NV	0.011(H^S)	NV	NV	NV	NV

Table 2: Elisa readings of Annexin A1 biomarker

S.no.	1	2	3	4	5	6	7	8	9	10	11	12
A	1.65	0.015(a^U)	0.013(i^U)	0.014(a^S)	0.018(i^S)	0.016(A^U)	0.017(I^U)	0.012(A^S)	0.010(I^S)	NV	NV	NV
B	1.09	0.014(b^U)	0.012(j^U)	0.012(b^S)	0.017(j^S)	0.018(B^U)	0.014(J^U)	0.016(B^S)	0.011(J^S)	NV	NV	NV
C	0.58	0.015(c^U)	0.011(k^U)	0.016(c^S)	0.019(k^S)	0.017(C^U)	0.016(K^U)	0.013(C^S)	0.010(K^S)	NV	NV	NV
D	0.42	0.013(d^U)	0.017(l^U)	0.012(d^S)	0.016(l^S)	0.016(D^U)	0.013(L^U)	0.011(D^S)	0.009(L^S)	NV	NV	NV
E	0.22	0.015(e^U)	0.019(m^U)	0.013(e^S)	0.015(m^S)	0.018(E^U)	0.018(M^U)	0.017(E^S)	0.016(M^S)	NV	NV	NV
F	0.18	0.014(f^U)	0.017(n^U)	0.012(f^S)	0.013(n^S)	0.014(F^U)	0.015(N^U)	0.016(F^S)	0.013(N^S)	NV	NV	NV
G	0.08	0.014(g^U)	0.014(o^U)	0.011(g^S)	0.011(o^S)	0.014(G^U)	0.011(O^U)	0.016(G^S)	0.011(O^S)	NV	NV	NV
H	B	0.012(h^U)	NV	0.011(h^S)	NV	0.016(H^U)	NV	0.011(H^S)	NV	NV	NV	NV

Table 3: Elisa readings of Annexin A2 biomarker

S.no.	1	2	3	4	5	6	7	8	9	10	11	12
A	1.82	0.012(a^U)	0.013(i^U)	0.018(a^S)	0.019(i^S)	0.019(A^U)	0.017(I^U)	0.016(A^S)	0.013(I^S)	NV	NV	NV
B	1.15	0.019(b^U)	0.010(j^U)	0.018(b^S)	0.017(j^S)	0.016(B^U)	0.017(J^U)	0.014(B^S)	0.019(J^S)	NV	NV	NV
C	0.65	0.011(c^U)	0.012(k^U)	0.011(c^S)	0.018(k^S)	0.015(C^U)	0.016(K^U)	0.019(C^S)	0.011(K^S)	NV	NV	NV
D	0.38	0.010(d^U)	0.010(l^U)	0.012(d^S)	0.017(l^S)	0.011(D^U)	0.012(L^U)	0.013(D^S)	0.009(L^S)	NV	NV	NV
E	0.27	0.016(e^U)	0.018(m^U)	0.013(e^S)	0.015(m^S)	0.014(E^U)	0.019(M^U)	0.016(E^S)	0.019(M^S)	NV	NV	NV
F	0.062	0.014(f^U)	0.016(n^U)	0.012(f^S)	0.012(n^S)	0.012(F^U)	0.018(N^U)	0.013(F^S)	0.013(N^S)	NV	NV	NV
G	0.036	0.012(g^U)	0.013(o^U)	0.011(g^S)	0.011(o^S)	0.011(G^U)	0.016(O^U)	0.012(G^S)	0.011(O^S)	NV	NV	NV
H	B	0.012(h^U)	NV	0.011(h^S)	NV	0.001(H^U)	NV	0.001(H^S)	NV	NV	NV	NV

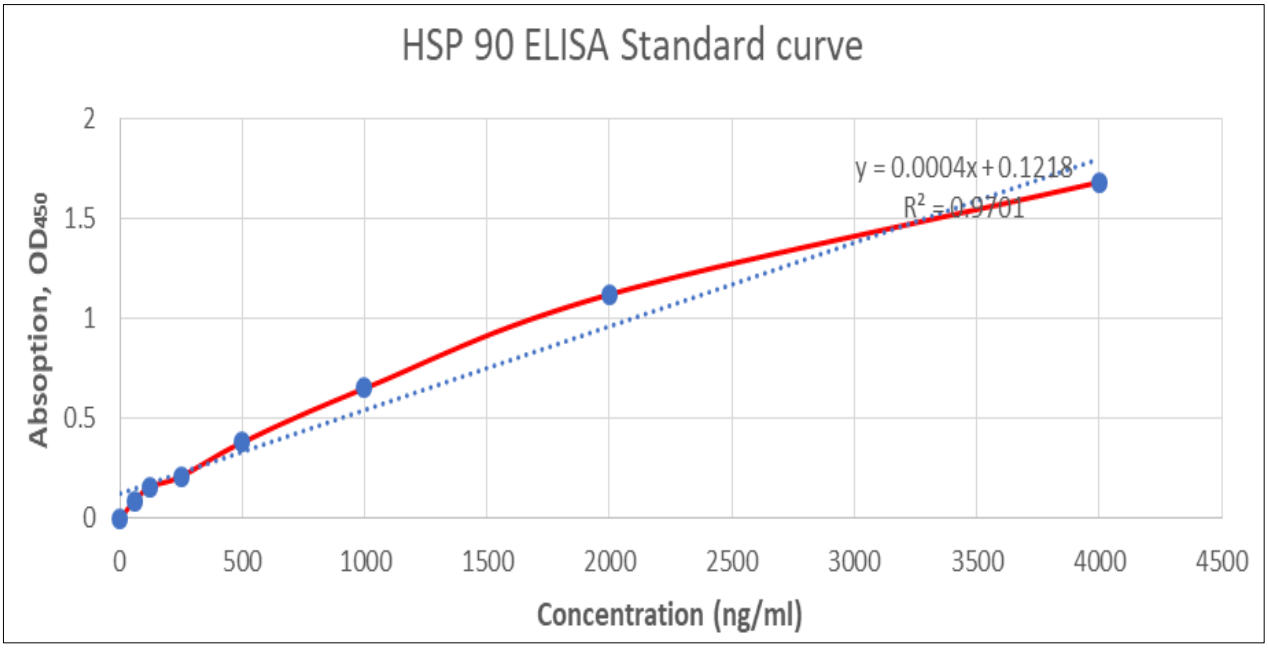

Fig 1: Standard curve of ELISA for HSP 90

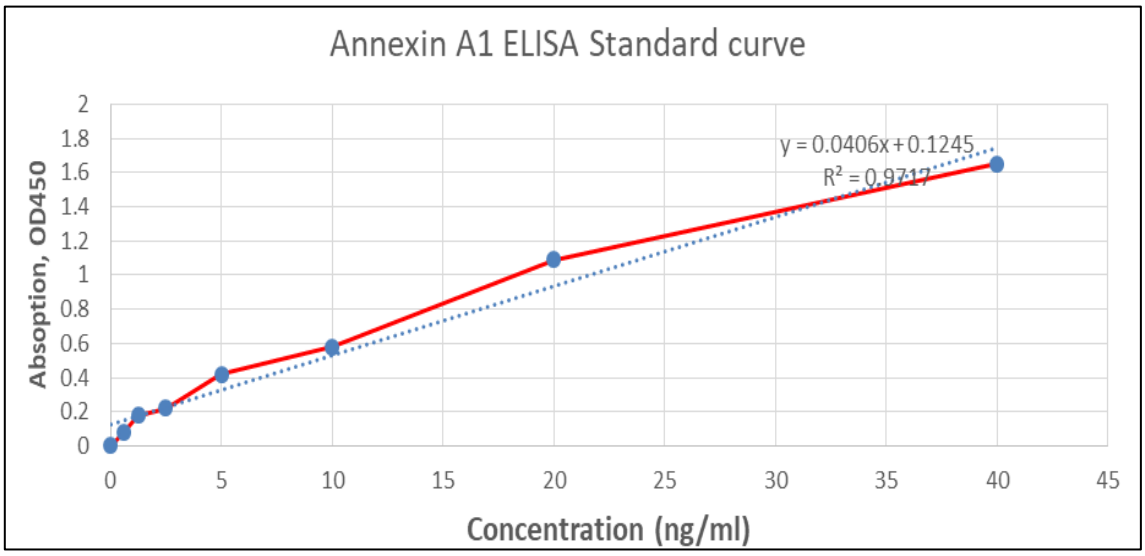

Fig 2: Standard curve of ELISA for Annexin A1

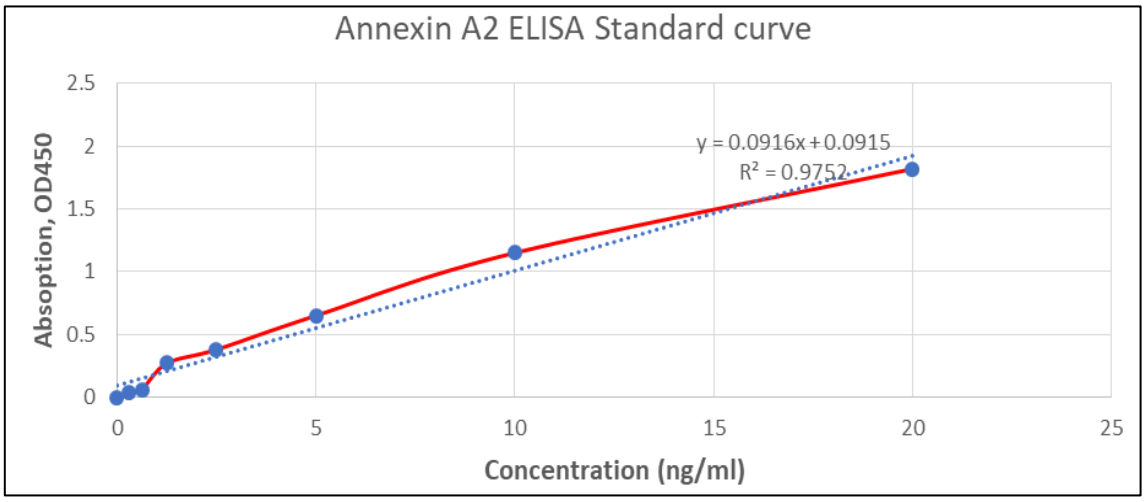

Fig 3: Standard curve of ELISA for Annexin A2

Conclusion

It can be concluded that the analysis of HSP-90, Annexin A1 and A2 biomarkers by using ELISA is not remarkable for the diagnosis of endometritis in mares.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

References

1. Troedsson MHT. Uterine clearance and resistance to persistent endometritis in the mare. Theriogenology. 1999;52(3):461-471.
2. LeBlanc MM, Magsig J and Stromberg AJ. Use of a low-volume uterine flush for diagnosing endometritis in chronically infertile mares. Theriogenology. 2007;68(3):403-412.
3. Overbeck W, Witte TS, and Heuwieser W. Comparison of three diagnostic methods to identify subclinical endometritis in mares. Theriogenology. 2011;75(7): 1311-1318.
4. McCue PM. The problem mare: management philosophy, diagnostic procedures, and therapeutic options. Journal of equine veterinary science. 2008;28(11):619-626.
5. LeBlanc MM and Causey RC. Clinical and subclinical endometritis in the mare: both threats to fertility. Reproduction in Domestic Animals. 2009;44:10-22.
6. LeBlanc MM. Advances in the Diagnosis and Treatment of Chronic Infectious and Post–Mating-Induced Endometritis in the Mare. Reproduction in domestic animals. 2010;45:21-27.
7. Bourke M, Mills JN and Barnes AL. Collection of endometrial cells in the mare. Australian veterinary journal. 1997;75(10):755-758.
8. Nielsen JM. Endometritis in the mare: a diagnostic study comparing cultures from swab and biopsy. Theriogenology. 2005;64(3):510-518.
9. Riddle WT, LeBlanc MM and Stromberg AJ. Relationships between uterine culture, cytology and pregnancy rates in a Thoroughbred practice. Theriogenology. 2007;68(3):395-402.
10. De Maio A, Santoro MG, Tanguay RM and Hightower LE. Ferruccio Ritossa's scientific legacy 50 years after his discovery of the heat shock response: a new view of

biology, a new society, and a new journal. Cell stress and chaperones. 2012;17(2):139-143.
11. Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA and Hightower LE. Guidelines for the nomenclature of the human heat shock proteins. Cell Stress and Chaperones. 2009;14(1):105-111.
12. Khan FA, De Amorim MD, Scholtz EL and Chenier TS. Qualitative differences in the uterine luminal fluid proteome between normal mares and mares with endometritis or endometrosis. Clinical Theriogenology. 2017, 9(3).
13. Marth CD, Young ND, Glenton LY, Noden DM, Browning GF and Krekeler N. Deep sequencing of the uterine immune response to bacteria during the equine oestrous cycle. BMC genomics. 2015;16(1):1-19.
14. Rescher U and Gerke V. Annexins–unique membrane binding proteins with diverse functions. Journal of cell science. 2004;117(13):2631-2639.
15. Futter CE and White IJ. Annexins and endocytosis. Traffic. 2007;8(8):951-958.
16. Grieve AG, Moss SE and Hayes MJ. Annexin A2 at the interface of actin and membrane dynamics: a focus on its roles in endocytosis and cell polarization. International journal of cell biology. 2012.
17. Flower RJ and Rothwell NJ. Lipocortin-1: cellular mechanisms and clinical relevance. Trends in pharmacological sciences. 1994;15(3):71-76.
18. Chapman LP, Epton MJ, Buckingham JC, Morris JF and Christian HC. Evidence for a role of the adenosine 5′-triphosphate-binding cassette transporter A1 in the externalization of annexin I from pituitary folliculo-stellate cells. Endocrinology. 2003;144(3):1062-1073.
19. Danielsen EM, Van Deurs B and Hansen GH. "Nonclassical" secretion of annexin A2 to the lumenal side of the enterocyte brush border membrane. Biochemistry. 2003;42(49):14670-14676.
20. Faure AV, Migne C, Devilliers G and Ayala-Sanmartin J. Annexin 2 "secretion" accompanying exocytosis of chromaffin cells: possible mechanisms of annexin release. Experimental cell research. 2002;276(1):79-89.
21. Morand EF, Hutchinson P, Hargreaves A, Goulding NJ, Boyce NW and Holdsworth SR. Detection of intracellular lipocortin 1 in human leukocyte subsets. Clinical immunology and immunopathology. 1995;76(2):195-202.
22. Movitz C and Dahlgren C. Endogenous cleavage of annexin I generates a truncated protein with a reduced calcium requirement for binding to neutrophil secretory vesicles and plasma membrane. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2000;1468(1-2): 231-238.
23. Perretti M & Gavins FN. Annexin 1: an endogenous anti-inflammatory protein. Physiology. 2003;18(2):60-64.
24. Sharma M, Ownbey RT and Sharma MC. Breast cancer cell surface annexin II induces cell migration and neoangiogenesis via tPA dependent plasmin generation. Experimental and molecular pathology. 2010;88(2):278-286.
25. Paradela A, Bravo SB, Henríquez M, Riquelme G, Gavilanes F, González-Ros JM and Albar JP. Proteomic analysis of apical microvillous membranes of syncytiotrophoblast cells reveals a high degree of similarity with lipid rafts. Journal of proteome research. 2005;4(6):2435-2441.
26. Kaczan Bourgois D, Salles JP, Hulling F, Fauvel J, Moisand A, Duga Neulat I and Chap H. Increased content of annexin II (p36) and p11 in human placentabrush-border membrane vesicles during syncytiotrophoblast maturation and differentiation. Placenta. 1996;17(8):669-676.
27. Benítez A, Vasconcellos R, Lombide P, Viotti H, Pérez W, Cazales N and Pedrana G. Heat shock protein HSP90 immunoexpression in equine endometrium during oestrus, dioestrus and anoestrus. Anatomia, Histologia, Embryologia. 2021;50(1):50-57.
28. Rehman S, Nadeem A, Javed M, Hassan FU, Luo X, Khalid RB & Liu Q. Genomic identification, evolution and sequence analysis of the heat-shock protein gene family in buffalo. Genes. 2020;11(11):1388.
29. de Amorim MD, Khan FA, Chenier TS, Scholtz EL and Hayes MA. Analysis of the uterine flush fluid proteome of healthy mares and mares with endometritis or fibrotic endometrial degeneration. Reproduction, Fertility and Development. 2020;32(6):572-581.