CONTRIBUTION AND PERSPECTIVES OF THE IMPACT OF PROTEOMICS IN STUDY OF PLACENTAS AND EMBRYONIC AND FETAL MEMBRANES

CONTRIBUIÇÃO E PERSPECTIVAS DO IMPACTO DA PROTEÔMICA EM ESTUDO DE PLACENTAS E MEMBRANAS EMBRIONÁRIAS E FETAIS

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REVIEW

SUMMARY

Proteomic approaches have revolutionized the analysis of biological samples by allowing the identification of non-characterized protein expression and its role on protein interactions and post-translational modifications during embryonic development or as a consequence of pathological states. This new frontier of knowledge is, nowadays, an essential contribution to the elucidation of biological problems, by providing information that can not be obtained by other target driven methods. This review highlights the new perspectives of proteomics studies based on bidimensional eletrophoresis (2D-PAGE) associated to mass spectrometry (MS) analytical methods of placenta, embryonic membranes and embryo evaluation, with emphasis on comparisons between normal and diseased tissues consequence of reproductive biotechnology manipulation.

KEY-WORDS: Protein analysis. Mass spectrometry. Bioinformatics. Bidimensional eletrophoresis.

RESUMO

Abordagens proteômicas revolucionaram a análise de amostras biológicas por permitir a identificação de padrões ainda não caracterizados de expressão de proteínas e/ou suas interações e modificações pós-traducionais em diferentes tecidos, como a placenta ou em embriões e fetos em diferentes estágios de desenvolvimento. Esta nova fronteira de conhecimento se constitui atualmente numa ferramenta fundamental para a elucidação de problemas biológicos, por fornecer informações que não podem ser obtidas por métodos mais focalizados, ou com alvos específicos. Essa revisão salienta as novas perspectivas geradas pela contribuição da proteômica baseada na eletroforese bidimensional (2D-PAGE) associada à espectrometria de massas (MS) para o estudo da placenta, das membranas embrionárias e do embrião, com ênfase nas comparações entre condições normais e aqueles provenientes de estados patológicos ou conseqüentes ao uso de biotecnologias reprodutivas.

PALAVRAS-CHAVE: Análise de proteínas. Espectrometria de massas. Bioinformática. Eletroforese bidimensional.

INTRODUCTION

While the genome represents the sum of all genes of an individual, the proteome, in contrast, is not a fixed characteristic of an organism. Proteome is the protein expression of the genome of a particular organism or tissue, changing according to tissue development stage or to environmental conditions in which the organism is. Thus, phenomena such as alternative splicing of RNA and protein post-translational modifications lead to the presence of more proteins in the proteome than genes in the genome, especially eukaryotes (DI CIERO & BELLATO; 2003).

The term proteome was coined by WILKINS & WILLIANS (1994) to describe the entire set of proteins expressed by a genome, or in the case of multicellular organisms the protein complement expressed by a tissue or differentiated cells (WILKINS et al., 1996).

After the euphoria caused by genome sequencing of several organisms, the scientific community realized that in order to understand the genes in all its fullness, an in depth study of the proteins expressed by them was mandatory. It was understood, that although very important, nucleotide sequencing do not always reflect a direct relationship with its protein expression and thus biological activity (GYGI et al., 1999).

The understanding of the proteome allows to identify, characterize and understand the functional interactions between proteins that are essential to the cell. Further on, this type of study was extended to other biomolecules. These efforts have led to approaches called "omics" a suffix used to denominate several areas such as transcriptomics, lipidomics and metabolomics, respectively, the study of transcripts, fatty acids and metabolites of a cell. Nowadays, metabolomics subsets such as lipidomics, glycomics and fluxomics are evolving to an integrated omics picture of the genes, the interactome (transcripts, proteins and metabolites) to fully describe the complexity of cellular functions (DENNIS, 2009).

The initial goal of a proteomic study is to identify all proteins present in a cell or tissue. Currently, proteomics has been used to analyze increasingly complex mixtures of proteins from cell lysates and tissue extracts, in order to detect quantitative and qualitative differences in global protein expression (WESTERMEIER & NAVEN, 2002).

In this context the study of the proteome of embryos and embryonic annexes using analytical methods such as bidimensional electrophoresis (2D) and mass spectrometry (MS) is unraveling the complexity of the embryo and unknown aspects of embryogenesis. The interest in these studies is due to several aspects. In ruminants, about 40% of early embryonic death occurs during pre-implantation, that is, between 8 and 17 days of gestation (HUMBLOT, 2001; THATCHER et al., 2001), period in which mother-fetus communication with the participation of *interferon tau* is essential (BAZER et al.,1997). Thus the proteome at this stage of the pregnancy, that is, analysis of protein post-translational modifications, protein-protein interactions, existence of isoforms, activity and structure of the proteins in embryonic tissues, and in embryonic and fetal membranes as well, contribute to the understanding of materno-fetal relationship and certainly, for advancing biotechnologies such as artificial insemination, embryo transfer, IVF and cloning. Therefore, proteomics should rapidly impact fertility assessment methods, infertility diagnosis in animals and humans, and lead to optimization of protocols related to the culture of embryos and oocytes, selection of oocytes for IVF and embryo transfer , and the use of biomarkers to diagnose infertility (FERREIRA et al., 2010).

In the study of embryonic placenta and membranes, this approach has shown the complexity of the structures responsible for embryo maintenance and development, from pre-implantation to the end of the pregnancy. The formation of the placenta, which connects the developing fetus to the mother's body, is an essential feature of pregnancy in mammals. Placenta development and its function are precisely regulated and coordinated to promote the exchange of nutrients and waste products between the maternal and fetal circulatory systems (WOODING et al., 1994). At the end of pregnancy, placenta undergoes rapid changes to prepare for birth, among these, placental maturation is seen as a prerequisite for partial separation and sub-release of fetal membrane (WILLIAMS et al., 1987; STALLMACH et al., 2001). Therefore, placentation plays an important role in the pathogenesis of some inherited disorders, it promotes teratogenic transplacental transmission of microbial agents, and contributes to failure in the gestation of cloned calves (HILL et al., 1999). Proteins control most cellular processes, which occur in great diversity, acting as enzymes, antibodies, hormones, structural components and cellular receptors (AEBERSOLD & MANN, 2003), certainly involved in understanding the pathogenesis of several processes such as those mentioned above. Moreover, understanding the involvement of proteins in organogenesis and cell differentiation should have application in the field of cell therapy, with the identification of involved proteins and transcription factors, which can also be used as biomarkers of this process (POTTEN et al., 1997).

EMBRYO PROTEOME

Contrary to what occurs with the relatively static and identical genome of somatic cells of an organism, embryo proteome is in a dynamic state, responding to external and internal stimuli (GODOVAC-ZIMMERMANN & BROWN, 2003). The great diversity of proteins controls cellular processes in the form of enzymes, antibodies, hormones, structural components, transcription factors and cell receptors (D'SOUZA et al., 2000; AEBERSOLD & MANN, 2003). Thus, studying the proteome of these biological samples from embryos would help ascertain the regulation effect of the gene expression that happens post-transcription and post-translation, providing

numerous explanations as to its biological functioning and involvement in the intracellular metabolic processes involved in tissue differentiation (HOOGLAND et al., 1999; FEY & LARSEN, 2001; ISFORT, 2002; WOLF et al., 2003; CHAZE et al., 2008; SUTOVSKY, 2009), which very often cannot be detected by quantitative analysis of mRNA (GYGI et al., 1999). It is expected that proteomics tools will provide a comprehensive characterization of biomarkers indicative of the status of embryonic development and relative to the health of the maternal-fetal environment (WOLF et al., 2003; RIDING et al. 2008 a; DEGRELLE et al., 2009; BELLIDO et al., 2010), to congenital diseases (NATH et al., 2009), embryotoxicity (USAMI et al., 2008) and embryo viability (KATZ-JAFFE et al., 2009; ROYÉRE et al., 2009, DEGRELLE et al., 2009).

PROTEOMICS

This article will be restricted to the description of proteomics strategy by using bidimensional polyacrylamide gel electrophoresis (2D-PAGE) combined with mass spectrometry (MS) with electrospray ionization source (ESI) and desorption ionization and laser assisted matrix (MALDI) (HAGER, 2004). The ESI and MALDI techniques are considered soft ionization forms of high molecular weight biological molecules. The term "soft ionization" means that the ions formed have low internal energy, which allows the observation of molecular ions with little or no fragmentation. After ionization, the charged molecules are separated according to their mass-charged values (m/z) in one or more mass analyzers such as time-of-flight (TOF) or quadrupolo (ROCHA et al., 2005).

Briefly, in MALDI type ionization, the solution containing the sample is mixed with a supersaturated solution of the organic matrix, placed on a metal plate and bombarded by a beam of high power laser with wavelength corresponding to the maximum absorption matrix, resulting in gas phase ions that will go to the mass analyzer (KNOCHENMUSS & ZENOBI, 2003). After that, the desorbed matrix ions are accelerated under high vacuum by applying a potential difference (15-30 kV). In the TOF analyzer, the time required to reach the detector is proportional to both applied voltage and ion m/z ratio (PIELES et al., 1993; DATTA; PIHUR, 2010).

In ESI ionization, the analytes solution is passed through a metal capillary where voltage is applied. Then, a spray containing highly charged (positive or negative) droplets is formed, which is then evaporated using a heated flow of nitrogen. After the solvent contained in the droplets is evaporated, the high charge density culminates in a coulômbica explosion event that "ejects" the ions to the gas phase (GROSS, 2004).

The 2D-PAGE electrophoresis is a traditional tool in proteomic studies that works by isolating protein spots according to their isoelectric point and molecular weight, in order to identify and further relate these proteins to their biological activity (O'FARRELL, 1975; ROCHA et al. 2005; GORG et al., 2004). The 2D-PAGE has a great resolution power when dealing with intact proteins and has also been used to distinguish isoforms and protein altered states (MOTOYAMA, 2008). In general, liquid chromatography is used as protein separation method prior to MS and ESI (THIEDE et al., 2005). Therefore, after protein separation using 2D-PAGE, the protein spots on the gel are cut out, purified and submitted to tryptic digestion to obtain peptides, which are further analyzed by MS, to identify and quantify accurately these proteins (PANDEY; MANN, 2000; GYGI et al., 1999; TYERS; MANN, 2003; DEGRELLE et al., 2009). Besides HPLC, new strategies for faster and more efficient separation have emerged, mainly due to the need to analyze complex samples, where a mixture of many proteins is present (THIEDE et al., 2005, MOTOYAMA, 2008).

One of the most efficient chromatographic separation techniques is ultra-performance liquid chromatography (UPLC), that allows efficient separation of small protein fragments for further MS analysis (WASHBURN et.al., 2001; PENG et.al., 2003). For UPLC ESI-MS, a complex mixture of peptides is loaded directly into a biphasic microcapillary column with an array of strong cation exchange and in sequence another reverse phase. The biphasic microcapillary column is placed directly in line with the mass spectrometer, featuring the fragmentation into peptides in MS tandem (HERNÁNDEZ, 2009). This is called shotgun proteomics (MOTOYAMA, 2008).

Sometimes the detection of mas spectra is made more than once, and then called UPLC MS/MS, MALDI-TOF MS/MS, or sometimes when the probes are repeated n times, it is called MS^E. The objective here is to further increase the accuracy of the method, especially for complex and small quantity samples.

PROTEOMICS OF EMBRYONIC AND FETAL PLACENTAS AND MEMBRANES

The proteomic analysis of placenta, embryonic annexes and the reproductive tract itself has been used for different purposes. There are several examples in the literature.

In cattle, the analysis of the protein contained in the amniotic fluid might be important to assess the maternal-fetal environment and the state of development of the fetus resulting from natural mating or from *in vitro* fertilization (IVF) protocols and nuclear transfer. Increased levels of bovine cathelicidin antimicrobial protein (CAMP) were identified and quantified by 2D-PAGE associated to mass fingerprinting MALDI-TOF MS in samples of fetuses resulting from IVF. This fact could be related to changes in placental function or fetal development (RIDING et al., 2008).

The proteomic approach was used to examine the differences in protein expression in samples of human placenta from fetuses generated by assisted reproductive technology procedures (ART) compared to normal fertilization. A total of 20 proteins were identified by MALDI-TOF MS, including proteins

involved in membrane transport, nucleic acid metabolism, stress response and cytoskeleton. In the ART group, it was identified anexina 3, heterogeneous nuclear ribonucleoproteins (hnRNP) C1/C2, nucleosome assembly protein 1 **(**SNAP 1), ferritin light polypeptide-like (FTL) and ATP synthase alpha-subunit gene (ATP5A) and related to changes in placental functions in this group (ZHANG et al., 2008).

In another study, an increase of 12 proteins in human placental villi in early pregnancy loss (EPL) was identified. These proteins appear to be related to the processes involved in network signaling and regulation of cellular activity, such as cell defense against reactive oxygen species (ROS), differentiation, proliferation, metabolism, transcription, apoptosis and proteolysis. Significant anomalies in the three-dimensional structure of some of these proteins were also observed (LIU et al., 2006).

Some results suggest that placenta malformation in cloned pigs may be caused by apoptosis of the placenta cells. A differential expression of 43 proteins was found, many of them related to apoptosis, showing the importance of proteomics to understand the pathophysiology of this condition (LEE et al., 2007).

In another study, neurokinin B NKB) was increased in the placenta in cases of pre-eclampsia. High NKB was able to reduce the expression of 20 proteins in cytotrophoblast cultures. Four of these proteins were involved in the antioxidant defense processes, while two work to inhibit intravascular coagulation, leading to the conclusion that NKB may contribute to the pathogenesis of pre-eclampsia (SAWICKI et al., 2003).

Samples of normal human placentas and of patients with pre-eclampsia were studied using 2D-PAGE and 2D-immunoblotting. From the results, dynactin p-50 protein related to cell turnover was identified and related to pre-eclampsia and its severity levels (MINE et al., 2007).

The practical application of animal cloning by somatic cell nuclear transfer (SCNT) has shown low success rate. A proteomic approach using 2D-PAGE and MS was used to evaluate a possible placental dysfunction in SCNT. Placentas SCNT that originated from post-natal death of the fetus were compared to normal placentas that originated from artificial insemination. From the comparison, 33 protein spots were differentially expressed in SCNT placenta. The protein TIMP-2 (Metalloproteinase inhibitor 2) was present in higher quantity in SCNT placenta, and this protein is related to the extracellular matrix and tissue remodeling during pregnancy. The results showed modified protein profiles in SCNT placentas, which resulted in fetal loss (KIM et al., 2005).

FINAL CONSIDERATIONS

Building a database of placenta proteomics at several stages of fetal development, as well as the characterization of the proteome of the embryo in early pregnancy are critical to understand the morphophysiological differences between normal placentas and those found in fetuses generated by assisted reproductive techniques, and should improve the latter. Although proteomic analysis is highly complex in cells and eukaryotic tissues, this approach has spread and contributed to the understanding of protein role in the placenta and embryo. The "omics" approach can relate to, reflecting cellular complexity and leading to elucidation of complex integration networks of metabolic pathways and the interactome of transcript genes, proteins and metabolites that are part of the cellular function.

REFERENCES

AEBERSOLD, R.; MANN, M. Mass spectrometry-based proteomics. **Nature**, v.402, p.198-207, 2003.

BAZER, F. W.; SPENCER, T. E.; OTT, T. L. Interferon-tau; a novel pregnancy recognition signal. **American Society for Reproductive Immunology**, v.37, p.137-143, 1997.

BELLIDO, M. L.; RADPOUR, R.; LAPAIRE, O.; DE BIE, I.; HÖSLI, I.; BITZER, J. MALDI-TOF Mass Array Analysis of RASSF1 and SERPINB5 Methylation Patterns in Human Placenta and Plasma. **Biology of Reproduction Papers in Press.** Published on January 14, 2010.

CHAMBERY, A; VISSERS J. P.; LANGRIDGE, J. I., LONARDO, E.; MINCHIOTTI, G.; RUVO, M.; PARENTE, A. Qualitative and quantitative proteomic profiling of cripto(-/-) embryonic stem cells by means of accurate mass LC-MS analysis. **Journal of Proteome Research,** v.8, p.1047–1058. 2009.

CHAZE, T.; MEUNIER, B.; CHAMBON, C.; JURIE, C.; PICARD, B. In vivo proteome dynamics during early bovine myogenesis. **Proteomics.** v.8, n.20, p.4236-4248, 2008.

DATTA, S.; PIHUR, V. Feature selection and machine learning with mass spectrometry data. **Methods in Molecular Biology**, v.593, p.205-229, 2010.

DE SOUZA, M. V.; FONTES, W.; RICART, C. A. O análise de Proteomas: O despertar da área pós-genômica. **Revista on-line Biotecnologia Ciência e Desenvolvimento**. p.12-14, 2003.

DEGRELLE, S. A.; BLOMBERG, L. A.; GARRETT, W. M.; LI, R. W.; TALBOT, N. C. Comparative proteomic and regulatory network analysis of the elongating pig conceptus. **Proteomics,** v.9, p.2678-2694, 2009.

DENNIS, E. A. Lipidomics joins the omics evolution. **Proceedings of the National Academy of Sciences U S A**. v.106, n.7, p.2089-2090, 2009.

DI CIERO, L.; BELLATO, C. M. PROTEOMA: Avanços Recentes em Técnicas de Eletroforese Bidimensional e Espectrometria de Massa. Revista on line - **Biotecnologia Ciência e Desenvolvimento**. n.29, p.158-164. 2003.

D'SOUZA, A. L.; MASUDA, K.; OTTEN, L. M.; NISHIDA, Y.; KNUDSON, W.; THONAR, E .J. Differential effects of interleukin-1 on hyaluronan and proteoglycan metabolism in two compartments of the matrix formed by articular chondrocytes maintained in alginate. **Arch Biochem Biophys**. v.374, n.1, p.59-65, 2000.

FENN, J. B.; MANN, M.; MENG, C. K.; WONG, S. F.; WHITEHOUSE, C.M. Electrospray ionization for mass spectrometry of large biomolecules. **Science.**, v.246, n.4926, p.64-71, 1989.

FERREIRA, C. R.; TURCO, E. G. L.; SARAIVA, S. A.; BERTOLLA, R. P.; PERECIN, F.; SOUZA, G. H. M. F.; MURGU, M.; GARCIA, J. S.; CORTEZZI, S. S.; MEIRELLES, F. V.; KLITZKE, C. F.; CABRAL, E. C.; MIGLINO, M. A.; PORCIUNCULA, P. M.; LEAL, C. L. V.; BORGES JR, E.; MARTINS, D. S.; AMBRÓSIO, C. E.; D´ALEXANDRI, F.; SMITH, C. S.; EBERLIN, M. N. Proteomics, metabolomics and lipidomics in reproductive biotechnologies: the MS solutions. **Acta Scientiae Veterinariae**. v.38, (Supl 2), p.s277-s289, 2010.

FEY, S. J.; LARSEN, P. M. 2D or not 2D. Two-dimensional gel electrophoresis. Curr. Opin. **Chemistry & Biology**, v.5, n.1, p.26-33, 2001.

GODOVAC-ZIMMERMAN, J. AND BROWN, L.R. Proteomics Approaches to Elucidation of Cellular Signalling. Curr. Opin. **Molecular Therapy,** v.5, p.241-249. 2003.

GORG, A.; WEISS, W.; DUNN, M. J. Current two-dimensional electrophoresis technology for proteomics. **Proteomics**, v.4, p.3665-3685, 2004.

GYGI, S. P.; RIST, B.; GERBER, S. A.; TURECEK, F.; GELB, M. H.; AEBERSOLD, R. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. **Nature**, v.17, p.994-999, 1999.

GROSS, J. H. **Mass spectrometry**: a textbook. Heidelberg, Germany: Springer Verlag, 518p. 2004.

HAGER, J. W. Recent trends in mass spectrometer development. **Analytical and Bioanalytical Chemistry,** v.378, n.4, p.845-850, 2004.

HERNÁNDEZ, L.G. **Análise proteômica aplicada à ontogenia, comportamento e aprendizagem em abelhas.** 2009. 81p. Tese de doutorado em Biologia Molecular, Universidade de Brasília, 2009.

HILL, J. R.; ROUSSEL, A. J.; CIBELLI, J. B.; EDWARDS, J. F.; HOOPER, N. L.; MILLER, M. W.; THOMPSON, J. A.; LOONEY, C. R.; WESTHUSIN, M. E.; ROBL, J. M.; STICE, S. L. Clinical and pathologic features of cloned transgenic calves and fetuses (13 case studies). **Theriogenology**. Jun; v.51, n.8, p.1451–1465. 1999.

HOOGLAND, C.; SANCHEZ, J. C.; WALTHER, D.; BAUJARD, V.; BAUJARD, O.; TONELLA, L.; HOCHSTRASSER, D. F.; APPEL, R. D. Two-dimensional electrophoresis resources available from ExPASy. **Electrophoresis**, v.20, n.18, p.3568-3571, 1999.

HUMBLOT, P. Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. **Theriogenology**, v.56, p.1417-1433, 2001.

ISFORT, R. J. Proteomic analysis of striated muscle. **Journal Chromatogr. B. Analyt Technol. Biomed. Life Science**, v.771, n.1-2, p.155-165, 2002.

KATZ-JAFFE, M. G.; MCREYNOLDS, S.; GARDNER, D. K.; SCHOOLCRAFT, W. B. The role of proteomics in defining the human embryonic secretome. **Molecular Human Reproduction**, v.15, n.5, p.271–277, 2009.

KIM, H. R.; KANG, J. K.; YOON, J. T.; SEONG, H. H.; JUNG, J. K.; LEE, H. M.; PARK, S. C.; JIN, D. I. Protein profiles of bovine placenta derived from somatic cell nuclear transfer. **Proteomics.** v.5, p.4264-4273, 2005.

KNOCHENMUSS, R.; ZENOBI, R. MALDI ionization: the role of in-plume processes. **Chem. Rev**., v.103, n.2, p.441-452, 2003.

KRÄMER-ALBERS, E.M., N. BRETZ, S. TENZER, C. WINTERSTEIN, W. MÖBIUS, H. BERGER, K.-A. NAVE, H. SCHILD, J. TROTTER. Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: Trophic support for axons? **Proteomics-Clinical Applications**., n.1, p.1446–1461. 2007.

LEE, S. Y., PARK, J. Y., CHOI, Y. J., CHO, S. K., AHN, J. D., KWON, D. N., HWANG, K. C., KANG, S. J., PAIK, S. S., SEO, H. G., LEE, H. T., KIM, J. H. Comparative proteomic analysis associated with term placental insufficiency in cloned pig. **Proteomics.** v.7, n.8, p.1303-15, 2007.

LI, G. Z.; VISSERS, J. P.; SILVA, J. C.; GOLICK, D.; GORENSTEIN, M. V.; GEROMANOS, S. J. Database searching and accounting of multiplexed precursor and product ion spectra from the data independent analysis of simple and complex peptide mixtures. **Proteomics,** v.9, p.1696–1719. 2009.

LIU, A. X.; ZHANG, W. W.; ZHOU, T. H.; ZHOU, C. Y.; YAO, W. M.; QIAN, Y. L.; HUANG, H. F. Proteomics analysis on the alteration of protein expression in the placental villous tissue of early pregnancy loss. **Biology of Reproduction,** v.75, p.414-20, 2006.

MINE, K. ; KATAYAMA, A. ; MATSUMURA, T. ; NISHINO, T. ; KUWABARA, Y. ; ISHIKAWA, G. ; MURATA, T. ; SAWA, R. ; OTSUBO, Y. ; SHIN, S. ; TAKESHITA, T. Proteome analysis of human placenta: pre-eclampsia versus normal pregnancy. **Placenta**. v.28, p.676-87, 2007.

MOTOYAMA, A. Multidimensional LC Separations in Shotgun Proteomics. **Analytical Chemistry,** v.80, p.7187–7193, 2008.

NATH, A. K.; KRAUTHAMMER, M.; LI, P.; DAVIDOV, E.; BUTLER, L. C.; COPEL, J.; KATAJAMAA, M.; ORESIC, M.; BUHIMSCHI, I.; BUHIMSCHI, C.; SNYDER, M.; MADRI, J. A. Proteomic-Based Detection of a Protein Cluster Dysregulated during Cardiovascular Development Identifies Biomarkers of Congenital Heart Defects. **PLOS ONE**, v.4, n.1, p.4221, 2009.

O'FARRELL, P. H. High resolution two dimensional electrophoresis of proteins. **Journal of Bioogical Chemistry**. v.250, p.4007-4021, 1975.

PANDEY, A.; MANN, M. Proteomics to study genes and genomes. **Nature,** v.405, n.6788, p.837-846, 2000.

PENG, J., ELIAS, J. E.; THOREEN, C. C.; LICKLIDER, L. J.; GYGI, S. P. Evaluation of Multidimensional Chromatography Coupled with Tandem Mass Spectrometry (LC/LCMS/MS) for Large-Scale Protein Analysis: The Yeast Proteome. **Journal of Proteome**, v.2, n.1, p.43-50, 2003.

PIELES, U.; ZÜRCHER, W.; SCHÄR, M.; MOSER, H. E. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a powerful tool for the mass and sequence analysis of natural and modified oligonucleotides. **Nucleic Acids Research,** v.21, n.14, p.3191-3196, 1993.

POTTEN, C. S.; WILSON, J. W.; BOOTH, C. Regulation and Significance of Apoptosis in the stem cells of the gastrointestinal epithelium. **Stem Cells**, v.15, n.2, p.82-93, 1997.

RIDING, G. A.; HILL, J. R.; JONES, A.; HOLLAND, M. K.; JOSH, P. F.; LEHNERT, S. A. Differential proteomic analysis of bovine conceptus fluid proteins in pregnancies generated by assisted reproductive **Technologies Proteomics**, v.8, p.2967–2982, 2008.

ROCHA, T. L.; COSTA, P. H. A.; MAGALHÃES, J. C. C.; EVARISTO, R. G. S.; VASCONCELOS, E. A. R.; COUTINHO, M. V.; PAES, N. S.; SILVA, M. C. M.; GROSSI-DE-SÁ, M. F. Eletroforese bidimensional e análise de proteoma. **Comunicado Técnico, Embrapa Recursos Genéticos e Biotecnologia**, n.136, p.1-12, 2005.

ROYÈRE, D.; FEUERSTEIN, P.; CADORET, V.; PUARD, V.; UZBEKOVA, S.; DALBIES-TAN, R.; TEUSAN, R.; HOULGATTE, R.; LABAS, V.; GUÉRIF, F. Non invasive assessment of embryo quality: proteomics, metabolomics and oocyte-cumulus dialogue. **Gynecol Obstet Fertil**, v.37, n.11-12, p.917-920, 2009.

SAWICKI, G.; DAKOUR, J.; MORRISH, D. W. Functional proteomics of neurokinin B in the placenta indicated a novel role in regulating cytotrophoblast antioxidant defenses. **Proteomics**. v.3, p.2044-51, 2003.

SILVA, J. C.; DENNY, R.; DORSCHEL, C. A.; GORENSTEIN, M.; KASS, I. J.; LI, G. Z.; MCKENNA, T.; NOLD, M. J.; RICHARDSON, K.; YOUNG, P.; GEROMANOS, S. Quantitative proteomic analysis by accurate mass retention time pairs. **Analytical Chemistry,** v.77, p. 2187-2200. 2005.

STALLMACH, T., HEBISCH, G., MEIER, K., DUDENHAUSEN, J. W., VOGEL, M. Rescue by birth: defective placental maturation and late fetal mortality**. Obstetrics & Gynecology,** v.97, p.505–509, 2001.

SUTOVSKY, P. Proteomic analysis of mammalian gametes and sperm-oocyte interactions. **Journal of reproduction and fertility,** v.66, p.103-116, 2009.

THATCHER, W. W.; GUZELOGLU, A.; MATTOS, R.; BINELLI, M.; HANSEN, T. R.; PRU, J. K. Uterine-conceptus interactions and reproductive failure in cattle. **Theriogenology,** v.56, p.1435-1450, 2001.

THIEDE, B.; HÖHENWARTERB, W.; KRAHA, A.; MATTOWC, J.; SCHMIDB, M.; SCHMIDT, F.; JUNGBLUT, P. R. Peptide mass fingerprinting. **Methods,** v.35, p.237–247, 2005.

TYERS, M.; MANN, M. From genomics to proteomics. **Nature,**. v.422, n.6928, p.193-197, 2003.

USAMI, M.; MITSUNAGA, K.; NAKAZAWA, K.; DOI, O. Proteomic analysis of selenium embryotoxicity in cultured postimplantation rat embryos. **Birth Defects Res. B. Dev. Reprod. Toxicol**., v.83, n.2, p.80-96, 2008.

WASHBURN, M. P.; WOLTERS, D.; YATES, J. R. Large-scale analysis of the yeast proteome by multidimensional protein Identification technology. **Nature Biotechnology,** v.19, p. 242- 247. 2001.

WESTERMEIER, R; NAVEN, T. **Proteomics in Practice**: A laboratory Manual of proteome analysis, Germany, Weinheim: Wiley-VCH Verlag-GmbH, p.329. 2002.

WILKINS, M. R.; PASQUALI, C.; APPEL, R. D.; OU, K.; GOLAZ, O.; SANCHEZ, J. C.; JAN, J. X.; GOOLEY, A. A.; HUGHES, E.; HUMPHERY-SMITH, I.; WILLIANS, K. L.; HOCHSTRASSER, D.

F. From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis. **Bio. Technology**, v.14, p.61-65, 1996.

WILLIAMS, W. F., MARGOLIS, M. J., MANSPEAKER, J., DOUGLASS, L. W., DAVIDSON, J. P. Peripartum changes in the bovine placenta related to fetal membrane retention. **Theriogenology. v.**28, p.312–323, 1987.

WOLF, E.; ARNOLD, G. J.; BAUERSACHS, S.; BEIER, H. M.; BLUM, H.; EINSPANIER, R.; FRÖHLICH, T.; HERRLER, A.; HIENDLEDER, S.; KÖLLE, S.; PRELLE, K.; REICHENBACH, H. D.; STOJKOVIC, M.; WENIGERKIND, H.; SINOWATZ, F. Embryo-Maternal Communication in Bovine – Strategies for Deciphering a Complex Cross-Talk. **Reproduction in Domestic Animals**, v.38, p.276–289, 2003.

WOODING, F. B., MORGAN, G., BRANDON, M. R., CAMOUS, S., Calcium transport and the localisation of calbindin-D9k in the ruminant placenta during the second half of pregnancy. **Cell and Tissue Research,** v.276, p.387–397, 1994.

ZHANG, Y., ZHANG, Y. L., FENG, C., WU, Y. T., LIU, A. X., SHENG, J. Z., CAI, J., HUANG, H. F. Comparative proteomic analysis of human placenta derived from assisted reproductive technology. **Proteomics.** v.8, n.20, p.4344-56, 2008.