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Oocyte developmental competence and embryo quality: distinction and new perspectives

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Abstract

In vitro embryo production is the cornerstone of infertility treatment in human and is increasingly used in cattle to propagate high genetic merit animals. To increase its efficiency, many different approaches have been tested all of which stem from the concepts of oocyte quality and developmental competence. Presented here are recently reported findings and perspectives related to bovine oocyte biology and analysis of blastocyst quality that addresses these concepts from a different angle supporting the complex nature of the very dynamic developmental window that encompasses late oogenesis up to blastocyst development. It was recently reported that the atypical nature of the oocyte is supported by extensive nurturing from the surrounding cumulus cells in the form of large cargo transfer as well as transfer of phosphocreatine as an alternate means of generating ATP to fulfill the oocyte's needs during the energy demanding process of maturation. It has been shown many times over that the determinants of early embryogenesis are embedded in the oocyte, however, transcriptome analysis dissociates embryonic yield from the concept of embryonic quality. Within the divergent gene expression, long non-coding RNAs represent a very functionally diverse class of transcripts that have yet been characterized. Taken together, it is clear that a clearer definition of both oocyte and embryonic quality are still needed to support the improvement of *in vitro* embryo production.

Keywords: embryo quality, oocyte competence, RNA transfer.

Introduction

Reproductive success can be broadly summarized as the birth of a viable and healthy offspring. This achievement relies on the completion of numerous complex and selective developmental steps occurring throughout the reproductive process. Interestingly, very few gametes ever get to contribute to the next generation, and reproductive success relies heavily on the quality of those gametes. Recent findings support an added contribution from the male gamete through the transfer of proteins (Saunders *et al.,* 2002) and RNA (Ostermeier *et al.,* 2004) at fertilization and also of an epigenetic legacy (Lambrot *et al.,* 2013) but the constitution of the early embryo is dependent upon the constitution of the egg. In addition to the maternal genome, the oocyte also provides the cytoplasmic components including RNA and protein reserves as well as the mitochondrial contingent all of which are necessary to sustain early embryo development.

To achieve reproductive success, the oocyte must display competence to resume meiosis, to cleave upon fertilization, to sustain early development (namely to activate its genome), to establish a pregnancy, and to sustain fetal growth and development until birth. It is well accepted that succeeding in the first events does not ensure the success of subsequent ones (Sirard *et al.,* 2006). It is this capacity to successfully complete these steps that is referred to as developmental competence. As it has already been discussed, developmental competence is ¨a convenient but biologically fuzzy concept¨ (Duranthon and Renard, 2001) since in its broadest sense, the impact of the oocyte is carried up to the birth of a healthy and fertile offspring. However, other factors, excluding the oocyte, have to be considered such as the reciprocal interaction between the conceptus and the endometrium in the establishment of a pregnancy. Generally, a narrower definition is used where the oocyte's intrinsic developmental competence is studied up until the blastocyst stage after which the requirement for the uterine environment becomes a confounding effect.

By comparing blastocyst rates when producing embryos either *in vitro* or *in vivo* there are three main processes undoubtedly affecting developmental outcomes; they are oocyte maturation, fertilization, and embryo culture. It has been shown that this shorter view of developmental competence is heavily influenced by the quality of the oocyte at the outset and completion of maturation (Rizos *et al.,* 2002). Still today, the characteristics defining oocyte quality are vague and subjective. Studies have focused on the morphology of the cloud of somatic cells surrounding the oocyte and the visual aspect of the gamete's cytoplasm (Blondin and Sirard, 1995). As a token of this, using these characteristics it is possible to choose a subset of cumulus-oocyte complexes (COCs) that will reach the stage of blastocyst *in vitro* in a greater proportion than the unselected population but the rates are seldom higher than 50% and some COCs that do not harbour

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the targeted criteria are able to produce a viable embryo.

In complement, it has been shown that the microenvironment to which the oocyte is submitted can have a profound impact on the proportion of oocytes reaching the blastocyst stage (Lequarre *et al.,* 2005). In order to improve *in vitro* embryonic yields, conditions such as oxygen tension (Quinn and Harlow, 1978; Olson and Seidel 2000; Correa *et al.* 2008) and media composition (Harper and Brackett, 1993a, b; Lonergan *et al.,* 1996; Baldoceda-Baldeon *et al.,* 2014) have been tested and shown to increase the number of blastocysts but still rarely over 50%. The bulk of these studies have been done using COCs aspirated from ovaries collected post-mortem. Using this source, it has been shown that collecting COCs immediately after death leads to low blastocyst rates whereas letting the ovaries incubate in warm saline for a few hours before COC aspiration improves blastocyst rates (Blondin *et al.,* 1997). The mechanism by which oocyte quality improves within the dying follicle is still unknown but it is again a good example of how the oocyte's microenvironment can influence the acquisition of developmental competence.

Considering the selected follicular sizes from these post-mortem ovaries are generally between 3 and 8 mm whereas a bovine preovulatory follicle can reach over 20 mm (Quirk *et al.,* 1986), the suboptimal embryo production could thus be associated with incomplete follicular growth or be representative of a situation where not all oocytes can acquire developmental competence. The latter was challenged when manipulation of the hormonal regimen during ovarian stimulation was shown to produce cohorts of immature oocytes capable of sustaining *in vitro* development to reach the blastocyst stage sometimes at a rate of 100% (Blondin *et al.,* 2002; Nivet *et al.,* 2012). Many reviews have already summarized these observations (Sirard *et al.,* 2006; Fair, 2010; Keefe *et al.,* 2015; Moussa *et al.,* 2015) but yet, the nature of the cues inducing the acquisition of developmental competence is unknown alike the distinctive characteristics harboured by a developmentally competent oocyte.

The need to understand and improve oocyte quality is fuelled by the application of assisted reproductive technologies (ART) both in human to palliate to infertility, and in livestock to increase the rate of selective breeding. In the field of *in vitro* embryo production (IVP) efforts have been directed towards the improvement of culture systems to increase blastocyst yields which have now lead to some concerns regarding the potential for these conditions to cause short and long term effects on embryo quality. These effects from culture conditions can be observed as: a shift in developmental kinetics (Holm *et al.,* 2002), a skew in male: female ratio (Kimura *et al.,* 2005, 2008), and lower tolerance to cryopreservation (Rizos *et al.,* 2003, 2008). It is also known that *in vitro* embryo metabolism varies according to culture conditions and differs from *in vivo* derived counterparts (Krisher *et al.*, 1999; Khurana and Niemann, 2000).

Concerns over the potential carry over impacts on the development of pathological phenotypes much later than the blastocyst stage were reported at the turn of the millennium. Embryo production *in vitro* was associated with the large/abnormal offspring syndrome in ruminants (Young *et al.*, 1998; McEvoy *et al.,* 2000) which was then paralleled in human with higher frequency of imprinted disorders namely the Beckwith-Wiedemann, Angelmann, Prader-Willi and Silver-Russell syndromes (reviewed by Jacob and Moley, 2005), in addition to higher abortion rate and higher fetal abnormality rate (Taverne *et al.*, 2002). Concerns over the long-term impact of ART now encompass all steps including the ovarian stimulation regimen (Denomme and Mann, 2012, 2013) for their potential extended effects on fetal development and even on disease development in adult life (Chen *et al.*, 2011; Hart and Norman 2013a, b; Chen *et al.*, 2014). These long-term impacts are believed to be carried by epigenetics and have so far been studied with most focus at the level of DNA methylation. These concerns bring forth the need to define embryonic quality and include a concept pertinent to embryonic health or "normalcy".

Genome wide gene expression has been used to describe embryos mostly as a mean to compare *in vitro* to *in vivo* produced counterparts. These studies have mainly been conducted with the perspective of increasing embryonic quality to increase developmental competence. Several recent reviews have evaluated the observations (Driver *et al.*, 2012; Gad *et al.*, 2012) and as expected, given a different environment, embryonic cells adapt their gene expression. The challenge remains to determine which genes or extent of gene expression are associated with embryonic quality or are rather deviances that will lead to poor phenotypes. Both the concepts of oocyte developmental competence and embryonic quality are closely intertwined and can also be distinct in nature. A better understanding of oocyte biology is necessary as a basis of defining what makes a "good" oocyte, alike a better understanding of embryogenesis is necessary to define the interval within which an embryo can be defined as "good". Recent observations add to the complexity of these concepts. We have used the bovine model for the potential application to produce more embryos from high genetic merit donor cows, as well as for its value as a model for human reproduction being a large mono-ovular mammal with similar follicular dynamics and kinetics of early embryogenesis.

Oocyte biology

It is known that oocyte developmental competence is acquired once the gamete reaches full size. In bovine, oocytes from <2 mm follicles have not reached full size and show little capacity to mature, whereas full size oocytes are found in 3 mm follicles

and develop to reach blastocyst stage at a rate around 30-35% (Motlik *et al.*, 1984; Fair *et al.*, 1995; Feng *et al.*, 2007). It is known that oogenesis is progressing in synchrony with folliculogenesis through the close interdependence on the bilateral communications occurring between the follicular cells and the gamete. The granulosa cells communicate through paracrine signalling (for example EGF-like peptides; Gilchrist, 2011), while the cumulus cells and more precisely the inner layers of the corona radiata which bear cellular extensions reaching through the zona pellucida (Fig. 1) communicate with the oocyte through the gap junctions found at the articulation between the projection's end and the oocyte plasma membrane.

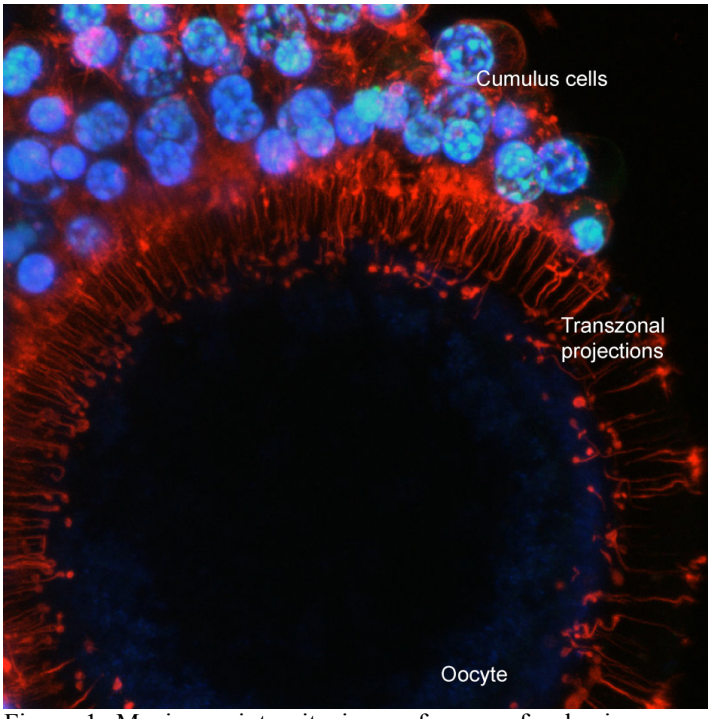

Figure 1. Maximum intensity image from confocal microscopy showing the transzonal projection. Numerous actin microfilaments in projections are coloured in red by the rhodamine phalloidin stain. Cumulus cells nuclei are stained in blue (Hoechst 33342).

Transferring cargo to the oocyte

Direct transfer from the somatic cell to the oocyte is known to occur where cyclic nucleotides control meiosis resumption. The importance of this communication has been the subject of recent reviews (Gilchrist, 2011). The use of gap junctions is also known to limit the size of transferred material to <1 kDa. This is unlike other animal models like *c. elegans* and *drosophila* where large material is delivered during oogenesis either from canals bridging oocytes together or from supporting nurse cells (Deng and Lin, 1997; Wolke *et al.*, 2007). We have recently reported that transzonal projections (TZPs) are very large channels made of actin filaments (Macaulay *et al.*, 2014). RNA granules were abundantly found within these structures and from a de novo labeling assay combined with a transcriptomic survey of the RNA found within the zona pellucida, it was proposed that cumulus cell transcripts are selectively and actively shuttled towards the oocyte. The transcriptome comprised of messenger RNAs as well as long non-coding RNAs (Macaulay *et al.*, 2014). Analysis of the interconnection showed that vesicles are secreted and contact is maintained until 9 h after initiation of maturation after which the connections detach and are completely broken at 22 h (Macaulay *et al.*, 2014). Comparison of transcriptomes before and after maturation allowed identification of mRNAs increasing in abundance in the gamete during maturation. The cumulus cells origin is supported by the known transcriptional silence occurring in the oocyte associated with chromatin condensation (Lodde *et al.*, 2008) in addition to the fact the increase was only observed in intact COCs not in denuded oocytes (Macaulay *et al.*, 2014). Large cargo transfer was further proven by reconstructing COCs; placing denuded oocytes into culture with transfected cumulus cells expressing a GFP fusion protein. Following reconstruction, both GFP coding transcripts and protein were detected in the oocyte (Macaulay *et al.*, 2014). From these results, it seems that the somatic cells continue to nurture the gamete even during its

transcriptionally quiescent period. The contribution of these transfers to oocyte quality and developmental competence remains to be established. Recent data indicates a subset of the transferred mRNAs could be translated and play a role in supporting maturation (Macaulay *et al*., 2015; Université Laval, Québec, Canada; manuscript submitted for publication). This opens a window of opportunity to influence oocyte quality through the cumulus cells. Additionally, it is known that early development relies on maternal stores of mRNAs where prior to genome activation, transcripts stabilized in ribonucleotide particles are recruited to support the protein synthesis demand (Sternlicht and Schultz ,1981). As such, cumulus cells could complement maternal reserves during the last stage of COC preparation.

Mitochondria and oocyte energy production

Cumulus cells are also known to support the oocyte by transferring energetic substrates such as lactate and pyruvate (Bilodeau-Goeseels, 2006). Typically, ATP production is sustained by glycolysis which produces pyruvate that is transported to the mitochondria to be metabolized by the Krebs cycle and the oxidative phosphorylation occurring in the cristae. However, the mammalian oocyte is an atypical cell where it was found that the glycolytic pathway is impaired missing the phosphofructokinase enzyme (Cetica *et al.*, 2002). As an alternative, pyruvate is pumped from the cumulus cells into the oocyte where it is metabolized to generate ATP (Conti *et al.,* 2002; Bilodeau-Goeseels *et al.,* 2007). However, the extent of this energy production can be questioned since the gamete's mitochondrial contingent is odd displaying a round/ovoid form with few cristae and that can be hooded or contain a vacuole (Bavister and Squirrell, 2000). It is believed that the low potential for oxidative phosphorylation per mitochondrion is compensated for by their large number (250,000-300,000; reviewed by (Sutton-McDowall *et al.*, 2010).

By exploring the transcriptome of polyribosomes during oocyte maturation, one main characteristic stood apart where adenylate kinases and creatine kinase where highly represented in this subpopulation of actively translated mRNAs (Scantland *et al.*, 2011, 2014). This highlighted a potential for the adenosine salvage pathway to contribute to the ATP pool of the oocyte. This process needs two enzymatic reactions. The first one implicates the adenylate cyclase that catalyzes the interconversion of adenine nucleotides, and the second one is performed with the creatine kinase that catalyzes the conversion phosphocreatine and ADP to generate creatine and ATP (Fig. 2).

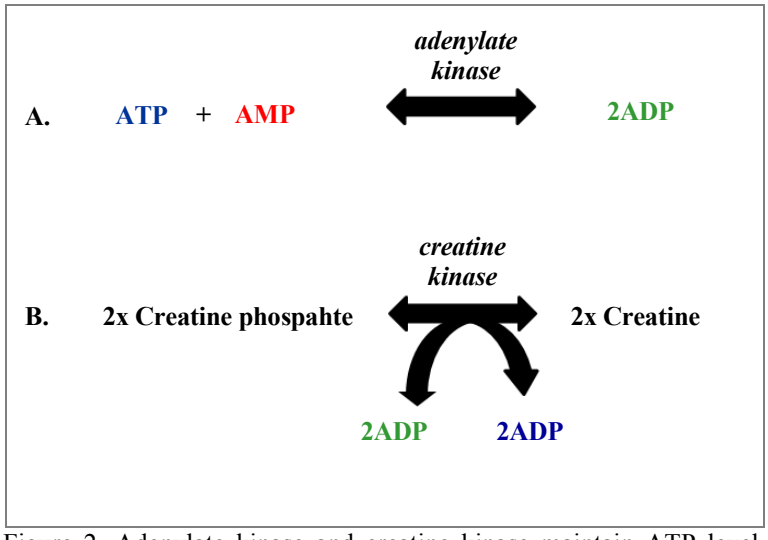

Figure 2. Adenylate kinase and creatine kinase maintain ATP level during oocyte maturation.

Through ATP content measurements during maturation and the addition of specific inhibitors and substrate, it was shown that the bovine oocyte is capable of phosphorylating AMP to produce ATP (Scantland *et al*., 2014). The proposed model is based on recycling the residuum generated by meiosis resumption. More precisely, the large quantity of cyclic AMP (cAMP) produced and transferred from the cumulus cells to the oocyte to control meiosis arrest is converted to AMP through the activity of phosphodiesterases when meiosis resumption is triggered leaving free AMP available for the adenosine salvage pathway. This alternative source of ATP may be important for oocyte quality to support protein synthesis and phosphorylation important for oocyte maturation (Stojkovic *et al.*, 2001). The mitochondrial impact can act on the long term due to their numerous functions beyond energy production. Indeed, mitochondria also play a central role in other processes underlying oocyte quality and also developmental competence. The mitochondria have

important roles in fertilization (being involved in intracellular calcium storage) and embryo development (being the control center of programmed cell death; Santos *et al.*, 2006).

It is at the 4 to 16-cell stage that the mitochondria elongate and the numbers of transverse cristae increase alongside higher demand for glucose and pyruvate (Thompson *et al.*, 1996; Bavister and Squirrell, 2000; Wilding *et al.*, 2009). It was shown that reduced efficiency of mitochondrial respiration and ATP content in the oocyte and early embryo are correlated to a decrease in embryo development, and that maternal aging further influences this relationship (Stojkovic *et al.*, 2001; Nagano *et al.*, 2006; Wilding *et al.*, 2009). Furthermore, maternal ageing has been associated with less metabolically active mitochondria which may confer long term impacts since the offspring are at greater risk of developing obesity and other metabolic syndromes (Wilding, 2015). These observations exemplify how oocyte quality can influence not only developmental competence and embryonic quality but also convey far-reaching health effects that persist into adulthood.

Embryo quality

In order to increase blastocyst rates, culture conditions have been modified extensively by the addition of compounds that would scavenger free radicals or increase cell survival by adding anti-apoptotic compounds (Brison and Schultz ,1997; Kolle *et al.*, 2002; Livingston *et al.*, 2004; Block *et al.*, 2008; Khalil *et al.*, 2013; Wang *et al.*, 2013) . Ultimately, the challenge remains to determine which embryos are "sound" for transfer. As such, different morphological characteristics are used to assess embryo quality such as developmental kinetics where faster cleavage rates (fast 2-cells) display better blastocyst rates similar to embryos displaying "normal" cell-cycle lengths or appreciating the embryonic structure looking for symmetrical cells, embryonic homogeneity, low fragmentation, less apoptotic cells, appropriate cell lineage distribution with the inner cell mass/ trophectoderm (ICM/TE) ratio, or cell to cell interaction where tighter gap junctions are preferred (Lee *et al.*, 1987; Van Soom *et al.*, 1997a, b; Holm *et al.*, 2002; Houghton *et al.*, 2002). These grading characteristics can be subjective, and variable results are often obtained in regard to their *in vivo* similarity or gestation success after transfer.

Consequences of *in vitro* culture systems

As mentioned, since the introduction of bovine *in vitro* embryo production, blastocyst rates have been improved through the use of more suitable environmental conditions including media composition and supplementation. However, embryo production from oocytes aspirated from ovaries collected at the local abattoir has reached somewhat of a plateau with 30 to 40% of oocytes developing into blastocysts. Improvement strategies have generally used blastocyst rates as the target metric where increased production of embryos equates to a better production system that is often also interpreted as one that produces better quality embryos. The observations of shifts in the sex ratio and the increased frequency of difficult parturition due to the offspring overgrowth raised questions regarding the impacts of culture conditions on embryonic quality. The presence of components from biological origins (serum, BSA, hormones) has been addressed, as their use is now known to represent an important source of variance due to contaminants that remain following purification (Kane, 1983; Batt and Miller, 1988; Rorie *et al.* 1994). For more than a decade, embryonic gene expression has been surveyed in order to determine the impacts of culture conditions as well as to attempt to provide a definition of embryonic quality (Wrenzycki *et al.*, 2007; Carter *et al.*, 2010; Clemente *et al.*, 2011). So far, it has been challenging to compare results between studies due to the use of distinct technological platforms, as well as the difficulty in interpreting results as at least two confounding effects arise from any modification to the embryonic culture medium where deviance in gene expression is part of the natural cellular response or perhaps a part of an ill fated response (For review see Robert, 2010; Robert *et al*., 2011). This is caused by the lack of a reference of quality where the comparison with *in vivo* collected embryos is somewhat inappropriate. Although it is the most natural reference, it is also expected that *in vitro* conditions should lead to the some deviance in gene expression since both types of embryos (*in vivo* and *in vitro*) are grown is a very different microenvironment. Thus, transcriptomic divergences are expected but to which extent these deviations become a sign of lower quality? Timing developmental stage between *in vivo* and *in vitro* is also a challenge since timing post-insemination differs from timing post-fertilization. It has also been challenging to delineate between treatment effects and deviance in gene expression caused by a shift in development kinetics leading to the comparison of blastocysts at different developmental stages or by a shift in sex ratio that would highlight the gender associated differences in gene expression (Bermejo-Alvarez *et al.*, 2010). These can become confounding factors but are also part of the treatment effect e.g. a treatment can induce a shift in developmental kinetics that will lead to the comparison of embryos at slightly different developmental stages (early blastocysts vs. expanded blastocysts); in such situation, comparing transcriptomes will identify deviations that may not be a sign that embryos are abnormal but may rather be representative that the embryonic cohorts are different.

In one of our studies, the embryonic transcriptome was compared across ten different *in vitro*

production systems (Table 1; Cote *et al.*, 2011). The initial premise was to collect embryos at a definite time post-fertilization, and compare blastocysts collected without selecting for embryonic morphology or gender as any skew in these factors is part of the treatment effect. Again, *in vitro* produced embryos have been compared to an *in vivo* embryos used as reference. Unexpected results showed that some treatments with poor embryonic yields generated transcriptomic signatures closer to the *in vivo* reference than the high yielding systems, and that these high yielding systems were characterized with very heterogeneous embryonic cohorts. To reconcile these observations we proposed that increasing embryonic rates necessitate more lenient conditions increasing developmental kinetics and allowing the survival of weaker embryos that would otherwise die. We raised the question that it may be necessary to eliminate the weak embryos to minimize the expression of poor phenotypes (Cote *et al.*, 2011). Although these embryos were not transferred, this comparative study suggests that it is possible to produce a lower number of better quality embryos therefore dissociating developmental competence defined as the maximum number of embryos produced from the concept of embryonic quality.

In a subsequent study, it was shown that when selecting a subset of blastocysts using a defined morphology at a fixed time post-fertilization, the impact of culture conditions on gene expression deviations are largely minimized (Plourde *et al*., 2012). This confirmed that previous findings are related to the structure of the embryonic cohort. This study also showed that the source of the oocytes either collected from abattoir ovaries or from ovum pick-up following an ovarian stimulation protocol has a more profound impact on blastocyst gene expression than the culture conditions (Plourde *et al*., 2012). This by contrast supports the close relationship that exists between oocyte quality and embryonic quality. In this study, it was also observed that one of the main impacts of *in vitro* embryo production across all treatments compared to *in vivo* blastocysts involved genes related to mitochondrial functions (Plourde *et al*., 2012). This again supports the theory that mitochondria play a central role in both oocyte quality and developmental competence. This is in accordance with the quiet embryo hypothesis where analysis of the metabolites from the culture media can be associated with embryo quality (Leese, 2012). This concept correlates the metabolism of individual preimplantation embryos to their subsequent viability where a low metabolism observed in regard to low glycolytic rate and amino acid turnover and their high antioxidant capacity characterize the best embryos (Leese, 2012). So, a healthy embryo will have "quiet" metabolism because it spend less energy to rectifying damage to the genome, transcriptome, and proteome, or has less injury compared to a non-viable embryo (Leese *et al.*, 2007).

Table 1. Comparison of ten different embryonic *in vitro* production systems.

Treatment no.	Oocyte maturation (IVM)	Embryo culture (IVC)	Blastocyst yield	Blastocyst morphology at Day 7	Distance of transcriptome from reference	Overall variance between replicates
1	*In vivo*	*In vivo*	N/A	N/A	Reference	++
2	SOF-BSA	SOF-BSA	+++	Fully expanded	++	+
3	SOF-serum	SOF-BSA	++	Fully expanded	++++	++
4	SOF-BSA	SOF-serum	+++	Fully expanded	+++	++++
5	SOF-serum	SOF-serum	+++	Fully expanded	++	++++
6	SOF-BSA	SOF-BSA *	+	Small and dark	+	+++
7	SOF	SOF	+	Small and dark	+++	++
8	SOF-BSA	SOF co-culture	+++	Fully expanded-hatching	++	++
9	TCM-serum	TCM co-culture	+++	Fully expanded-hatching	+	++
10	TCM-serum	SOF-BSA	+++	Expanded but darker	++++	++
11	TCM-serum	TCM-serum	+	Small and dark	+++	+

*Serum was added on the last 2 days of embryo culture and thus after embryonic genome activation in an attempt to determine whether fully active embryonic cells could better use this hormone-rich supplement. [+]Subjective appreciation of distance from reference and variance within treatment. Adapted from Cote *et al*., 2011.

Mining embryonic trancriptome: the role of the long non-coding RNA

During the transcriptomic surveys, it was found that bovine embryonic cells express a large contingent of uncharacterized transcripts (Cote *et al.*, 2011; Plourde *et al.,* 2012). Sequence analysis indicate that these transcripts do not contain an open-reading frame meeting the minimal international standard of 300 nucleotides in length (100 amino acids; Dinger *et al.*, 2009) and that they are generated from both the nuclear and the mitochondrial genome (Cote *et al.*, 2011; Plourde *et al.*, 2012). These long non-coding RNA (lncRNA) constitute the largest class of non-coding RNA. By definition, they are longer than 200 nucleotides, and do not serve as a template for protein synthesis. Thus far, approximately 15,000 human lncRNA genes have been identified (Derrien *et al.*,

2012). Evidence demonstrating that they have various biological functions and play critical roles in the embryonic stem cell regulatory circuit began to emerge through the mouse model only a few years ago (Guttman *et al.,* 2010; Sheik Mohamed *et al.,* 2010). However, understanding the role of lncRNA is challenging and only 1% of all human non-coding transcripts have been associated with a function (Perkel, 2013).

When comparing transcriptomes to identify differential gene expression associated with culture conditions, it was shown that some of these lncRNA molecules are highly responsive to the embryonic microenvironment (Cote *et al.*, 2011; Plourde *et al.*, 2012). So far, their roles have not been characterized. Since non-coding RNAs are part of epigenetics as some of them can regulate gene expression (Bouwland-Both *et al.*, 2013; Bhan and Mandal, 2014) it was anticipated that some of these lncRNAs could be indicative of epigenetic perturbations caused by the culture conditions (Le Bouc *et al.*, 2010; Santos *et al.*, 2010). To investigate this, three candidates have been further characterized and were found surprisingly in the cytoplasm rather than in the nucleus and also to be associated with the polyribosomes (Caballero *et al.*, 2014). Moreover, one of them was found amongst the transcripts isolated from the TZPs between cumulus cells and the oocyte (Caballero *et al.,* 2014). Knocking down this transcript in matured oocytes unexpectedly accelerated embryo developmental kinetics (Caballero *et al.,* 2014). By overlaying the transcriptome and DNA methylome analyses, the knock down was shown to impact on four concordant genes (Coxsackie virus and adenovirus receptor (CXADR), advillin (AVIL), CD9 molecule, and plasminogen activator urokinase (PLAU)) where DNA methylation and transcript abundance follow the rule that increasing methylation results in lower gene expression and vice versa. The last two identified genes are known to be associated with the demise and the survival of blastocysts during the pre-implantation period, respectively (El-Sayed *et al.* 2006). The roles of these lncRNAs in early embryogenesis have yet been elucidated. Like the proteins from the mRNAs, it is possible that lncRNAs are involved in numerous aspects of cellular functions.

Conclusions

It is clear that both concepts of oocyte developmental competence and embryonic quality are closely related, evidenced by the possibility to increase the number of blastocysts produced *in vitro* by modulating follicular conditions. It is conversely true that it is possible to modulate culture conditions to increase the number of low quality blastocysts. Therefore, blastocyst rate may not be a sufficient metric to improve *in vitro* embryo production. As others have proposed for more than a decade it seems clear that a common denominator between embryonic developmental potential and quality is associated to the presence of healthy mitochondria (Van Blerkom, 2008, 2009, 2011). This is the basis of the therapeutic intervention done to increase fertility of the oocyte of aged women (St John, 2012; Tilly and Sinclair, 2013). Although promising any development in the field of embryo production still is in need for the definition of a good quality oocyte and of a top quality embryo. Given the impact of the epigenome and the sensitivities of the oocytes and early embryos to their surrounding environment, the broader definition of developmental competence must be all-encompasing when the goal of embryo production is to induce gestation with healthy offspring as the end result.

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