Anim. Reprod, v.7, n.3, p.177-186, Jul./Sept. 2010

Reproductive biology in the "omics" era: what can be done?

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Abstract

The term "omics" have been widely used in science nowadays. The current nomenclature of "omics" sciences includes genomics for DNA, transcriptomics for mRNA, proteomics for proteins, lipidomics for lipids, metabolomics for intermediate products of metabolism and more recently interactome for the whole set of molecular interactions in cells. All these "omics" are encompasses in the high-dimensional biology approach (HDB), which refers to the simultaneous study of the different biological levels of an organ, tissue or an organism. HDB fundamental premise is that the complexity of biological systems renders them difficult to comprehensively understand using only a reductionist approach, studying just units of the whole. The integration of "omic" techniques can be called Systems Biology and it aim is to define the interrelationships of several or, if possible, all the elements in a biological system. The HDB and System Biology have been used widely for biomarker discover in several diseases, once they allow for a global description of changes in biological systems and do not require a specific hypothesis. Unfortunately despite the fact that achieves HDB studies is not so difficult nowadays, the use of this approach together with systems biology is rare in the reproductive biology field. The main goal of this review is to introduce the reader to the HDB and mass spectrometry and how they could benefit the reproductive biology field.

Keywords: high dimensional biology, lipidomics, mass spectrometry, proteomics.

Introduction

The term "omics" can be considered as a neologism that designates broad fields of biology with a name that end with the suffix "omes". This term probably has its origins in the word biomes in the late 20th century and became very popular with the large study of the total human and other organisms genes, called genome. The "omic" approaches propose a global characterization of specific classes of target biomolecules in uni- or multi-cellular systems as a strategy to achieve comprehensive understanding of biological functions.

The genomics, aimed at performing the entire genetic sequencing of organisms, represented the seminal step towards the understanding of the complex logic that orchestrates the function of all organisms or the defects leading to diseases (Stratton *et al.*, 2009; Shuldiner and Pollin, 2010). However to express the phenotype, information needs to flow from DNA via carrier biomolecules through processes that have being addressed by new "omic" sciences such as the transcriptomics, proteomics, metabolomics, glycomics, lipidomics, fluxomics and interactomics.

Transcriptomics (Blow, 2009c; Transcriptomics, 2009), aimed to perform the study of all set of RNA molecules (mRNA, rRNA, tRNA and non-coding RNA) in a single cell or organism. How transcriptomics reflects the genes that have being active expressed at any given time in the cell, it is also referred as expression profiling. The main technique used to address this "omic" approach is the RNA and DNA microarray.

Proteomics (Cravatt *et al.*, 2007) is, together with genomics, the most well know "omic" approach. The proteomics era is coincident with significant developments in mass spectrometry (MS), as well as fast bioinformatics tools, database search engines, and fulfillment of genome sequencing efforts. Proteomics involves not only the identification of gene products and their abundances, but also the use of protein interactions for analysis of protein complexes, protein-protein interaction networks, and the dynamic behavior of the networks as a function of time or experimental condition. The major breakthrough of MS-based proteomics for biomarker search is the possibility to quantify a wide spectrum of proteins, and the easiness of assembling multiplex detection in a single measurement, an approach believed to lead to personalized medicine and treatments in the future (Pan *et al.*, 2009).

Glycomics (Blow, 2009a; Ly *et al.*, 2010) and lipidomics (Blanksby and Mitchell, 2010; Quehenberger *et al.*, 2010), aimed to perform the study of the complete profile of sugar and lipids produced in a biological system, respectively, also studying the pathways and networks involved in each specific class of compounds.

Fluxomics (Tang *et al.*, 2009; Zhang *et al.*, 2010) aimed to study the dynamic change of molecules within a cell over time. Basically it is described as the flux balance analysis of a large and wide systematic framework.

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Metabolomics (Carroll *et al.*, 2010; Cuperlovic-Culf *et al.*, 2010; Zhao *et al.*, 2010) has the ambitious aim of study changes of the higher number possible of different small-molecules metabolites in a cell, tissue, organ or organism. Differently from transcriptomics and proteomics, metabolomics profiling can give a snapshot of the physiology of the cell and have been used widely nowadays.

Interactome (De Las Rivas and Fontanillo, 2010; Marras *et al.*, 2010; Sandhu, 2010) can be defined as the whole set of molecular interactions in cells. It is easy to realize that this technique is based in the integration of the other "omic" techniques and is heavily dependent on software and bioinformatics tools.

To address an "omic" analysis a high throughput technique is required due to the high amount of data that have to be analyzed. For genomes large gene sequencers are used with an actual unbelievable capacity of sequencing a human genome in a manner of days (Schatz *et al.*, 2010). For the other "omics" the use of magnetic nuclear resonance (MNR) and other analytical techniques such as infrared and ultraviolet spectroscopies are common used. However, mass spectrometry is nowadays the most powerful technique for the structural characterization of biomolecules, and has become the central technique for the "omic" sciences.

Mass spectrometry

MS is a unique analytical technique which has seen incredible growth over the past 25 years, evolving to the forefront of analytical techniques. With this technique is possible to count and measure the mass of a great variety of isolated gaseous atoms and molecules in ionized forms in a fast, selective, highly sensitive and reliable way.

MS differs therefore from other spectroscopic techniques such as ultraviolet, infrared, and NMR spectroscopies, which are based on the measurement of physical events resulting from the interaction of organic molecules with electromagnetic radiation. Currently, MS is a fundamental technique for characterization and quantitation of atoms and molecules in chemistry, biology and medical sciences. MS instrumentation has increased approximately 5-fold in sensitivity every three years, allowing to study biomolecules in inconceivable ways if compared to a quarter of century ago (Zhou and Veenstra, 2008). Even single cell studies have been performed in the last few years (Hjelle *et al.*, 2010; Wang and Bodovitz, 2010).

Using revolutionary ionization techniques such as electrospray (ESI) and matrix-assisted laser desorption ionization (MALDI), a wide range of biomolecules such as peptides, proteins, lipids and sugars are efficiently transferred in intact ionized forms to the gas phase for MS analysis. The development of ESI-MS and MALDI-MS has been awarded the Nobel Prize for Chemistry in 2002, rocketing the application of MS in the "omic" sciences. More recently, ambient ionization MS techniques, such as desorption electrospray ionization (DESI) and easy ambient sonic-spray ionization (EASI), have been developed for ionization in the open atmosphere, in a workup free and high throughput fashion directly from sample in their original environments.

Important applications of MS in biological sciences include the structural characterization of biomolecules such as carbohydrates, nucleic acids and steroids, sequencing of peptides and proteins and oligosaccharides, drug metabolism determination and quantification. MS can be used even to produce chemically selective images by monitoring the patterns of distribution of (bio)molecules in tissues via a technique known as MS imaging (Feng *et al.*, 2008).

The atomic or molecular species analyzed by MS must be electrically charged and be in the gas phase to allow for manipulation inside the mass spectrometer, which weights each individual gaseous species by their mass-to-charge (*m/z*) ratios. These intact species can also be fragmented to access information of molecular connectivity (structure). MS allows therefore for the identification, quantification, and also elucidation of molecular structure.

The equipments used for MS analysis are the mass spectrometers and they are generally composed of three fundamental parts: a) the ionization source, b) the mass analyzer, and c) the detector. In the MS analysis flow, ions from the (bio)molecules of interest are generated via an appropriate ionization technique, separated according to the *m/z* values in a mass analyzer, and detected and counted (ion current) via an ion detector. The detector "counts" the ions by measuring the ion current and transforms such current into an electric pulse (a peak in the mass spectra). The data generated (mass spectrum) include the *m/z* of the ionic molecules or atoms on the abscissa and their relative abundance on the ordinate (peak heights) after normalization to the most abundant ion (Siuzdak, 1994). It is not the focus of this article an overview of all types of mass spectrometers and ionization techniques. However some deserve special attention due to their large use in the "omics" approaches and will be addressed briefly here.

Initially, the application of MS was restricted to the analysis of gas, volatile, and thermally stable molecules due to limitations of the first ionization techniques such as electron ionization (EI) and chemical ionization (CI). In the 90's, however, two revolutionary techniques were developed for the ionization of large and labile biomolecules ionization. One of these was named electrospray ionization (ESI), which ionizes molecules and biomolecules in solutions and then "ejects" the solution ions into the gas phase. ESI is considered one soft ionization technique, once this kind of ionization allows the formation of ions without

fragmentation of the original compound. ESI was first proposed as a source of gas phase ions by Dole *et al*., 1968. However, the success of this technique began when Fenn *et al*. (1989) used ESI in mass spectrometry and demonstrated that multiply charged ions were obtained from proteins. As elegantly summarized by the Nobel laureate John Fenn, "ESI makes molecular elephants fly". ESI has therefore allowed the coupling of MS to liquid chromatography: LC-MS (Fenn *et al*., 1989).

In summary, ESI-MS involves the spraying of the analyte solution through a thin metal capillary and the application of a strong electric field on the capillary tip. A very fine spray of highly charged (either positively or negatively charged) droplets will be formed and desolvated by a heated inert gas up to the point where the repulsive coulombic forces approach the magnitude of the surface tension forces (Kebarle and Tang, 1993) causing coulombic explosion and "ejection" of the analyte ions to the gas phase.

Other called soft ionization techniques that can handle analytes in solution such as atmospheric pressure chemical ionization (APCI; Horning *et al.*, 1973; Caroll *et al.*, 1975) or atmospheric pressure photoionization (APPI; Locke *et al.*, 1982; Robb *et al.*, 2000) can be also coupled to LC for "omic" studies, but they rely on the evaporation of neutral molecules from the spray droplets and post gas phase ionization.

The second revolutionary ionization technique was named matrix-assisted laser desorption ionization (MALDI; Karas and Hillenkamp, 1988). For MALDI-MS, the sample can be only analyzed off-line and normally a small volume (1 or 2 µl) of its solution is mixed with an organic matrix on a metallic target plate. Due to its pre-selected chemical nature, the matrix preferentially absorbs the laser beam energy and is desorbed rapidly carrying the analyte molecules to the gas phase. Within this high density plume of gaseous neutral and ionic species, a series of ion/molecule reactions promote analyte ionization. MALDI promotes selective heating of the matrix molecules and thus can handle heavy and nonvolatile biomolecules such as proteins, polymers and oligonucleotides (Gluckmann *et al*., 2001; Knochenmuss, 2006).

Modern mass analyzers include quadrupole mass-filters (Q), 3D ion-traps (IT), linear ion-traps (LIT), ion cyclotron resonance (ICR) traps, orbitraps, and time-of-flight (TOF) analyzers. Each analyzer displays a set of advantages and drawbacks. Some display ultrahigh resolution and accuracy for the *m/z* measurements. Nonetheless, due to their high cost and more demanding maintenance, other mass spectrometers may be more suitable because of being less expensive, robust, compact and easier to operate at the cost of low resolution and accuracy for the *m/z* measurements.

Some mass spectrometers are perfect for quantitation (Triple quadrupoles or Q-Traps, and hybrid instrument that aggregate the best of the triple quadruples and the power of fragmentation of an ion trap) whereas others display their best performance for high throughput analysis or the best cost/benefit ratio for *de novo* molecular characterization. Some of these analyzers can also be connected in sequential arrangements to allow for multi-stage MS, such as in triple (QqQ) or even pentaquadrupoles (QqQqQ) of TOF-TOF instruments (Eberlin, 2007).

Hybrid instruments are particularly clever arrangements since they try to combine two analyzers using each member of the pair to compensate the drawbacks of the other (MSn capabilities of one analyzer with high resolution and accuracy for the other) hence hybrids provide overall the best MS performance. Most successful hybrids are the Q-traps (triple quadrupoles with a quadrupole that can be operated also as an LIT), IT-TOF, Q-TOF, Q-ICR and LIT-ICR, which also confer the advantage of structural analysis of biomolecules by dissociation via tandem (MS/MS) or multi-stage (MSn) mass spectrometry experiments (Gluckmann *et al.*, 2001). Mass spectrometric data are highly dependent on the sample introduction strategy, on the method of ionization and on the mass analyzer used. Therefore, instrumentation is an important issue for best performance according to each application (Milne *et al.*, 2006). Since a mass spectrometer separates, measures and detects ions of different masses, the different isotopes of a given element or different isotopologues of a given molecule are easily detected in the same abundances that they occur in nature, providing therefore typical isotopic "signatures" for atoms and molecules. For instance, organic molecules will display corresponding isotopologue ions with characteristic relative abundances due to ^{13}C (1.1% of natural abundance) as a function of the number of carbon atoms (11.0% for C_{10}, for instance) or monobrominated molecules will display a typical pattern of two peaks of about the same abundance separated by 2 *m/z* units (^{79}Br and ^{81}Br of the same natural abundance). Therefore, the mass spectrum provides typical isotopic patterns (isotopic signatures) that are very useful for analyte characterization and the identification of the presence of elements in the molecule composition, particularly for those elements with a rich and characteristic isotopic diversity, such as Cl, Br, S, Si, B and most of the metals (Glish and Vachet, 2003). Monoisotopic elements such as F and I also provide a clue since they produce no isotopic diversity. Other relevant MS information is the overall mass defect of the molecule. The mass of a particular atom, except the ^{12}C of exactly 12 Da by convention, slightly deviates from integral masses, that is, is not exactly equal to the sum of the masses of the individual neutrons, protons, and electrons of which the atom consists. This mass defect, due to different nuclei stabilities, can be measured if high resolution and accuracy mass spectrometers such as FT-ICRs are used. These instruments are capable of determining the "exact

mass" of an ion at sub-ppm accuracy and hence to determine undoubtedly its full composition. Such data is also able to distinguish between isobars, which are molecules with the integral nominal mass (30 Da) but different exact masses such as C_2H_6 (30.04695), CH_2O (30.01056), and NO (29.99799). This feature is particularly useful for complex mixtures such as crude oil analysis (Alberici *et al.*, 2010). Metabolomics strategies take great advantages of high resolution and accuracy MS to get molecular composition and to differentiate small molecule isobars (Glish and Vachet, 2003). The ultra-high resolution and accuracy for the *m/z* measurement and the possibility to fragment the ions allow structural analysis and confident attribution of the lipid species.

LC-MS/MS

For the more complex samples, the coupling with chromatographic techniques such as liquid chromatography (LC) or gas chromatography (GC) as well as the use of tandem MS (LC-MS/MS and GC-MS/MS) has allowed comprehensive mixture characterization of major biomolecules.

An initial limitation in the development of a combined LC-MS method was the interface between the HPLC (High-performance liquid chromatography) and the mass spectrometer. However, with the development of MS ionization techniques such as ESI and APCI, biomolecules like lipids can now be analyzed directly from aqueous samples. A major advantage of this approach is the simplification of sample preparation (Murphy *et al.*, 2005). Another advantage is the possibility of relative and absolute quantitation of practically any kind of biomolecules that can be found a in commercial standard and an appropriate internal standard. As a result, liquid chromatography tandem mass spectrometry (LC/MS/MS or LC/MS^n) has become one of the most powerful techniques to identify and quantify large numbers of biomolecules in a variety of biological matrices (Newman *et al.*, 2002; Masoodi and Nicolaou, 2006; Schmelzer *et al.*, 2006; Deems *et al.*, 2007; Blewett *et al.*, 2008). The HPLC separates the compounds based upon physical properties, followed by unambiguous identification based upon the characteristic product ions in the MS. This is generally achieved by scanning in selective reaction monitoring (SRM) mode. The acquisition rate, sensitivity and selectivity provided by SRM enables the acquisition of high quality data for more compounds at faster acquisition rates. The advent of ultra performance liquid chromatography (UPLC) methodologies will further increase this process based on increased chromatographic capacity (Wilson *et al.*, 2005). LC-MS/MS has consequently become the only platform capable of performing concurrent quantification of the large numbers of analytes required for studying the biological role of certain biomolecules, like oxylipins, a class of lipids involved in innumerous cellular functions (Lundstrom *et al.*, 2009; Haeggstrom *et al.*, 2010).

High dimensional biology approach

All "omics" techniques are encompassed in the high-dimensional biology approach (HDB), which refers to the simultaneous study of the different biological levels of an organ, tissue or an organism. HDB fundamental premise is that the evolutionary complexity of biological systems renders them difficult to comprehensively understand using only a reductionist approach, studying just units of the whole. The better way to try to understand a biological system is to archive the greatest number of measurements of different variables in the different levels of the system. This approach can be archived by the use of HDB approach.

Nowadays it has been seen that it is not individual genes but rather biological pathways and networks that drive an organism's response to a wide range of stimuli and the development of the range of phenotypes we observe. Many biologically significant networks, including metabolic networks, signal transduction networks and transcriptional regulatory networks, among others are involved in practically all what happens in the cells. In this scenery the HDB approach has provided very good results and helped to understand the complexity of biological systems.

Systems Biology

Certainly, between all the terms used in the "omics" field, systems biology is the most challenging to explain. It attempts to integration of all different "omic" techniques to produce a fully view of the function of a cell, tissue, organ or organism. Basically it is a biology based interdisciplinary study field that focuses on complex interaction in biological systems. Systems biology seeks to understand how system properties emerge from the non-linear interactions of multiple components (Kitano, 2002a, b; Aderem, 2005; Diez *et al*., 2010). The connections and interactions between individual constituents including genes, proteins, and metabolites are examined at the level of the cell, tissue, and organ to ultimately describe the entire organism or system (Barabasi and Oltvai, 2004; Ahn *et al.*, 2006). The intent is to identify the biological networks that connect the differing system elements, thereby defining the characteristics that describe the overall system. This information can then be used to derive mechanistic information on biological processes as well as identify potential target sites for therapeutic intervention (Hood and Perlmutter, 2004; Hood *et al.*, 2004). In the opposite side of the reductionism science, where the researchers try to explain different small parts of the whole, systems biology integrate the same idea of HDB approach. This field is heavily dependent on

bioinformatics tools and models and possibly had its origins with names like Ludwing von Bertalanffy, Alan Lloyd Hodgkin and Andrew Fielding Huxley, being the last two winners of the Nobel Prize. In the last few years several papers and reviews about this theme have been published and this review will not go further to this issue.

Tools for data analysis

One the most important steps in the HDB approach is the data analysis. Once high amount of data will be generated, the classical statistical analysis is not enough to data analysis. Since the generation of high-quality data depends on a number of factors, the steps of a HDB approach must be accompanied by rigorous assessment of the data quality. As such, the implementation of independent samples for quality control, rather than the data themselves, is necessary to identify potential flaws during the pre-processing workflow. Typical tools include a combination of diagnostic statistics and representations such as histograms, similarity plots in the form of heat maps or dendrograms, or multivariate data analysis such as principal component analysis (PCA) and orthogonal projection on latent squares (OPLS). Bioinformatics programs like Cytoscape and Ingenuity software are largely used to produce maps of interaction of different genes and to produce metabolic pathway maps that can explain the integration of different biological systems.

Different platforms for analysis of specific classes of compound have been developed and are considered important tools for data analysis. In the case of lipids analysis the Kyoto Encyclopedia for Genes and Genomes (KEGG; Wheelock, Goto *et al.*, 2009) and the LIPIDS Maps (Fahy *et al.*, 2009) can provide a wide range of bioinformatics tools for the analysis of different lipids classes.

The accumulation of large amounts of biological data from "omics" projects is providing the foundation for the development of systems biology. Accordingly, the new challenge is to combine information from multiple high-throughput experiments involving multiple platforms and formats and extract the relevant system properties (Bornholdt, 2005; Joyce and Palsson, 2006). A common approach to the visualization and examination of "omics" data involves the generation of a network of all the individual components of a given set of experiments (Bell and Lewitter, 2006; Han, 2008). These approaches are not novel and methods for analyzing systems and networks have already been developed in other fields, for example social and information networks. Network theory is widely used to analyze and visualize systems level relationships without losing detailed relations between components of the system. Network theory, or more generally graph theory, is a branch of mathematics devoted to the study of networks (graphs), which are mathematical structures used to model pairwise relations between objects from a ''collection''(Huber *et al.*, 2007). In a biological context, a collection could be the proteome of a cell and the relations are defined by their interactions (Blow, 2009b).

"Omics" in reproductive biology: What can be done?

Embryo and oocyte analysis

To our knowledge, a number of projects, experiments and new applications involving MS-based "omic" strategies specifically designed for embryo and gamete biotechnology area are underway or have their suitability already reported.

The use of MS fingerprinting for controlling the quality and evaluating the stability of culture media used for bovine embryo *in vitro* production (Ferreira *et al.*, 2009) has contributed with improving the routine for producing and storing commercial culture media. In these studies MS analysis enabled the identification of subtle changes in the chemical profile of embryo and oocyte culture media caused by temperature fluctuations, further demonstrating how "omics" can be used to establish new strategies of media storage and delivering.

MALDI-MS lipid fingerprinting of individual oocytes and embryos is a fast and high-throughput technique (Ferreira *et al.*, 2010), which can add valuable information to experiments aimed at optimizing embryo *in vitro* culture systems for cryopreservation. Also the detection of the "ideal" lipid profiles obtained from *in vivo*-derived embryos can be used as an indicative of embryo cryosensitivity.

For semen analysis, MALDI-MS lipid fingerprinting (Fuchs *et al.*, 2007; Fuchs and Schiller, 2008) can become a tool for the early evaluation of bull semen, especially if lipid biomarkers related to sperm cryoresistence or cryosensitivity can be characterized. Also, the characterization of the swine and bovine follicular fluid environment regarding the protein, amino acids and lipid content can contribute with the development of new synthetic media for embryo *in vitro* production allowing higher embryo development and viability, with increase in pregnancy rates and cryopreservation success. Since lipids are involved in membrane dynamics, cell energy homeostasis and regulation of the molecular machinery, they may serve as an excellent source of information when accelerated cell growth occurs and how is the stability and viability of cloned cells. A recent shotgun lipidomic MS study was successful to characterize and describe the detailed metabolic pathway of 250 lipids (corresponding to 21 lipid classes) from *Saccharomyces cerevisiae* membranes, which corresponded to ~95% of the lipid variety present in the yeast. Changes in entire lipidomic profiles were observed when culture temperature varied,

or after genomic deletion of 3-ketoacyl-CoA synthases (Ejsing *et al.*, 2009).

Several lipid classes such as glycerophospholipids, sphingolipids, lysolipids, and oxidized fatty acids have been reported to be altered in cancer conditions. Due to the major advantages regarding sensitivity, accuracy, reproducibility and applicability in detection and quantitation of a large array of lipid molecules, LC-MS/MS is being employed in the search of cancer biomarkers based on individual lipids, a class of lipids, or a unique lipid fingerprint (Fernandis and Wenk, 2009). ESI is considered the ionization technique of choice for MS lipidomic studies, but MALDI is as useful for lipid fingerprinting of biological samples (Schiller *et al*., 2004; Fuchs *et al*., 2007, 2008; Schiller *et al*., 2007; Fuchs and Schiller, 2008). We have used MALDI-MS to obtain direct lipid fingerprinting of individual oocytes and embryos of various species (van Meer, 2005). Due to the sensitivity and easiness of lipid ionization by MALDI, no sample extraction was needed. Embryos and oocytes were simply placed on a target plate, and covered with the MALDI matrix. PCs, SMs and TAGs were detected in few seconds. Oocytes and embryos displayed different lipid profiles, and embryos cultured in low oxygen atmosphere (5%) and without fetal calf serum as a supplement, showed PCs with less palmitic acid (a saturated fatty acyl residue) and more oleic acid (an unsaturated fatty acyl residue; Ferreira *et al.*, 2010).

Also related to the proteomics field a new "omic" technique is emerging with high potential in the analysis of embryo and oocytes. With the name Toponomics, the fluorescent robot imaging technology multi-epitope-ligant-cartography image system has revolutionized the field of proteomics/functional genomics, due to its ability to locate and decipher functional protein networks of hundreds of different proteins in a single cell or tissue section (Schubert *et al.*, 2008; Pierre and Scholich, 2010). This technique uses a light fluorescent microscope coupled to a robot capable to add and remove the fluorochrome-labeled tags at controlled temperature to avoid any displacement of the sample and objective. In each cycle a tag is added and the images are acquired using a high-resolution charged device (CCD), after what the samples are washed and the new tag is added. At the end of this process the different fluorescent images produced are aligned and processed to produce maps of protein location and possible protein-protein interaction. The main application of this technique so far was basically the neuroscience field. In animal reproduction this technique could be used to analyze the interaction of proteins in the embryo in development as well as in the analysis of the uterus, ovary and any other tissue.

To our knowledge the same approach has been tried using MALDI mass spectrometer to generate mass images, with the possible advantage to expand the analysis to other classes of compounds.

Animal cloning

Another interesting perspective for the "omics" approaches is related to the need of increasing commercial success of animal cloning. Somatic cell nuclear transfer (SCNT) where the nucleus of a differentiated cell is introduced into an oocyte from which its genetic material has been removed by a process called enucleation, have been tried widely in animal cloning. Cloning by nuclear transfer has potential applications in agriculture and biomedicine, but is limited by low efficiency. Cattle were the second mammalian species to be cloned after Dolly the sheep, and it is probably the most widely used species for SCNT experiments (Ross and Cibelli, 2010). This is, in part due to the high availability of bovine oocytes and the relatively higher efficiency levels usually obtained in cattle. Recently Smith and Yoo (Smith and Yoo, 2009) showed that the study of histone PTM can help to understand the control of cellular gene expression and repression patterns, which have been extensively associated to lower success rates in animal cloning. In this field transcriptomics, proteomics and lipidomics profile analysis of the oocyte types that will receive the nucleus compared to the donor cell could increase the efficiency of this method. The basic concept behind this idea is that the higher similarity between the two cells can increase the success of this method.

Mass spectrometry imaging

Mass spectrometry imaging (MSI) allows the rapid detection, localization, and identification of many molecules from the most complex, biological sample surfaces. It emerged as a response to the demand for spatial information about biomolecules detected by conventional mass spectrometry. The MSI instrumentation, methods, and protocols have been developed to study the spatial distribution of endogenous compounds such as lipids or proteins and exogenous compounds such as polymers or pharmaceutical compounds on complex surfaces (Chughtai and Heeren, 2010). It is a label free technique that can deliver detailed understanding of biological processes on different length scales, from subcellular to multicellular level and from organs to whole biological systems. With the introduction of the MALDI-MSI in 1997 by Caprioli *et al.* (1997) rapid developments of methodologies, instrumentation, and software used for imaging of biological samples started. Now, peptide and protein profiling directly from biological tissue samples is almost routine. Another method that can be used for mass spectrometry imaging is the desorption electrospray ionization (DESI), developed by R.G. Cooks in 2004 (Takats *et al.*, 2004), and which can be used for MSI analysis in an ambient environment. Contrary to MALDI, which operates under high or ultrahigh vacuum conditions, DESI is employed under

atmospheric pressure (AP). DESI is a combination of two MS ionization methods: electrospray ionization (ESI) and desorption ionization (DI). Instead of a laser beam used in MALDI, DESI uses energetic, charged electrosprayed solvent droplets to desorb the molecules from the sample surface. Some results from our laboratory in publication used DESI to create a peptide profile from different kinds of meat, being also possible the identification of contaminants such as high doses of hormones in the samples. Owing to MALDI-MS imaging technology, precise spatial and temporal differences in phospholipid composition during embryo implantation have also been revealed (Burnum *et al.*, 2008, 2009).

Both techniques can be applied in single cells or in tissue from the reproductive system. The main limitation of both techniques is the formation of ions for the mass spectrometry analysis. In the case of MALDI-MSI the use of different matrices can be necessary and for DESI different solvents can be used for different compound analyses.

Biomarker discovery

Between all the applications of "omics" techniques in animal and human reproduction, the biomarker discovery field can be considered as the one with more development potential.

Successful markers are compounds that could be objectively measured and evaluated as indicators of biological and/or pathological processes. Biomarkers can be anatomic, physiologic, or biochemical in nature and must be associated with a biological state of a cell, organ or system. To be scientifically useful, a biomarker must be detectable and measurable with objective techniques such as physical examination, imaging, or an analytical measurement. Biochemical markers are endogenous compounds that are either not present in a normal physiological state (certain tumor markers) or present within certain range of concentrations (intermediates and products of metabolic pathways). Biomarkers are important because accurate diagnoses and treatment monitoring make the foundation for successful outcomes. Moreover they might serve for early diagnostic needs, as indicators of severity of diseases, response to a treatment, recurrence of the diseases or to determine patient's prognosis (Kushnir *et al*., 2010).

The applications of tandem mass spectrometry to biomarkers discovery could be subdivided into two categories: screening and target analysis. Screening methods are intended to detect multiple biomarkers in diseases, drugs, or toxins, without previous knowledge of the compounds that will be detected. Also known as untargeted metabolomics this approaches needs a high resolution mass spectrometer which allows the unequivocal identification of the molecular mass of each compound. After that, the data obtained must be analyzed using software programs with huge libraries of different classes of molecules. In this approach, the goal is to achieve high throughput of testing and a low false-negative rate.

In target analysis, the main focus is on accurate and precise quantitation of a very well known number of analytes. Known as target metabolomics the use of LC-MS/MS or GC-MS/MS approaches are the most common used. Due to the combination of chromatography to mass spectrometry an unequivocal identification of the target compounds can be achieved. Despite the fact that target metabolomics does not need huge molecules libraries for screening, this approach needs the use of commercial standards with a very well known concentration and the use of internal standards (normally deuterated standards) to achieve accurate and reliable results in terms of concentration (Newman and Watanabe, 2002).

In all analytical applications measurement accuracy is important. Errors encountered in the clinical diagnostics, however, are especially costly compared to other fields, because they might lead to a misdiagnosis, mistreatment, patient injury, and even to the loss of life (Plebani and Carraro, 1997; Plebani, 2006).

The challenges of biomarkers discovery are related to the complexity of the biological samples, the large diversity of classes of molecules present in the samples, variability of the sample matrices among individuals, and the wide range of concentrations of the constituents in the samples.

The application of target and untargeted metabolomics for biomarkers discovery in animal reproduction is practically unlimited. As mentioned previously Ferreira *et al*. (2010) achieved an untargeted lipidomic profile of embryo and oocyte, and also analyzed the culture media for in vitro production of bovine embryos (Ferreira *et al*., 2009).

How different matrices can be analyzed follicular fluid, uterine flushing and different reproductive tissues (e.g. uterine and ovarian epithelium) can be analyzed. With different quantitation methods for steroids hormones and different classes of lipids the reproduction biology in animal and human could be better understood.

Conclusion

Modern and powerful MS methods are revolutionizing the fields of proteomics, lipidomics and metabolomics. This MS revolution has encouraged researchers to value the individual characterization and quantitation of biomolecules in living systems, as well as the need of a systemic molecular biological view. As an illustrative example, for embryo *in vitro* production studies, MS can bring key contributions by monitoring the highly dynamic metabolic changes during cell replication and differentiation, response to temperature, or oxidative stress.

Hand portable mass spectrometers are also being now commercialized decreasing the cost and operation requirements of MS machines, which are in unprecedented manner facilitating the spread and routine use of MS in research and commercial settings. The power and universality of MS in the whole biological sciences is now becoming to be fully realized. The fusion of data from different "omics" approaches must be considered the future way to analyze complex biological matrices and complex biological problems. However it is necessary to have in mind that in most of the cases the results obtained from an "omic" technique should be tested by the very well known and classic biological approaches.

In Brazil, where MS competence is internationally recognized, human resources training and the establishment of multiuser MS centers are the main challenges for the successful introduction of "omics" sciences in the reproductive biotechnology field.

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