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and one treatment per NP at concentrations of $2 \times LC_{50}$ were considered, for a total of four groups. Each group included about 20 individuals. Groups were monitored for ten days and then sacrificed, fixed in a 4% paraformaldehyde solution in 0.1 M phosphate buffered-saline pH 7.4 at 4°C, and embedded in a Technovit 8100 kit. Classic histochemical staining included Periodic acid-Schiff (PAS), Alcian Blue (AB) at pH 2.5 and High Iron Diamine (HID). Lectin-binding experiments were performed with FITC-labelled lectins (SBA, PNA, WGA, ConA, Paradoxical ConA, UEA-I, LTA, AAA). *Pelophylax kl. esculentus* presents paired cement glands whose secretion was intensely PAS positive and negative to AB pH 2.5 and HID. Lectin-binding was intense with WGA, moderate with SBA and weak with PNA and UEA. PNA and SBA binding were more intense in the treatments. UEA binding decreased in the iron treatment. It is concluded that the secretion is made by neutral mucins with several glycosaminylated residuals and small amounts of galactosyl/galactosaminylated and fucosylated residuals. Galactosyl/galactosaminylated residuals increase in treatments and in the iron treatment only fucosylation is reduced. Thus, nanoparticles treatments affect the glycosylation patterns of cement gland secretion with possible consequences on the adhesion properties of mucus, which need further investigations.

NEGATIVE PROGNOSTIC VALUE OF INTRA-DUCTAL FAT INFILTRATE IN BREAST CANCER

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Recent studies showed a correlation between Body Mass Index and both breast cancer occurrence and progression¹. Nevertheless, at the state of art no study reported an accurate evaluation of intra-ductal fat infiltrate. Therefore, the main aim of this study was to evaluate the putative association between intra-ductal fat infiltrate (IDFi) in breast cancer subtypes by using digital pathology. We retrospectively collected 200 breast biopsies. Paraffin serial sections were used for haematoxylin and eosin staining and immunohistochemical evaluation of the following markers: oestrogen receptor (ER), progesterone receptor (PR), Ki67 and c-erb2. Three haematoxylin and eosin sections for each paraffin block were digitalized. Digital slides were used to evaluate the areas of IDFi. Five randomized areas were evaluated for each slide. By using GraphPad software IDFi areas was correlated with a) breast cancer histotype, b) presence of microcalcifications and c) biomarkers expression (a and b, Mann Whitney test; c, Pearson correlation). Breast biopsies were classified as follow: 20 normal breast, 50 benign lesions (hyperplasia) and 140 malignant lesions (30 lobular infiltrating carcinomas; 50 ductal *in situ* carcinomas; 60 ductal infiltrating carcinomas). Statistical analysis showed a significant increase of IDFi in malignant lesions as compared to both normal breast and benign lesions. We noted higher IDFi in breast ductal carcinomas as compared to lobular lesions. Not significant differences were observed between breast lesions with microcalcifications respect to lesions without calcifications. Noteworthy, we also found a positive association between IDFi and the expression of both ER and Ki67. Results of our study highlighted the possible role of fat infiltrated in breast cancer progression suggesting a negative prognostic value of IDFi.

Reference

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HISTOCHEMISTRY AS SUPPORTING TOOL IN GRASSLAND ECOSYSTEM MANAGEMENT: APELIN SYSTEM DETECTION IN EWE REPRODUCTIVE APPARATUS

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Adipokines are molecules involved in energy metabolism and represent important links between nutritional status, neuroendocrine axis and healthy pregnancy¹. We analysed the presence of apelin (APLN) and its receptor (APLNR) in the reproductive apparatus of the sheep, into a research project aimed to achieve a sustainable grassland productive ecosystem management. 15 adult female ewes (Comisana x Appenninica) in dry stage were fed with fresh hay from June to the pasture maximum flowering (MxF). From this period to maximum dryness, the control group (Cnt) was fed with fresh hay while, the experimental group (Exp) was also supplemented with 600 g/day/head of barely and corn (1:1). Ovary, ampulla and uterus samples were collected at each time and processed to perform RT-PCR, morphological and immunohistochemical analysis. Samples for molecular biology were frozen in liquid nitrogen. Samples for histochemical procedures were fixed in 10% neutral buffered formalin, included in paraffin wax and treated with polyclonal rabbit anti-APLN and anti-APLNR antibodies². Positive staining for APLN and APLNR were observed in the ovary corpus luteum. RT-PCR evidenced both transcripts in the examined organs. As the genital tract concerns, the highest levels were detected in the Cnt group ewes in the luteal phase compared to the MxF group in the anoestrous phase. APLN was detected in the epithelium lining the ampulla and uterus and in the uterine glands. APLNR was showed in the ampulla secreting cells, in the epithelium lining the uterus and uterine glands. APLN showed a high expression in the Cnt group compared to Exp one. The distribution and expression of the apelin system in the reproductive apparatus suggest its involvement in the ewe reproductive functions. Differences evidenced could be mainly related to the cyclic activity of organs, that seems to be affected also by the diet. Data suggest that a more energetic diet can anticipate the beginning of the oestrous cycle.

References

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METHYLATION AND DEMETHYLATION, AN ULTRASTRUCTURAL STUDY ON THE CELL NUCLEUS

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Methylation and demethylation are two epigenetic processes of a big relevance for different biological pathways. The two events happen on the carbon in position five of the cytosine belonging to the so called CpG island. The methylation implies the addition of a methyl group on the cytosine, forming the 5-methylcytosine (5mC) thanks to enzymes called DNMT (DNA-Methyltransferase). After, when required, the methyl group is oxidized or demethylated by a family of enzyme called TET, forming the 5-hydroxymethylcytosine (5hmC). The role of the 5mC is generally correlated with gene expression repression, while the 5hmC function must be clarified. In this context, in order to elucidate the hypothetical role of these markers we decide to investigate at