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Reproductive technologies and the porcine embryonic transcriptome☆

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ABSTRACT

The domestic pig is not only an economically-important livestock species, but also an increasingly recognized biomedical animal model due to its physiological similarities with humans. As a result, there is a strong interest in the factors that affect the efficient production of viable embryos and offspring in the pig using either *in vivo* or *in vitro* production methods. The application of assisted reproductive technologies (ART) has the potential to increase reproductive efficiency in livestock. These technologies include, but are not limited to: artificial insemination (AI), fixed-time AI, embryo transfer, cryopreservation of sperm/oocytes/embryos, *in vitro* fertilization and somatic cell nuclear transfer (cloning). However, the application of ART is much less efficient in the pig than in many other mammalian species such as cattle. Until recently, the underlying causes of these inefficiencies have been difficult to study, but advances in molecular biology techniques for studying gene expression have resulted in the availability of a variety of options for gene expression profiling such as microarrays, and next generation sequencing technologies. Capitalizing on these technologies the effects of various ARTs on the porcine embryonic transcriptome has been determined and the impact on the related biological pathways and functions been evaluated. The implications of these results on the efficiency of ARTs in swine, as well potential consequences for the developing embryo and resulting offspring, are reviewed.

1. Introduction

The domestic pig is an economically-important livestock species, with pork constituting 40% of the world's meat consumption, making it the most important global meat source (Dang-Nguyen et al., 2010). However, pigs are also a well-recognized biomedical animal model (Rogers et al., 2008; Chorro et al., 2009; Vilahur et al., 2011) due to its physiological similarities with human (Abeydeera, 2002; Betthauser et al., 2000). As a result, there is a strong interest in the factors that affect the efficient production of viable embryos and offspring in this species using either *in vivo* or *in vitro* production methods. As an agricultural species, domestic swine have been raised and selectively bred to produce pigs with desired characteristics for centuries. The application of assisted reproductive technologies (ART) increases reproductive efficiency and genetic gains in this and other livestock species by facilitating the dissemination of genetics from superior sires and dams. From a biomedical and biotechnology point of view, the pig was initially utilized as a model for developing and testing surgical procedures. Later, advances in the area of molecular biology along with the development and

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application of ART formed the basis for genetic manipulation of swine in order to create porcine models that mimic human conditions and disease, among other applications (Whyte and Prather, 2011) or use them as bioreactors (Dyck et al., 2003). Recent research has focused on using the pig as a medical model for renal transplantation (Giraud et al., 2011), cardiovascular-related diseases (Zaragoza et al., 2011), atherosclerosis (Vilahur et al., 2011) and Cystic Fibrosis (Rogers et al., 2008). As well, advances in induced pluripotent stem cell (iPSCs) technologies (Esteban et al., 2009; Roberts et al., 2009; West et al., 2010) make the pig an attractive model for regenerative medicine and stem cell research. Despite the importance of this species for these varied purposes the application of ART in swine is much less efficient in the pig than in other livestock species (Gajda, 2009; Kikuchi et al., 2002). Although a full gambit of ART have been applied in pigs, for research and biotechnology purposes, the regular application of many ART for agricultural purposes is less common in swine production than in species such as cattle. Therefore, an improved understanding of the effects of ART on embryonic gene expression and development in swine will have both agricultural and biomedical benefits.

1.1. ARTs in swine

Intellectual and technological advances in the area of reproductive physiology in the past 60 years has resulted in the development of four generations of ART, which are often considered to be either "sperm-based" or "embryo-based" technologies (Bertolini and Bertolini, 2009). The first to third generations of ART are general considered to include: (1) Artificial insemination, sperm and embryo freezing. (2) Induced and/or multiple ovulations, ultrasonography as well as embryo transfer. (3) *In vitro* embryo production and culture, as well as semen and embryo sexing. While the fourth generation of these technologies, which is considered to be more experimental and is still evolving, includes: transgenesis, stem cell biology and "cloning" procedures. The fourth generation of ARTs have the potential to greatly enhance the influence of superior animals on production, as mentioned above, commercial use has been limited to biomedical and biotechnological applications in the pig.

1.2. Gene expression profiling analysis and the porcine embryonic transcriptome

The success of ART in swine varies dramatically across these 4 generations. Following artificial insemination, almost 100% of all the fertilized embryos in the pig can develop into blastocysts *in vivo* (Geisert and Schmitt, 2002). However, porcine embryos derived from *in vitro* ART manipulation systems, such as *in vitro* fertilization and culture, cloning, and parthenogenesis, are less competent than their *in vivo* counterparts, showing slower development, lower cleavage and blastocyst formation rate.

Until recently, the underlying causes of these impacts and inefficiencies have been difficult to study. Initial studies in this area relied on morphological evaluations of early embryos, but the lack of "diagnostic" tools to study embryos limited progress in this area (Alexopoulos and French, 2009; Coy and Romar, 2002; Crosier et al., 2001; McEvoy et al., 2001; Sinclair et al., 1999). Advances in molecular biology techniques for studying gene expression have resulted in high-throughput platforms for gene expression profiling such as next generation sequencing (NGS) technologies and microarrays (Pariset et al., 2009). This, coupled with the ability to amplify the minute amount of genetic material present in the early conceptus, has enabled scientists to better study early embryo development.

Next generation sequencing systems, such as RNA-seq, are considered state-of-the-art in this field, as it allows for the precise quantification of transcript levels in tissues or cells of interest and facilitates data mining and identification of transcript isoforms. This technology allows for massive amounts of information to be gathered from minute samples, but the amount of data generated can be problematic and requires sophisticated computational and bioinformatics approaches. Microarrays also represent a powerful method of gene expression profiling, which can analyse expression of thousands of predetermined transcripts at the same time (Hornsh et al., 2009). As a result, microarrays are quite efficient when a large number of different samples are to be studied and work well in a multiuser environment, but they are limited by the gene and sequence knowledge required for the microarray development and data interpretation. As well, although gene expression microarray platforms are available for various species (including the pig), most of these platforms have been designed based on somatic cell gene expression profiles. It has been shown that the embryonic transcriptome differs significantly from that of somatic cells (Vallee et al., 2009) and, therefore, these somatic cell based platforms are not well suited for studies in embryos. This has prompted the development of embryo-specific microarray platforms for both cattle (Robert et al., 2011) and the pig (Tsoi et al., 2012) in order to better study this critical period of development in these important livestock species. There have been efforts to characterize the gene expression profile of *in vivo* developed porcine embryos and early embryos produced after *in vitro* manipulations using next generation sequencing (NGS) and microarray platforms. This new ability to study early porcine development has created an environment in which scientists can collaborate with livestock and biotechnology industries to evaluate the effect of various ARTs on the porcine embryonic transcriptome and determine their impact on the related biological pathways and functions. For example, *in vitro* culture and *in vitro* production of porcine embryos has proven to be a challenge, however extensive transcriptome profiling of oocyte maturation and early embryonic development has provided insights into the metabolism of the porcine embryo and molecular factors that control its development, which in turn has allowed for dramatic improvements in these techniques (Prather et al., 2013). Despite this, there is still a great deal that is unknown regarding the impact of ARTs on the porcine embryonic transcriptome. Evaluating these impacts, will allow us to better understand the mechanisms involved in early embryonic development in the pig and, in