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Chapter 5

Molecular Regulation of Preimplantation Development of Farm Animals

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Background

Preimplantation period is the early series of events required to establish pregnancy. Therefore, it is one of the main research focus all over the world. The early classic studies was based on *in vivo* model to monitor the success of fertilization based on superovulation and embryo flushing at different time points. However, application of *in vitro* embryo production has advanced our understanding on the molecular regulation of the events encompass this crucial period. Indeed, different approaches and molecular techniques have been performed to get deep insights into the molecular mechanisms controlling early events of preimplantation embryo development of several mammalian species. Semi- and quantitative real-time polymerase chain reaction was first introduced as gene-by-gene approach to study spatiotemporal regulation of well-known candidate genes and their involvement in determining the quality of oocytes and embryos. Another interesting focus of research was to identify the alterations of transcript abundance of candidate genes in response to various biological conditions like *in vivo* and *in vitro* culture conditions. The polyadenylation pattern during oocyte maturation and

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embryonic development was the key molecular mechanism that was investigated deeply in many laboratories. Recently, genome-wide assessment strategies of omics approaches (transcriptomics, miRNAomics, proteomics, lipidomics and metabolomics) have been applied to profile all detailed structural and functional changes of the whole genome in attempt to use this knowledge in downstream applications and practices. Although, the great efforts have been done in this field during the last decades, the applications are still in the infancy stage. In this chapter, more highlights will be focused on all attempts has been made in this field.

1. Introduction

The reproductive performance of a herd is the biggest factor affecting production and product quality of livestock. Thus, a decline of either the female or the male fertility represents a dramatic economic loss in beef and dairy industries. Therefore, it would be of great importance to define and manage the biological factors contributing to livestock fertility. In large animal farm management, individual male could serve thousands of females worldwide through application of artificial insemination. Therefore, a genetic mutation related to certain phenotypic trait(s) of one male will transmitted to have a global economic impact. Thus, a single male has a larger impact on herd productivity than fertility of a single female. Additionally, sperm viability is a determinant factor, which affects success rate of fertilization, and embryo production [1]. Subsequently, the expression of compromised genetic information from the spermatozoa can impair embryo quality [2]. A study done in bovine bulls has detected novel genetic loci harboring ITGB5 gene, which associated with the bull reproductive performance by controlling sperm-oocyte interaction, and resulted embryo development [3]. Expression profile of selected genes was used as a good indicator for the physiochemical parameters of semen collected from farm animals' males of different species [4, 5 and 6]. This confirms the involvement of sperm genes in various reproductive processes such as spermatogenesis, sperm motion [7]. fertilization of ova and subsequent preimplantation development [8].

On the female side, animal breeders normally select dairy cows based on milk record as the main phenotypic (productive) trait, which resulted in fertility decline of their progeny during next generations [9]. This attitude has resulted in compromising fertility of highly milk producing which was measured by the reduction of first service conception rate from approximately 65% in 1951 to 40% in 1996 [10]. Buffalo breeding is faced with the low fertility rate, which leads to high economic loss under heat stress conditions [11]. In this regard, cumulus-oocytes complexes are highly affected when the female is exposed to environmental heat stress during follicular development, ovulation and in vivo events of oocyte maturation [12, 13, 14]. Buffalo cumulus-oocyte complexes that were collected during hot season had a high percentage of arrested oocytes in metaphase I stage of nuclear progression after in vitro maturation [15]. Interestingly, bovine oocytes that have been exposed to increased temperatures at 40.0°C and 41.0°C recorded lower rates of nuclear and cytoplasmic maturation that was

linked with reduced in vitro embryo development [16]. In support to previous observation, experimental exposure of COCs to heat shock during the maturation has revealed reduced cleavage rate as well as blastocyst development [17]. In vivo experiments have reported reduced pregnancy rate by 25% for each °C elevation in body temperature, which is due to the negative impact of heat stress on preimplantation development embryos [18]. The expression of stress indictor gene known as HSP70 was increased in buffalo oocytes collected under heat stress condition compared to those collected in cold season [19]. [20] have reported greater expression profile of genes related to thermal stress (HSP 70.1 and HSP 70.2) and cell death inducing genes (CASPASE-3, BID and BAX) in buffalo embryos (8-16-cell and blastocyst stages) exposed to 39.5°C and 40.5°C than that in control group. This implicates that exposure of buffalo preimplantation stages of development to heat stress even for short duration reduced embryo development rate and this was regulated by increase cell death genes and embryo try to resist this negative effect by upregulation of heat shock genes.

The key biological factor that is behind the reduced fertility either in high milk producing cows and under heat stress is the poor oocyte, which compromise embryonic development qualities [21, 18]. It was established that the molecular mechanisms linked with low developmental potential of bovine oocytes is highly complex and may be reliant on many small changes in the RNA levels of many genes [22,23]. There is a clear relationship between altered transcript abundance patterns and some aspects of embryo quality (i.e. cryotolerance), which render the embryo capable of establishing a pregnancy, if transferred fresh, but incapable of withstanding cryopreservation [24,25]. The main factors implicated in embryo and fetal loss can be categorized as those of intrinsic genetic problems, environmental factors like heat stress, failures in maternal physiological environment, and endocrine related problems like unsuccessful embryo-maternal communication [26,27,28]. It has been stated, that aberrant gene expression either in the uterine endometrium [29] or in the embryo [29-32] is the major cause of pregnancy failure in cattle. Early embryogenesis depends on a tightly choreographed succession of gene expression patterns involved in different biological processes, which define normal development [33, 34]. Even a defect in a single gene is sufficient to cause implantation failure [35]. In this regard, all HSP40 family genes were found to be up-regulated in degenerate embryos versus blastocysts [36]. In addition, heat shock protein gene Hsp70.1 was up-regulated in blastocysts produced in vitro compared to in vivo embryos [37]. Identification of preimplantation gene regulation and functional analysis of key expressed molecular markers is crucial to understand and control important events ecompassed this period to improve farm animals' fertility. Therefore, this chapter aims to highlights research has been done in the molecular regulation of preimplantation development.

1.1. Approaches of large-scale expression analysis in bovine

The advent of high-throughput analyses of gene sequence and transcriptional regulation

has advanced our understanding of cellular and molecular activity of certain biological process. In particular, array technology is nowadays a powerful gene expression profile tool. Researchers have used gene-by-gene approach to identify gene expression before the availability of genome wide approaches such as microarray and next generation sequencing (NGS). Traditional polymerase chain reaction (PCR) has been performed to profile the expression of housekeeping genes during in vitro maturation of buffalo oocytes [38, 39]. While, quantitative real/time PCR was use to compare gene expression of key regulatory genes in bovine embryos produced either in vitro and in vivo [37]. Global approaches have been introduced to get deep insights into the molecular regulation of specific event during preimplantation development. For example, differential display and suppressive subtractive hybridization were used to define transcript abundance of genes associated with developmental competence of bovine oocytes [40]. cDNA array approach was done to identify genes differentially regulated during bovine oocyte maturation using human specific array [41]. Nevertheless, significant research efforts have been devoted to the development of cDNA resources in all major livestock species in the last few years.

Gene expression analysis using microarrays is a promising approach enabling global gene profiling to define the big picture of early embryo development. However, mRNA analysis in oocytes/embryos has to overcome many technical hurdles caused by the limited quantity of materials available and the biology of tissue studied [42]. Many universities and research institutes have tried to generate their own specific array as platform for global gene expression analysis. A large bovine microarray containing over 18,000 EST clones was developed [43]. Although this array covers a significant portion of the bovine genome, transcripts of oocyte origin may be under-represented, since the expressed sequence tags (ESTs) used for construction of this large array were derived from libraries of mixed tissue origin. A collaborative program named GINGER (Gene Index for Gene Expression profiling in Ruminants) has constructed a ruminant cDNA array with 1896 clones collected from non-normalised cDNA libraries of three tissues (muscle, embryo and mammary gland). This array was developed to be resource for gene expression profiling in ruminant tissues involved in reproduction and production (milk and beef) traits. Bovine cDNA array namely BlueChip containing ~2000 randomly selected clones was constructed from four different subtraction suppressive hybridizations (SSH) between bovine embryos and somatic tissues [42] is one of the bovine preimplantation specific array. A cDNA microarray with over 2000 randomly selected cDNA clones was generated from bovine oocyte library and was used to identify genes highly expressed in fetal ovary (an enriched source of oocytes) relative to adult spleen and liver tissues [44]. Investigation in gene expression at the level of the mRNA in mammalian reproductive tract during early embryonic development may help to identify genes, which are involved in embryo-maternal communications [45]. In the last few years both cDNA and oligonucleotide microarray technology have been successfully applied to study endometrial gene expression (reviewed in Giudice 2003). In order to, identify

embryo induced transcriptome changes in bovine endometrium a combination of SSH and cDNA microarray have been applied to compare the gene expression between uterine samples from pregnant and no pregnant heifers [46].

Functional genomics studies of oocyte competence was conducted using bovine cDNA array containing expressed sequence tags (ESTs) representing approximately 15 200 unique genes [47]. Affymetrix bovine-specific DNA microarray is the biggest available array (with >23,000 transcripts) was used for global transcription analysis in matured bovine oocytes and 8-cell embryos [48]. Global gene expression profiles of mouse and human preimplantation embryos from the GV oocyte to blastocyst stages have been established via microarray analyses using in vitro-transcribed antisense RNA as amplified target material [49]. In addition, cDNA microarrays contained 932 bovine EST clone inserts has identified a range of mRNA transcripts that are differentially expressed between bovine blastocysts derived from in vitro versus in vivo culture [50]. The transcriptome dynamics throughout preimplantation development o bovine was done using NGS to find out distinct cluster of gene regulators [51]. Furthermore, a multi-species cDNA microarray containing 3,456 transcripts from three distinct oocyte-libraries from bovine, mouse and Xenopus laevis were constructed to identify genes expressed in oocytes and conserved in these distant species [52]. Recently, the integrated interaction of ovarian miRNA and mRNA was performed in sheep to detect genes regulating prolificacy trait using NGS [53, 54].

2. Sperm RNA Population

Sperm is a differentiated cell, has a specific function which deliver the haplotype to the oocyte, it seems a simple mission, among species sperm phenotype shows a high degree of variation such as sperm size [55], studies documented that sperm not only delivers the DNA to egg but also complex RNA which it is difficult to explain [56,57]. During the replacement protamines with histones to compact DNA, a lot of changes occur in chromatin so RNA originates not from DNA transcription [56,58] and not dismiss from sperm formation process for two reasons, firstly RNA stored in sperm cell used as a substrate to activate RNA translation process [56], secondly RNA share during fertilization and embryo development [56,57,59].

The sperm has a large RNA population which is identified in many species including insects [56] and plant [60] and mainly localized in sperm head (Johnson et al. 2015) including messenger RNA (mRNA), micro- RNA (miRNA), interference RNA (iRNA), and antisense RNA [61]. and also can be characterized as coding RNAs, long non-coding RNAs (lncRNAs) and small non-coding RNAs (sncRNAs) [62] (Das et al. 2013; Jodar et al. 2013; 2015, Sendler et al. 2013; Pantano et al. 2015; Selvaraju et al. 2017; Zhang et al. 2017). Sperm RNA transcripts heat shock proteins, cytochrome P450 aromatase and wide range of receptors [61]. Large number of non-coding RNAs has potential biological functions (Jodar et al. 2013), whereas

a large number of sperm-specific non-coding RNAs have been identified, including intron retained regions and short expression regions (Jodar et al. 2013, Sendler et al. 2013, Selvaraju et al. 2017). Around 880 sperm lncRNAs seem conserves between human and mouse, however their functions are unknown (Zhang et al. 2017).

The RNA-sequencing technology (RNA-seq) is a useful tool in studying of spermatozoa ribosomal RNAs (rRNA) and has revealed that ribosomal RNAs (rRNAs) represent in from human, pig, stallion and bull sperms (around 80% of total RNA), but in mice the rRNAs represent only 30% of total RNA (Das et al. 2013; Jodar et al. 2013; Johnson et al. 2015; Selvaraju et al. 2017; Gòdia et al. 2018a). The detection of mature sperm RNA explains the role of coding RNA fermentation in transcriptional shutdown during this stage. The spermatozoon's intact transcripts selectively protected from degradation and it may have a vital role during spermatid maturation, fertilization and early embryogenesis (Sendler et al. 2013; Selvaraju et al. 2017).

2.1. Transcription and RNA storage during spermatogenesis

During spermatids maturation the absence of protein synthesis synchronize with a progressive decline in stored RNA. DNA transcription to RNA is also canceled in spermatids before and during the DNA condensation onto protamines [56], [58] because the DNA-protamine is condensed at least six times efficient than DNA-histone (minimization the volume of the nucleus) [63] and DNA toroid structures formation occur when protamine DNA becomes super condensed in later stage of spermatids formation. This toroid structure can be microscopically visualized approximately 50,000 base pairs of DNA [64] instead of the 146 base pairs of DNA for coiled twice [65] and linked to other toroids to form (DNase sensitive toroid) linked with region in which the DNA remains folding with histones [66]. The DNA toroids have not enough space to allow for RNA storage in opposite during spermatid chromatin condensation. The toroids links with the periphery of the nucleus and called (the peri-nuclear theca) [67]. These toroid structures protect the DNA from mutation, toxies and free radical [68]. The DNA amount is wrapped to protamines showed a different percentage for most mammalian species >98% of total DNA [69, 70], but for human sperm approximately 90% [68]. The super condensation minimize the volume leading to cellular process silencing and this is required for maintaining the sperm ability as a specialized cell deliver the haploid to the oocyte.

2.2. Biological roles of sperm molecules delivers to the oocyte

The microgamete sperm cell with delivers only the haploid genome to the oocyte which is called a macrogamete to contribute the cellular organelle required for embryogenesis as cytosol [71] and mitochondria because sperm structures are destroyed by the fertilized oocyte [72]. But it was observed that the meiosis II division activation in the fertilized oocyte [71] related to the sperm's centrosome (non-genomic) which is critical factor for embryo development in mammals not in rodents [73], [74] and prove that the sperm can deliver more than genomic

material [75].

Also sperm cell transports specific phosphor-lipase C-z to the oocyte, which is present in pre-nuclear theca of head and necessary for embryonic development activation [76], [77].

The increase of Ca2+ during fertilization induces the oocyte to complete meiosis and subsequently to begin in embryonic development. The signals required for oocytes calcium oscillations begin after fertilization and showed as PLC-z [76,78]. In addition, peri-nuclear theca proteins coding other molecules working as a signals involved in different protein kinase pathways but other transcriptional factors and structural proteins [79,80], activate the oocyte meiosis activation process and pronuclear development [57] need to be deeply studied. Some of sperm components are believed that slowly degrade such as mitochondria and others preserve until late embryonic stages [57,81].

2.3. RNA transfer to the oocyte and its functions

At early studies on sperm RNA, there was a belief that sperm RNA is non-functional and remain from the spermatid gene expression, on the other side, it was predicted that sperm RNA has a role after fertilization in formation of male pronucleus [82,83]. Recently among other cellular factors, is supposed that RNA affect on embryo development [84]. The first time that the three different types of sperm mRNA can be identified, the fertilizing sperm can transfer mRNA into the oocyte, which can be intact for at least 3 h post-fertilization. At least five sperm specific mRNA were recovered in oocytes post-fertilization, although they were absent in unfertilized oocyte [85].

2.4. mRNA without function

Some mRNA is specific and only expressed during spermatid differentiation such as mRNA encoding protamine-2 which expressed before, and during the DNA condensation phase when histones are replaced to protamine-2. Also protamine-2 role in chromatin coiling synchronizes with spermatid differentiation by encoding mRNA could be involved in differentiation process. But the protamine mRNA degradation is very rapid in fertilized ova explaining its deleterious expression after fertilization [86]. Other sperm cell mRNA may be residual from the last spermatid expression phase. Recently, there is a thought that the analysis of sperm mRNA composition may be an indicator for male fertility [87, 88]. A group of genes known as GA17, COX5B and TFAM mRNAs exist in large quantities in all individual human ejaculates [89] but they are non-functional and cannot use in translation or trans-membrane in mature sperm [90]. GA17 coding for a putative fusogenic protein that may be important for sperm–oocyte interaction [91,92] COX5B is a subunit of the terminal mitochondrial respiratory transport enzyme and the mitochondrial transcription factor A (TFAM) genes are coded in nuclear but located in mitochondria. COX5B and TFAM mRNA imported to the mitochondria

matrix can be translated by the ribosomes then their products will be exported to the cytosol but this suggestion is not correct because the last described phenomena have not been occurred in the mitochondria of any cell type [93]. A more suitable explanation would be the presences of some remnant cytosolic ribosomes responsible for detecting the mRNA translation. RNA is absent in the sperm mitochondria and in the mid-piece [82]. This study agree with the suggestion that sperm mitochondria proteins do not involved in transcription and translation [94].

2.5. Sperm-egg binding

The oocyte plasma membrane consists of two regions, a microvilli-free region and a microvilli-rich region. The sperm fuses the oocyte in the microvilli-rich region [95,96]. The interaction between the sperm and oocyte involves sperm cell-oocyte cell adhesion, followed by the fusion of two gametes membranes [97]. The inner acrosomal membrane of the sperm comes into the oocyte membrane [98] through the equatorial region [99,100].

Many molecules present in sperm and oocytes and are crucial for successful gamete binding as Fertilin α or ADAM1, fertilin β or ADAM2 and cyritestin or ADAM3. The role of ADAM1, ADAM2 and ADAM3 appear clearly in sperm oocyte binding [97]. Fertilin β is not essential for plasma membrane binding and fusion but poor adhesion to zona pellucida was observed in fertilin β and cyritestin knockout mice [101,102].

Oocytes integrins which found on the membrane surface are thought to be ADAMs sperm receptors. Some studies discovered that α6β1 integrin is an oocyte receptor for fertilin β [103,104] but other studies revealed that α9β1 integrin is a receptor for fertilin β [105,106]. CD46 express in rodents on the sperm acrosomal membrane [107] and maintain the membrane stability [108]. In human cells, CD 46 directly interacts with β1 integrin and indirectly with tetraspanins [109].

CD9 exists in oocytes membrane surface, one of tetraspanin protein family and is important for sperm-oocyte interactions [110]. The role of CD9 in sperm oocyte fusion has been detected in a numerous studies with CD9-null oocytes which the ability for strong sperm adhesion was reduced [111] the after fertilization reorganize microvilli distribution to enhance membrane block (Żyłkiewicz et al., 2010). CD9 deficiency in mice caused a reduction in fertility [112,113] also CD81 is another member, expressed on the surface of oocyte, its specific role is not fully studied until now but it interacts with CD9 [114]. CD81 gene silencing caused a reduction in fertility [115,116].

CRISP1 is a sperm protein expressed by the cumulus cells surrounding the oocyte, induces sperm direction through the modification of sperm hyper-activation and it may regulate Cat Sper function [117] and sperm chemo-attraction in female reproductive tract to help in

successful in-vivo insemination (Blasco, 2014).

IZUMO is a specific protein, expressed in sperm and essential for sperm-oocyte plasma membrane binding and fusion [118]. IZUMO interacts directly with some oolema molecules. Helical dimer of fragments of IZUMO N-terminus domain involved in sperm-oocyte fusion [119]. Dimers are formed by the connection between IZUMO and other proteins via its N-terminal domain [118]. Moreover, IZUMO considered a key role for organizing and stabilizing of protein-like complex which is crucial for membrane fusion. Also protein, angiotensin protein which has the ability to convert enzyme 3 (ACE3) on the sperm acrosomal cap, capable of interacting with IZUMO [120]. On the oocyte membrane, Juno belongs to the folate receptor family and recognizes the sperm IZUMO to facilitate fertilization. It has been demonstrated that mice lacking Juno on the surface of their egg cells are infertile female mice with lacking Juno failed in normal sperm fusion. The rapid absence of Juno from the oocyte membrane immediately after fertilization implies the essential role of shedding Juno in the fertilization process to prevent poly-spermy occurrence in mammals [121].

Trypsin-like acrosin and spermosin proteases are two different molecules involved in the first physical contact of the oocyte and sperm, it was suggested that aproteasome system participate in helping the sperm to penetrate the oocyte or in the process of sperm binding proteins [116]. Although sperm hyaluronidases are believed to be a limiting factor for fertilization in mammals, and sperm-specific SPAM1 and HYAL5 hyaluronidase have been suggested to paticipate in sperm-ZP binding in mice, the recent researches proved that hyaluronidases are not essential requirement for fertilization [122,123].

The oviduct tube which is the fertilization environment and secretions also play an essential role in the transport and interaction between male and female gametes. The lactoferrin expression in a human oviduct regulates polyspermy prevention process but in vitro inhibits gametes interaction (Yoon, 2014), also causes a modification in sperm function by decreasing sperm α-D-mannose binding sites and increasing the tyrosine phosphorylation of sperm proteins (Zumoffen, 2013).

3. Oocyte Transcriptomics

In the natural reproductive cycle, around 80% of the ovulated oocytes will be fertiliyed and developed to blastocyst [124]. A sharp drop in development of bovine embryo occurred when in vivo recovered oocytes are continue matured under in vitro condition compared with their counterparts that are matured in vivo [125,126]. It is supposed that COCs that are matured in vivo have accumulated all molecules such as RNA, proteins that are required for orchestrating early-cleaved embryos (Hyttel et al., 1997). During the initial cleavage divisions, embryonic development is supported by maternal mRNAs and proteins synthesized and stored during oogenesis [44]. Oocyte-expressed genes are not only important for follicular growth

and development but also are crucial for early embryogenesis however, our understanding of composition of the oocyte transcriptome and the identity of key oocyte-expressed genes with important regulatory roles in folliculogenesis and early embryonic development is far from complete [44]. In addition, investigation on the molecular characteristics of oocytes of poor developmental competence is critical to form a foundation for the development of future classification criteria for the selection of oocytes with superior developmental capacity [47]. Using oocyte-specific cDNA microarray, six genes were overexpressed in fetal ovary relative to [ribosomal protein L7a, dynein light chain, Doc2$_{\alpha}$, calmodulin, leucine-rich protein, and the novel gene clone (Begg20_H6)] [44]. Following cross-species hybridizations (bovine, mouse and Xenopus laevis), 268 transcripts were reported to be preferentially expressed in the oocyte of these three species [52]. In this study, transcripts of SMFN (Small fragment nuclease), Spin (Spindlin), and PRMT1 (Protein arginine methyltransferase 1) were identified in oocytes and conserved in three evolutionarily distant species. Recent report has evidenced that reduced transcript abundance for follistatin is associated with poor developmental competence of bovine oocyte [47].

Analysis of the relative abundance of transcripts during oocyte maturation will help to identify potential marker genes for bovine oocyte competence. Using heterologous human cDNA array, approximately 300 genes were expressed in the bovine oocyte, of which 70 were differentially expressed during meiotic maturation, the expressed genes were associated with cell cycle regulation (CCNB1 and CDC2), DNA transcription (TIF1 and GTF2H) and apoptosis regulation (DAD1, CASP4, FASTK and BCL2L1) [41]. In array study conducted by [48], has showed that genes controlling DNA methylation (DNMT1 and DNMT2), transport (IGF2R, VDP and ATP2B1) and metabolism (HSD11B2, MUT, SLC3A2 and PLCG1) were up-regulated in matured oocytes compared to 8-cell stage embryos. Many genes (polyA, CPSF) and CPEB) related to cytoplasmic polyadenylation element (CPE)-dependent polyadenylation complex machinery were found to be expressed in bovine oocytes pre- and post-resumption of meiotic maturation. Furthermore, the differential expression of the majority of these genes further underlines the tight temporal control of protein synthesis required for oocyte maturation and in preparation of subsequent fertilization and early embryo development [127].

Previous studies have shown that development of early embryos to the blastocyst stage was greater when oocytes were obtained during follicular growth/stagnation phase (G/S) than in the dominance/regression phase (D/R) [58,128,125, 2,129,130]. The dominant follicle exerts a direct inhibitory effect on the development of subordinate follicles in cattle [131]. causing them to undergo atresia [132], which may lead to lower in vitro developmental competence compared to their counterparts at growth phase (as measured by blastocyst rate) [128]. Moreover, blastocysts derived from oocytes collected from both medium and small follicles at growth/stagnation G/S stage or dominance/regression D/R stage were reported to be different

in relative abundance of developmentally related transcripts (Cx43) [133]. However, the molecular properties of these oocytes in bovine have not yet been investigated. Therefore, in one of our studies we have compared the transcript abundance of bovine oocytes retrieved from small follicles at growth and dominance phases of the first follicular wave using custom cDNA microarray. Comparative gene expression analysis of oocytes from growth and dominance phases and subsequent data analysis using Significant Analysis of Microarray (SAM) revealed a total of 51 genes to be differentially regulated. Hierarchical clustering and heatmap was performed to show the general and magnitude expression of differentially regulated genes **(Figure 1)**. Furthermore, gene ontology (GO) has classified expressed transcripts functionally into different clusters based on their molecular functions **(Figure 2)**. Accordingly, differentially regulated genes were found to represent transcripts with known function [70% (36/51)], with unknown function [12% (6/51)] and novel transcripts [18% (9/51)] **(Figure 2)**. Transcripts with known function showed to be involved in protein biosynthesis (18%), transcription (10%), cytoskeleton (8%), cell cycle (8%), NADH dehydrogenase activity (4%), calcium ion binding (4%), nucleotide binding (4%) and other molecular functions (10%) **(Figure 2)**. Quantitative real-time PCR has confirmed the expression profile of 80% (8/10) in independent oocyte populations from both growth and dominance phases to be in the same trend of microarray data **(Figure 3).** The reported differences in developmental capacity of bovine oocytes derived from small follicles at growth and dominance phases of follicular development are also accompanied by differences in the relative abundance of transcripts related to the various molecular events and processes governing oocyte growth and follicular development.

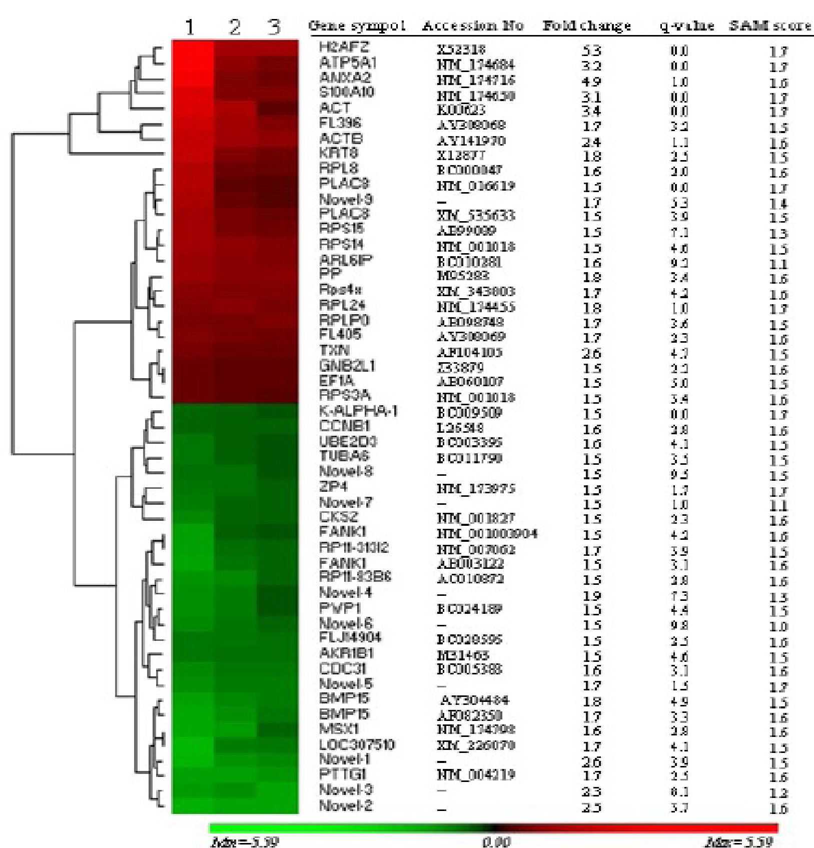

Figure 1: Hierarchical clustering and heatmap of 51 differentially expressed genes. The red blocks represent up-regulated genes while the green blocks represent down-regulated genes in oocytes recovered at growth phase.

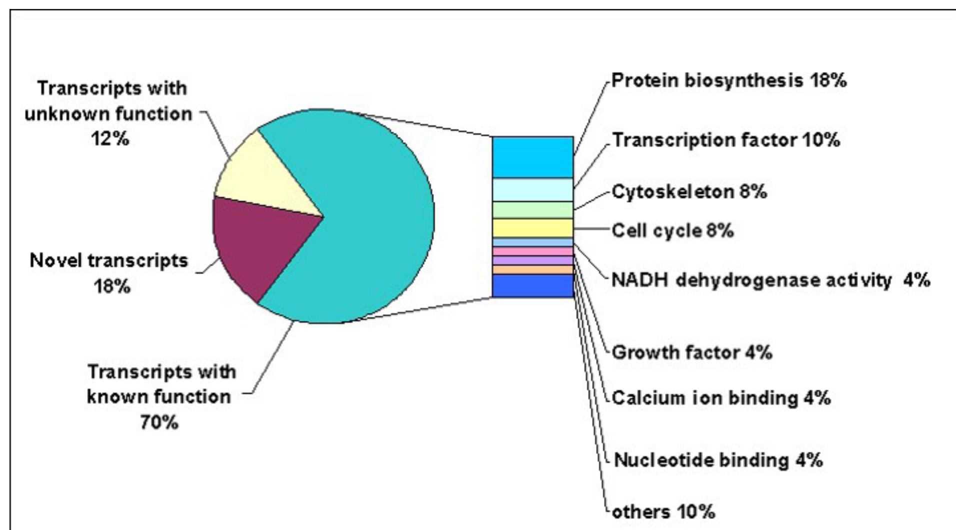

Figure 2: Differentially expressed genes as classified based on the Gene Ontology Consortium classifications (http://www.geneontology.org).

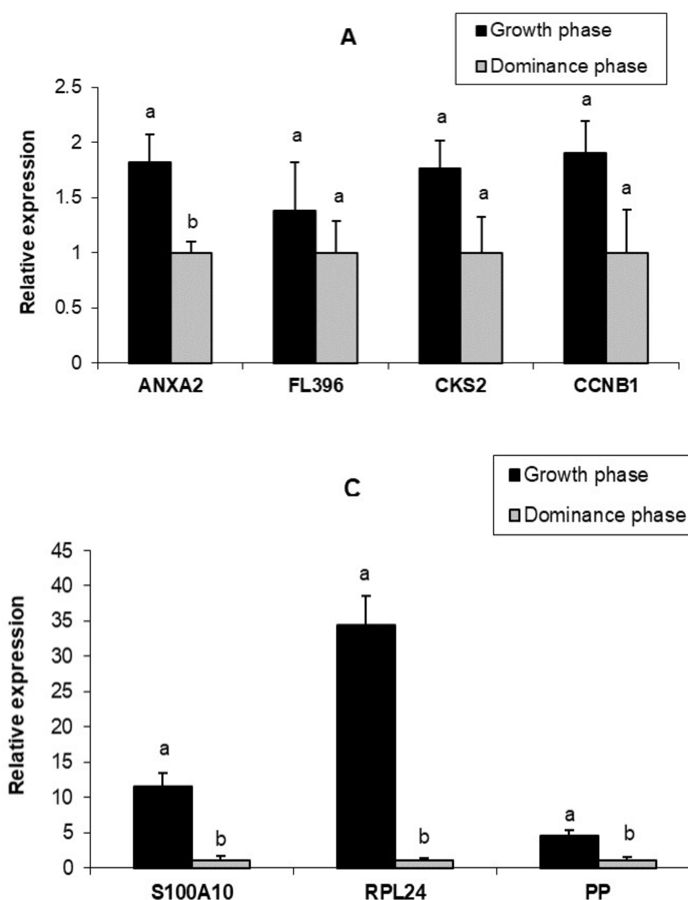

Figure 3: Quantitative real-time PCR validation of 10 differentially expressed genes in bovine oocytes recovered at growth phase (Day 3) vs. dominance phase (Day 7) as identified by microarray analysis (A, B, C). The relative abundance of mRNA levels represents the amount of mRNA compared to the calibrator (with the lowest normalized value). Bars with different superscripts (a, b) are significantly different at $P < 0.05$.

3.1. Transcriptome profile of cumulus cells

It is well established that integrated bilateral communication between the cumulus cells and its enclosed oocyte is key biological factor in acquisition of developmental competence to support preimplantation embryogenesis. Therefore, the molecular messages stored in cumulus cells are possible indicators of the further developmental fate of the oocytes until term.

Several studies have been conducted using farm animals and human granulosa cells to identify genomic signature(s) as molecular markers of developmental potential of oocyte to support embryogenesis and to establish pregnancy [134-136]. Suppressive subtractive hybridization combined with microarray were used to identify several potential cumulus cell markers of bovine COCs quality that includes several growth differentiation factor GDF9 downstream target genes like GREM1, HAS2, PTGS2, and TNFAIP6 [134]. In addition, epidermal growth factor receptor (EGFR), inhibin beta a (INHBA), betacellulin (BTC) and CD44 molecule was among candidate molecular markers that induce good potential of COCs. In another study, the transcript abundance of genes controlling of cumulus expansion process such as TNFAIP6 and nuclear maturation of oocyte like INHBA and FST were up-regulated in bovine in these cells as potential predictors of COCs quality when matured under *in vivo* [136]. A study using prepubertal bovine calf oocytes as a model of poor oocyte competence; microarray analysis detected genes encoding the cathepsin family of cysteine proteinases (CTSB, CTSS and CTSZ) are linked with reduced competence of bovine oocytes [135]. In search of potential markers of human granulosa cells, a study done by Feuerstein et al., (2007) has identified steroidogenic acute protein (STAR), prostaglandin-endoperoxide synthase 2 (PTGS2), stearoyl-co-enzyme A desaturase 1 and 5 (SCD1 and SCD5) and amphiregulin (AREG) are crucial regulators of nuclear maturation and their profile are increased after meiosis resumption. Noteworthy, reduced expression of connexin 43 (CX43) in cumulus is a good marker for nuclear maturation and further embryonic development upto blastocysts (Feuerstein et al. 2007).

3.2. Changes in gene expression during cleavage stages

In in vitro derived bovine embryos, estimates of total RNA content indicate that it declines from the mature oocyte to the morula stage, only to increase again at the blastocyst stage [137]. These estimates are based on Northern blot hybridization using probes for 28S and 18S rRNA, with the abundance of 5S rRNA following a similar pattern [137]. This pattern of RNA loss and reaccumulation mimics the patterns observed in the mouse although in the mouse the increase occurs by the 8- to 16-cell stage likely owing to the earlier onset of zygotic gene activation in this species (Piko and Clegg, 1982). In both species, the timing and the increase in abundance of specific mRNA transcripts occurs in a transcript specific manner. Examples of changes in steady state levels of various

During early development, the embryonic genome is inactive and the embryo relies on maternal messenger ribonucleic acid (mRNA) for protein synthesis (Thelie et al. 2009). The recruitment mechanisms by which dormant RNA is either targeted for translation or decay are still largely uncharacterized. The current model involves lengthening of the poly(A) tail, which triggers binding of the poly(A) binding protein and binding of translation initiation factors (Memili and First 2000; Groisman et al. 2002). RNA concentration is highest in the germinal vesicle stage oocyte and from then until the 8-cell stage, RNA is gradually depleted (Gilbert

et al. 2009; Vallée et al. 2009). Evidence in the mouse suggests that this decline is important for activation of the embryonic genome (Li et al. 2010). Depletion of maternal argonaute 2 (Ago2), which encodes a catalytic RNA hydrolase (RNase), disrupts gene expression and the 2 cell embryo fails to become a blastocyst (Li et al. 2010). In the bovine, embryonic genome activation (EGA) occurs at the 8 to 16-cell stage (Memili and First 2000) through an unknown mechanism.

3.3. Embryo transcriptomics

In mammals, maternally inherited transcripts stored within the oocyte regulate the earliest stages of embryogenesis. After fertilization, the embryonic genome becomes transcriptionally active and the expression some of embryonic genes begins in a stage-specific manner, which contribute to the early development process [137]. In cattle, global embryonic genome activation (EGA) begins during the 8-cell to 16-cell stage and it is the most critical event in early embryonic development. However, the identities of embryonic genes expressed and the mechanism(s) of EGA are poorly defined in the bovine. In addition, understanding of EGA will contribute to our understanding of nuclear reprogramming in somatic cell nuclear transfer experiments. Transcripts expressed at the 8-cell stage (EGA) include regulators of different molecular functions like transcription (NFY and USF2), cell adhesion (DSC2 and COL12A1), signal transducers (PTGER4) and transporter (CRABP1), metabolism (NEU3) and immune response (CXCL6) [48].

Accurate assessment of embryo viability is crucial for successful establishment and maintenance of pregnancy following embryo transfer. Evaluation of embryo quality is in particular of high impact for in vitro-produced embryos as these embryos differ in many aspects from their in vivo derived counterparts. The temporal or spatial and qualitative or quantitative shifts in the well-orchestrated expression patterns of developmentally important genes have been investigated in preimplantation bovine embryos following in vitro embryo manipulation. So far, studies in bovine embryos indicate that many of the differences in quality of in vitro- and in vivo-derived blastocysts can be related to culture environment-induced changes in mRNA abundance. The post-fertilization embryo culture environment has a dramatic effect on the pattern of gene expression in embryos, which in turn has serious implications for the normality of blastocyst development [138, 139]. This is the case, not only when one compares in vitro and in vivo culture systems, but also comparing different in vitro culture systems [140]. Recently, it was reported that in vitro-cultured embryos showed down-regulation of genes that are involved in transcription and translation (CCR4-NOT, EEF1G, PABPN1, FOXO3A, HMG2, GNB2L1 and DOT1L) events compared with in vivo counterparts, suggesting that in vitro-derived embryos are of inferior quality compared with in vivo-derived embryos due to a deficiency of the machinery associated with transcription and translation [50].

Imprinted genes appear to be more susceptible to alterations in epigenetic modifications [141]. especially after IVP of ovine [142] or bovine embryos [143]. Significant differences in the expression of non-imprinted genes have also been reported in bovine IVP and somatic nuclear transferred (sNT) derived embryos compared to their in vivo counterparts [144]. Differences in gene expression patterns between IVP and sNT-derived embryos and their in vivo counterparts may originate from all steps of the manipulation procedures, including in vitro maturation, in vitro fertilization, and in vitro culture and, for sNT, from different somatic nuclear transfer protocols [145]. In a recent study, it was found that there is a reduced levels of many genes required for the viability of every cell in the nuclear transferred (NT) embryo's cellular machinery [146]. In this study, transcripts of mitochondria (12S and 16S rRNA and cytochrome C oxidase I), cytoskeletal (TUBB, TPX2, KRT18, TMSB4X and ACTB), protein biosynthesis (EEF1A1, EFG2, BWZ1 and EIF5A) and ribosomal proteins (RPL4, RPL5, RPL21 and RPS20), protein binding/folding (GORASP2, HSPA9B, HSPA5 and LGALS3) and etabolism/biosynthesis (ADH5, PTGS2, SCP2, ACSL3, NDUFA1) were decreased in NT compared to in vitro produced blastocysts.

There is a clear relationship between altered transcript abundance patterns and some aspects of embryo quality (i.e. cryotolerance), which render the embryo capable o f establishing a pregnancy, if transferred fresh, but incapable of withstanding cryopreservation [147], [25]. Various studies in both mice and bovine have shown that the in vitro production of embryos under specific culture environments resulted in not only altered gene expression of transcripts related to metabolic and growth but also altered conceptus and fetal development following transfer [148,149]. Despite the fact that data on transcriptional analysis of transferable blastocysts of various origins have been accumulated, so far no direct connection of gene expression and developmental competence has been established. However, a well-established biopsy technique is needed to obtain cells from embryos before transfer without any lethal effect on the embryo during further development. For this, one study conducted in our laboratory to establish connection between transcriptional profile of embryos and the pregnancy success based on gene expression analysis of blastocyst biopsies taken prior to transfer to recipients [150]. Microarray data analysis revealed a total of 52 and 58 genes **(Figure 4 and 5)** were differentially regulated during comparison between embryo biopsies resulting in no pregnancy (G1) vs. calf delivery (G3) and those resulting in resorbed embryos (G2) vs. calf delivery (G3). Ontological classification has showed different functional clusters of genes **(Figure 6 and 7)**. In addition, differentially regulated genes represent genes with known functions (77%), ESTs (11.5%), and novel transcripts (11.5%) (**Figure 4 and 5**). Biopsies resulted in calf delivery were enriched with genes necessary for implantation (COX2 and CDX2), carbohydrate metabolism (ALOX15), growth factor (BMP15), signal transduction (PLAU), and placenta-specific 8 (PLAC8). Biopsies from embryos resulting in resorption are enriched with transcripts involved protein phosphorylation (KRT8), plasma membrane (OCLN), and glucose metabolism (PGK1

and AKR1B1). Biopsies from embryos resulting in no pregnancy are enriched with transcripts involved inflammatory cytokines (TNF), protein amino acid binding (EEF1A1), transcription factors (MSX1, PTTG1), glucose metabolism (PGK1, AKR1B1), and CD9, which is an inhibitor of implantation. The expression of those unknown and novel ESTs showed profiles similar to those of the annotated genes, as determined by tree hierarchical clustering analyses **(Figure 4 and 5)**. Quantitative real-time PCR has validated the expression of 87% (13/15) of the genes generated from the array hybridization. Thus, several genes identified in this experiment may be associated with embryo loss or survival in blastocysts during preimplantation period.

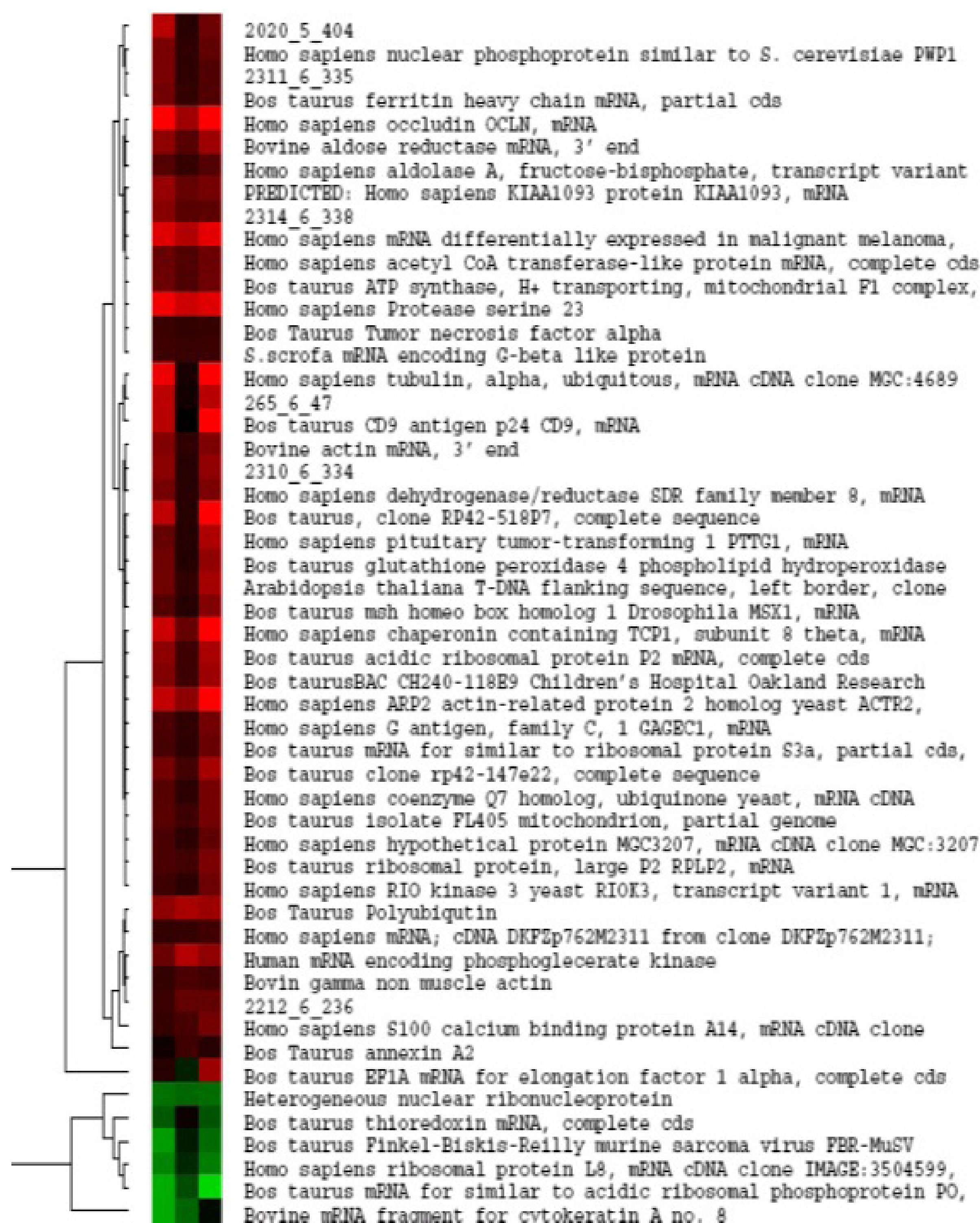

Figure 4: Hierarchical clustering for the differentially expressed genes between biopsies derived from blastocysts resulted in no pregnancy (G1) and calf delivery (G3). The columns represent the replicates. The rows represent 52 genes found to be differentially regulated between G1 and G3.

Bos taurus mRNA for similar to translationally controlled tumor
Bovine aldose reductase mRNA, 3' end
Bos taurus ribosomal protein, large P2 RPLP2, mRNA
Sus scrofa G-beta like protein GNB2L1, mRNA
Homo sapiens dehydrogenase/reductase SDR family member 8, mRNA
Human mRNA encoding phosphoglcerate kinase
Homo sapiens heat shock 60kDa protein 1 chaperonin, mRNA cDNA
Homo sapiensProtease serine 23
Heterogeneous nuclear ribonucleoprotein A1
Bos taurus polyubiqutin
Homo sapiens proteasome prosome, macropain 26S subunit, non-ATPase,
Homo sapiens DEAD Asp-Glu-Ala-Asp box polypeptide 5 DDX5, mRNA
Homo sapiens acetyl CoA transferase-like protein mRNA, complete cds
Bos taurus clone RP42-351K5, complete sequence
Homo sapiens ribosomal protein S5 RPS5, mRNA
Homo sapiens stromal cell-derived factor 2, mRNA cDNA clone
Bos taurus ATP synthase, H+ transporting, mitochondrial F1 complex,
Bos taurus mRNA for similar to ribosomal protein S14, partial cds,
Bovine mRNA fragment for cytokeratin A no. 8
Bos taurus annexin A2 ANXA2, mRNA
Homo sapiens chloride intracellular channel 1 CLIC1, mRNA
Bos taurus mRNA for similar to ribosomal protein S12, partial cds,
Homo sapiens ARP2 actin-related protein 2 homolog yeast ACTR2,
Bovine actin mRNA, 5' end
Homo sapiens occludin OCLN, mRNA
Homo sapiens membrane interacting protein of RGS16, mRNA cDNA clone
Homo sapiens actin, gamma 1 ACTG1, mRNA
Bos taurus acidic ribosomal phosphoprotein PO mRNA, partial cds
Homo sapiens proteasome prosome, macropain 26S subunit, non-ATPase,
Bos taurus isolate FL396 mitochondrion, partial genome
Homo sapiens coenzyme Q7 homolog, ubiquinone yeast, mRNA cDNA
B.taurus mRNA, alternative polyadenylation signals
Homo sapiens ribosomal protein S15 RPS15, mRNA
Bos taurus isolate 65 NADH dehydrogenase subunit 1 ND1, NADH
Primery structure of bovine 1.715 satelite DANN
Bos taurus mRNA for similar to ribosomal protein L26, partial cds,
Homo sapiens tubulin, alpha, ubiquitous, mRNA cDNA clone MGC:4689
Homo sapiens Hypothetical protein FLJ23320
1630_4_420
Bos taurus homeo box B7
Bovine mRNA for histone H2A.Z
Homo sapiens nucleoplasmin 2 variant 2 NPM2 mRNA, complete cds
Homo sapiens testes development-related NYD-SP20 NYD-SP20, mRNA
Homo sapiens placenta-specific 8 PLAC8, mRNA
Homo sapeins caudal type homeo box transcription factor 2
Bos taurus BTAB2MDS3 beta-2-microglobulin B2M gene, 3'UTR
Bos taurus bone morphogenetic protein 15 mRNA, partial cds
Homo sapiens LSM5 homolog, U6 small nuclear RNA associated S.
2271_6_295
1131_1_1020
Homo sapiens chromosome 5 clone RP11-425E13, complete sequence
Bos taurus thioredoxin mRNA, complete cds
Bos taurus ornithine decarboxylase ODC1, mRNA
Bos taurus arachidonate 15-lipoxygenase ALOX15, mRNA
Bos taurus urokinase-type plasminogen activator PLAU mRNA, complete
Homo sapiens RAN, member RAS oncogene family, mRNA cDNA clone
Sus scrofa mRNA for destrin, complete cds
Bos taurus prostaglandin G/H synthase-2 PGHS-2 mRNA, complete cds

Figure 5: Results of hierarchical clustering for the differentially expressed genes between biopsies derived from blastocysts resulted in resorption (G2) and calf delivery (G3) identified by microarray analysis. The columns represent the replicates.The rows represent 58 genes found to be differentially regulated between the two groups of biopsies.

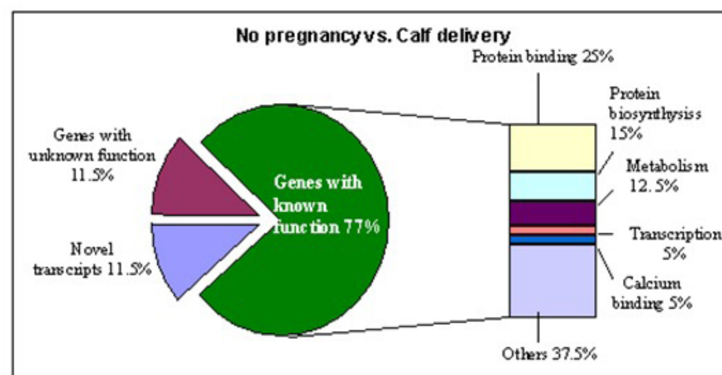

Figure 6: Ontology classification for differentially expressed transcripts between biopsies derived from blastocysts resulted n no pregnancy and calf delivery. The known genes were classified functionally based on the Gene Ontology Consortium classification (http://www.geneontology.org)

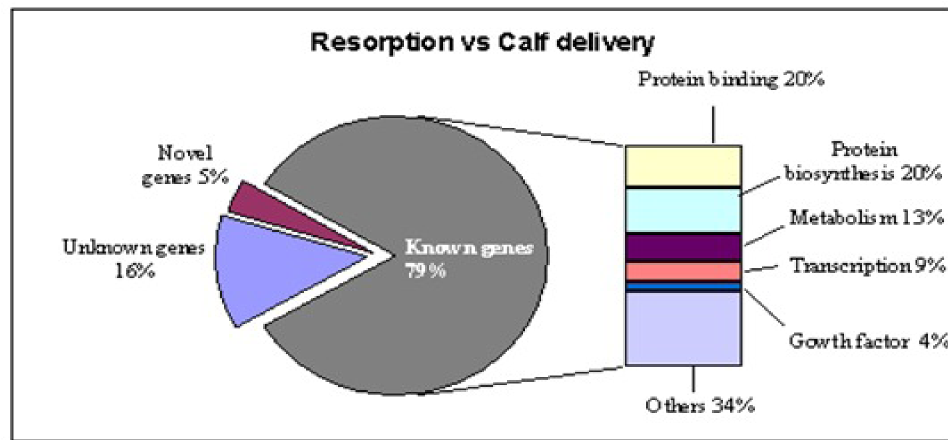

Figure 7: Ontology classification for differentially expressed transcripts between biopsies derived from blastocysts resulted in resorption and calf delivery. The known genes were classified functionally based on the Gene Ontology Consortium classification (http://www.geneontology.org).

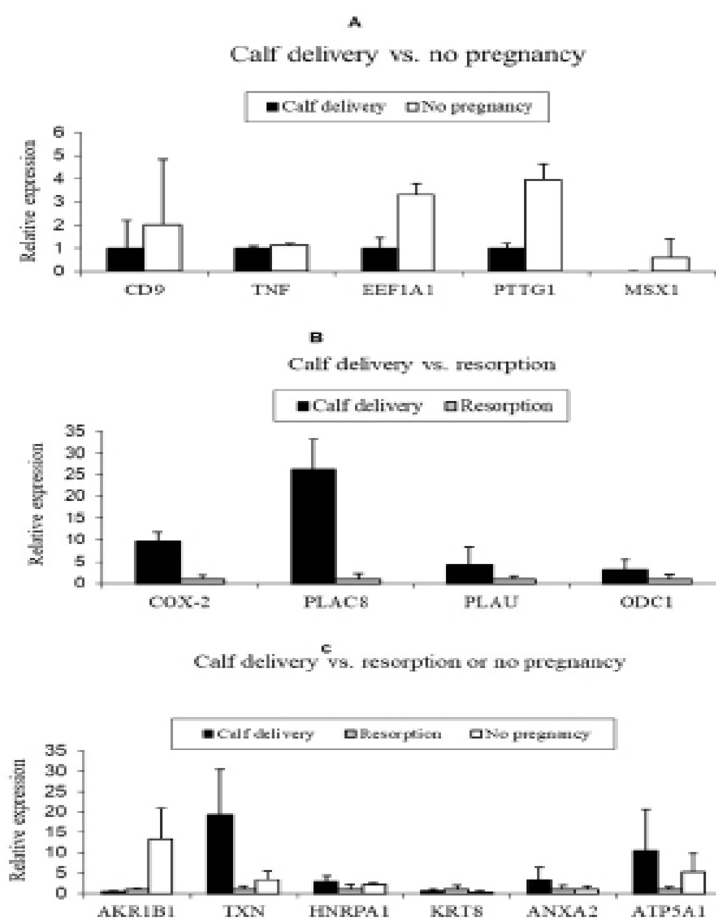

Figure 8: Quantitative real time PCR confirmation of selected transcripts between biopsies from blastocysts resulted in calf delivery versus no pregnancy (A) and calf delivery versus resorption group (B) and those resulted in calf delivery versus resorption or no pregnancy (C).

4. Characterization and Functional Analysis of Candidate Genes

To correlate transcript level with corresponding protein level and to elucidate embryonic cellular function, detailed characterisation is necessary at the protein level. Oogenesin gene is expressed during oogenesis and early embryogenesis in mouse and its protein is synthesised from the oocyte to four-cell embryo stages, suggesting a possible role during oocyte maturation and/or embryonic genome activation [151].

In one of our studies, MSX1 was selected as candidate gene for protein localization in early embryonic development and throughout follicular turnover. MSX1 protein was found to be localized at higher levels in the oocytes cytoplasm than in the surrounding cumulus cells **(Figure 9d, h, p)** or other cellular layers of the growing follicle **(Figure 9b, f, n)** at all stages of follicular development except at growth phase **(Figure 7j, l).**

The MSX1 protein was found to be dispersed in the cytoplasm of immature and matured oocytes and early zygote stages **(Figure 10a, b, c)** but tends to be localized around the nucleus at advanced zygote, 2-cell, 4-cell and 8-cell cleavage stage embryos **(Figure 10d, e, f, g)**. Comparative analysis of protein signals between oocytes showed that fluorescence signals were reduced after maturation **(Figure 10b).** Moreover, the in situ hybridization experiment results showed that MSX1 mRNA was localized in the oocytes, cumulus cells and follicular wall during the periods of follicular turnover under investigation **(Figure 11).**

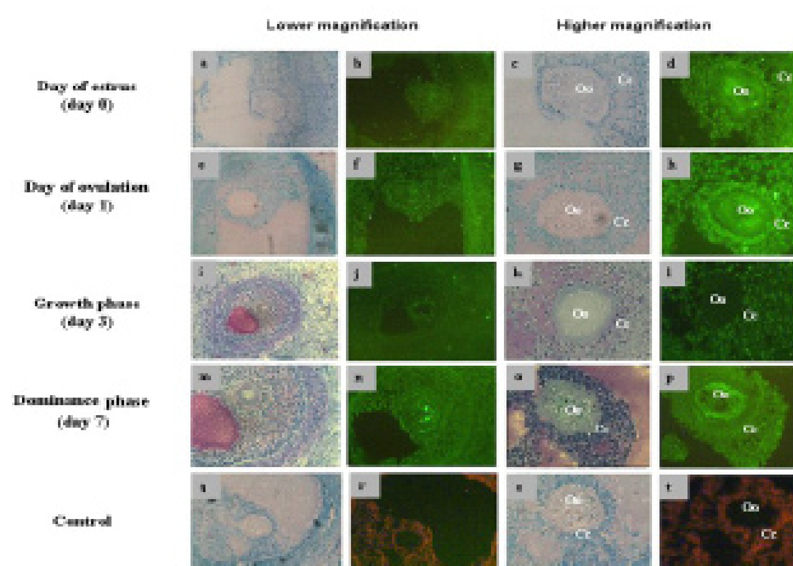

Figure 9: Immunohistochemical localisation of MSX1 protein in bovine ovarian sections at day of estrus (b, d), day of ovulation (f, h), growth phase (j, l), dominance phase (m, q). Cumulus cells are marked with Cc and oocytes are marked with Oo. Negative controls were processed without addition of primary anti-MSX1 antibody (r, t). Sections were counterstained with toluidine blue (a, c, e, g, i, k, m, o, q and s). Images from the same ovarian sections were captured with lower and higher magnification.

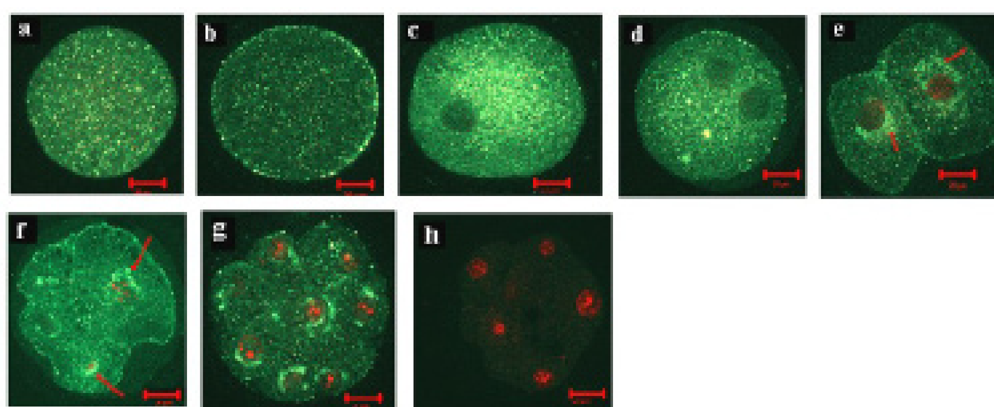

Figure 10: Subcellular localization of bovine MSX1 protein in bovine oocytes and early cleavage embryonic stages [immature oocyte (a), matured oocyte (b), zygote (c), advanced zygote (d), 2-cell (e), 4-cell (f) and 8-cell (g)]. Negative control (h) was processed without addition of primary anti-MSX1 antibody. Nuclei are stained with propidium iodide (red). Scale bars represent 20 µm.

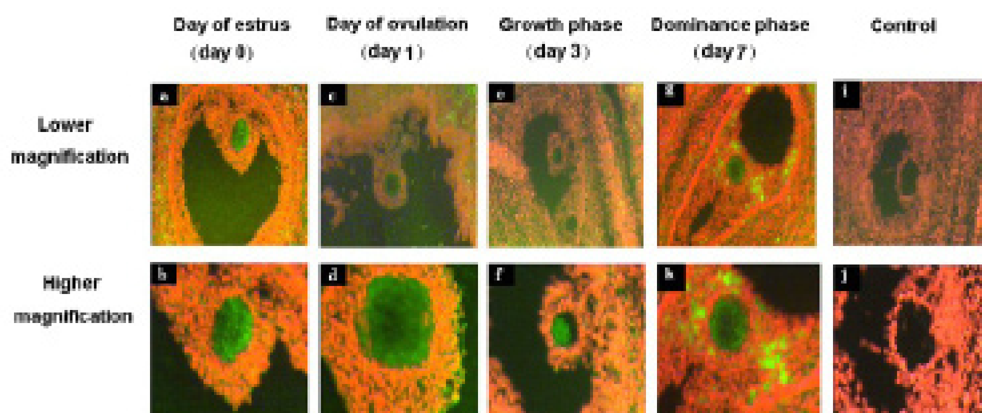

Figure 11: Fluorescent in situ hybridization of MSX1 mRNA conducted with DIG labelled RNA antisense probe in bovine ovarian sections at day of estrus (A, B), day of ovulation (B, C), growth phase (D, E), dominance phase (F, G). Cytoplasms of the oocytes (Oo) are darkly stained with green fluorescent compared to cumulus cells (Cc). Negative controls were hybridized with DIG labelled RNA sense probe (H, I). Images from the same ovarian sections were captured with lower and higher magnification.

5. References

1. Le Naour F, Rubinstein E, Jasmin C, Prenant M and Boucheix C: Severely reduced female fertility in CD9-deficient mice. Science 287: 319-321, 2000.

2. Leibfried-Rutledge ML (1999): Factors determining competence of in vitro produced cattle embryos. Theriogenology 51, 473-485.

3. Flesch FM, Gadella BM. Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochim Biophys Acta 2000; 1469: 197–235.

4. Kadivar A, Heidari Khoei H, Hassanpour H, Ghanaei H, Golestanfar A, Mehraban H et al. Peroxisome proliferator-activated receptors (PPARα, PPARγ and PPARβ/δ) gene expression profile on ram spermatozoa and their relation to the sperm motility. Vet Res Forum 2016; 7: 27-34.

5. Kim KU, Pang WK, Kang S, Ryu DY, Song WH, Rahman MS et al. Sperm solute carrier family 9 regulator 1 is correlated with boar fertility. Theriogenology 2019; 126:254-60.

6. Parthipan S, Selvaraju S, Somashekar L, Arangasamy A, Sivaram M, Ravindra JP. Spermatozoal transcripts expression levels are predictive of semen quality and conception rate in bulls (Bos taurus). Theriogenology, 2017; 98:41-49.

7. Bissonnette N, Lévesque-Sergerie JP, Thibault C, Boissonneault G. Spermatozoal transcriptome profiling for bull sperm motility: a potential tool to evaluate semen quality. Reproduction 2009;138: 65–80.

8. Boerke A, Dieleman SJ, Gadella BM. A possible role for spermatozoa RNA in early embryo development. Theriogenology 2007; 68:147–55.

9. Macmillan KL, Lean IJ, Westwood CT (1996): The effects of lactation on the fertility of

10. Lucy, MC (2001): Reproductive loss in high-producing dairy cattle: Where will it end?.J Dairy Sci. 2001 Jun;84(6):1277-93.

11. Marai, I.F.M and Haeeb A.A.M. (2010). Buffalo's biological functions as affected by heat stress - a review. Livest. Sci.,(127):89 - 109.

12. Al-Katanani YM, Paula-Lopes FF, Hansen PJ. Effect of Season and Exposure to Heat Stress on Oocyte Competence in Holstein Cows J Dairy Sci. 2002; 85(2):390-6.

13. Gendelman M, Roth Z. Seasonal effect on germinal vesicle-stage bovine oocytes is further expressed by alterations

in transcript levels in the developing embryos associated with reduced developmental competence. Biol Reprod. 2012 Jan 16;86(1):1-9.

14. Sadeesh EM, Sikka P, Balhara AK, Balhara S. Developmental Competence and Expression Profile of Genes in Buffalo (Bubalus Bubalis) Oocytes and Embryos Collected Under Different Environmental Stress. Cytotechnology. 2016; 68(6):2271-2285.

15. Abdoon AS, Gabler C, Holder C, Kandil OM, Einspanier R.Seasonal variations in developmental competence and relative abundance of gene transcripts in buffalo (Bubalus bubalis) oocytes. Theriogenology. 2014 Nov;82(8):1055-67.

16. Maya-Soriano MJ, López-Gatius F, Andreu-Vázquez C, López-Béjar M. Bovine Oocytes Show a Higher Tolerance to Heat Shock in the Warm Compared With the Cold Season of the Year. Theriogenology. 2013; 79(2):299-305.

17. Edwards JL, Hansen PJ. Differential responses of bovine oocytes and preimplantation embryos to heat shock. Mol Reprod Dev. 1997; 46(2):138-45.

18. Nabenishi H, Ohta H, Nishimoto T, Morita T, Ashizawa K, Tsuzuki Y. The effects of cysteine addition during in vitro maturation on the developmental competence, ROS, GSH and apoptosis level of bovine oocytes exposed to heat stress. Zygote. 2012; 20(3):249-59.

19. Armon L, Ben-Ami I, Ron-El R and Eisenbach M: Human oocyte-derived sperm chemoattractant is a hydrophobic molecule associated with a carrier protein. Fertil Steril 102: 885-890, 2014.

20. Yadav A, Singh KP, Singh MK, Saini N, Palta P, Manik RS, Singla SK, Upadhyay RC, Chauhan MS. Effect of physiologically relevant heat shock on development, apoptosis and expression of some genes in buffalo (Bubalus bubalis) embryos produced in vitro. Reprod Domest Anim. 2013 Oct;48(5):858-65.

21. Snijders SEM, Dillon P, O'Callaghan D, Boland MP (2000): Effect of genetic merit,

22. Donnison M, Pfeffer PL (2004): Isolation of genes associated with developmentally competent bovine oocytes and quantitation of their levels during development. Biol Reprod 71, 1813-1821.

23. Ghanem N, Hölker M, Rings F, Jennen D, Tholen E, Sirard MA, Torner H, Kanitz W, Schellander K, Tesfaye D, 2007: Alterations in transcript abundance of bovine oocytes recovered at growth and dominance phases of the first follicular wave. BMC Dev Biol 7, 90.

24. Rizos D, Ward F, Duffy P, Boland MP, Lonergan P (2002): Consequences of bovine

25. Rizos D, Gutiérrez-Adán A, Pérez-Garnelo S, de la Fuente J, Boland MP, Lonergan P (2003): Bovine embryo culture in the presence or absence of serum: implications for blastocyst development, cryotolerance, and messenger RNA expression. Biol Reprod 68, 236-243.

26. Rodriguez-Zas S, Schellander K, Lewin H, 2008: Biological interpretations of transcriptomic profiles in mammalian oocytes and embryos. Reproduction 135, 129-39.

27. Morris RL, Brown HM, Wright BD, Sharp DJ, Sullivan W, Scholey JM, 2001: Microinjection methods for analyzing the functions of kinesins in early embryos. Methods Mol Biol 164,163-172.

28. Geisert RD, Morgan GL, Short EC Jr, Zavy MT, 1992: Endocrine events associated with endometrial function and conceptus development in cattle. Reprod Fertil Dev 4, 301-305.

29. Salilew-Wondim D, Hölker M, Rings F, Ghanem N, Ulas-Cinar M, Peippo J, Tholen E, Looft C, Schellander K, Tesfaye D, 2010: Bovine pretransfer endometrium and embryo transcriptome fingerprints as predictors of pregnancy success after embryo transfer. Physiol Genomics 42, 201-218.

30. El-Sayed A, Hoelker M, Rings F, Salilew D, Jennen D, Tholen E, Sirard M-A, Schellander K, Tesfaye D, 2006: Large-scale transcriptional analysis of bovine embryo biopsies in relation to pregnance success after transfer to recipi-

ents. Physiol. Genomics 28, 84-96.

31. Gad A, Besenfelder U, Rings F, Ghanem N, Salilew-Wondim D, Hossain MM, Tesfaye D, Lonergan P, Becker A, Cinar U, Schellander K, Havlicek V, Hölker M, 2011: Effect of reproductive tract environment following controlled ovarian hyperstimulation treatment on embryo development and global transcriptome profile of blastocysts: implications for animal breeding and human assisted reproduction. Hum Reprod 26,1693-1707.

32. Ghanem N, Salilew-Wondim D, Gad A, Tesfaye D, Phatsara C, Tholen E, Looft C, Schellander K, Hoelker M, 2011: Bovine blastocysts with developmental competence to term share similar expression of developmentally important genes although derived from different culture environments. Reproduction 142, 551-564.

33. Niemann H, Carnwath J, Kues W, 2007: Application of DNA array technology to mammalian embryos. Theriogenology 68, S165-177.

34. Ruddock-D'Cruz NT, Hall VJ, Tecirlioglu RT, French AJ, 2007: Gene expression analysis of single preimplantation bovine embryos and the consequence for developmental potential. Soc Reprod Fertil Suppl 64, 341-363.

35. Copp AJ (1995): Death before birth: clues from gene knockouts and mutations. Trends Genet 11, 87-93.

36. Zhang B, Peñagaricano F, Driver A, Chen H, Khatib H, 2011: Differential expression of heat shock protein genes and their splice variants in bovine preimplantation embryos. J Dairy Sci 94, 4174-4182.

37. Niemann H, and Wrenzycki C, 2000: Alterations of expression of developmentally important genes in preimplantation bovine embryos by in vitro culture conditions: implications for subsequent development. Theriogenology 53, 21- 34.

38. Aswal APS, Raghav S, De S, Thakur M, Goswami SL, Datta TK, 2007a: Expression stability of two housekeeping genes (18S rRNA and G3PDH) during in vitro maturation of follicular oocytes in buffalo (Bubalus bubalis). Anim Reprod Sci 103, 164–171.

39. Aswal APS, Datta TK, Raghav S, De S, Yadav P, Goswami SL, 2007b: Development of a competitive quantitative PCR strategy for evaluating the expression stability of 18s rRNA during in vitro maturation of buffalo (Bubalus bubalis) follicular oocytes. Reprod Domest Anim 42, 195–201.

40. Robert C, Gagne D, Bousquet D, Barnes FL Sirard, MA (2001): Differential display and suppressive subtractive hybridization used to identify granulosa cell messenger RNA associated with bovine oocyte developmental competence. Biol Reprod 64, 1812-1820.

41. Dalbiès-Tran R, Mermillod P (2003) Use of heterologous complementary DNA array screening to analyze bovine oocyte transcriptome and its evolution during in vitro maturation. Biol Reprod 68, 252-261.

42. Sirard MA, Dufort I, Vallee M, Massicotte L, Gravel C, Reghenas H, Watson AJ, King WA, Robert C (2005): Potential and limitations of bovine-specific arrays for the analysis of mRNA levels in early development: preliminary analysis using a bovine embryonic array. Reprod Fertil Dev 17, 47-57.

43. Suchyta SP, Sipkovsky S, Kruska R, Jeffers A, McNulty A, Coussens MJ, Tempelman RJ, Halgren RG, Saama PM, Bauman DE, Boisclair YR, Burton JL, Collier RJ, DePeters EJ, Ferris TA, Lucy MC, McGuire MA, Medrano JF, Overton TR, Smith TP, Smith GW, Sonstegard TS, Spain JN, Spiers DE, Yao J, Coussens PM. (2003): Development and testing of a high-density cDNA microarray resource for cattle. Physiol Genomics 15, 158-164.

44. Yao J, Ren X, Ireland JJ, Coussens PM, Smith TP, Smith GW (2004): Generation of a bovine oocyte cDNA library and microarray: resources for identification of genes important for follicular development and early embryogenesis. Physiol Genomics 19, 84-92.

45. Wolf E, Arnold GJ, Bauersachs S, Beier HM, Blum H, Einspanier R, Fröhlich T, Herrler A, Hiendleder S, Kölle S, Prelle K, Reichenbach HD, Stojkovic M, Wenigerkind H, Sinowatz F 2003 Embryo-Maternal communication in bovine-Strategies for deciphering a complex cross-talk. Reprod Dom Anim 38, 276-289.

46. Bauersachs S, Ulbrich SE, Gross K, Schmidt SEM, Meyer HHD, Wenigerkind H, Vermehren M, Sinowatz F, Blum H, Wolf E 2006 Embryo-induced transcriptome changes in bovine endometrium reveal species-specific and common molecular markers of uterine receptivity. Reproduction 132, 319-331.

47. Patel OV, Bettegowda A, Ireland JJ, Coussens PM, Lonergan P, Smith GW (2007): Functional genomics studies of oocyte competence: evidence that reduced transcript abundance for follistatin is associated with poor developmental competence of bovine oocytes. Reproduction 133, 95-106.

48. Misirlioglu M, Page GP, Sagirkaya H, Kaya A, Parrish JJ, First NL, Memili E (2006): Dynamics of global transcriptome in bovine matured oocytes and preimplantation embryos. Proc Natl Acad Sci 103, 18905-18910.

49. Kocabas AM, Crosby J, Ross PJ, Out HH, Beyhan Z, Can H, et al. 2006. The transcriptome of human oocytes. Proc Natl Acd Sci USA. 103:14027-32.

50. Corcoran D, Fair T, Park S, Rizos D, Patel OV, Smith GW, Coussens PM, Ireland JJ, Boland MP, Evans ACO, Lonergan P (2006): Suppressed expression of genes involved in transcription and translation in in vitro compared to in vivo cultured bovine embryos. Reproduction 131, 651-660.

51. Kues WA, Sudheer S, Herrmann D, Carnwath JW, Havlicek V, Besenfelder U, Lehrach H, Adjaye J, Niemann H. Genome-wide expression profiling reveals distinct clusters of transcriptional regulation during bovine preimplantation development in vivo. Proc Natl Acad Sci U S A. 2008 Dec 16;105(50):19768-73.

52. Vallée M, Robert C, Méthot S, Palin M-F, Sirard M-A.(2006): Cross-species hybridizations on a multi-species cDNA microarray to identify evolutionarily conserved genes expressed in oocytes. BMC Genomics 7,113.

53. Hu X, Pokharel K, Peippo J, Ghanem N, Zhaboyev I, Kantanen J, Li MH (2016): Identification and characterization of miRNAs in the ovaries of a highly prolific sheep breed. Animal Genetics 47(2):234-239.

54. Pokharel K, Peippo J, Honkatukia M, Seppälä A, Rautiainen J, Ghanem N, Hamama TM, Crowe MA, Andersson M, Li MH, Kantanen J (2018) : Integrated ovarian mRNA and miRNA transcriptome profiling characterizes the genetic basis of prolificacy traits in sheep (Ovis aries).BMC Genomics, 29;19(1):104.

55. Pitnick, S. et al. (2009) Sperm morphological diversity. In Sperm Biology: An Evolutionary Perspective (Birkhead, T.R. et al., eds), pp. 69–149, Academic Press

56. Fischer, B.E. et al. (2012) Conserved properties of Drosophila and human spermatozoal mRNA repertoires. Proc. R. Soc. B 279, 2636–2644 8 Hecht, N.B. (1998) Molecular mechanisms of male germ cell differentiation. Bioessays 20, 555–561 9.

57. Miller, D. et al. (2005) The controversy, potential and roles of spermatozoal RNA. Trends Mol. Med. 11, 156–163

58. Hagemann LJ, Weilert LL, Beaumont S, Tervit HR (1998): Development of bovine embryos in single in vitro production (sIVP) systems. Mol Reprod Dev 51, 143-147.

59. Boerke, A. et al. (2007) A possible role for sperm RNA in early embryo development. Theriogenology 68S, S147–S155

60. Bourc'his, D. and Voinnet, O. (2010) A small-RNA perspective on gametogenesis, fertilization, and early zygote development. Science 330, 617–622

61. Dadoune, J-P. (2009) Spermatozoal RNAs: what about their functions? Microsc. Res. Tech. 72, 536–551. dairy cows. Aust vet J. 73, 141-147.

62. Krawetz SA. Paternal contribution: new insights and future challenges. Nat Rev Genet 2005; 6:633–42.

63. Gardener, A. and West, S.A. (2009) Green beards. Evolution 64, 25–38.

64. Curry, E. et al. (2011) Differential expression of porcine sperm microRNAs and their association with sperm mor-

phology and motility. Theriogenology 76, 1532–1539

65. Yan, W. et al. (2008) Birth of mice after intra cytoplasmic injection of single purified sperm nuclei and detection of messenger RNAs and MicroRNAs in the sperm nucleus. Biol. Reprod. 78, 896–902

66. Liu, W-M. et al. (2012) Sperm-borne microRNA-34c is required for the first cleavage division in mouse. Proc. Natl. Acad. Sci. U.S.A. 109, 490-494.

67. Gapp, K. et al. (2014) Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. Nat. Neurosci. http:// dx.doi.org/10.1038/nn.3695

68. Hamilton, W.D. (1964) The genetical evolution of social behavior I & II. J. Theor. Biol. 7, 1–52.

69. Pomiankowski, A. (1999) Intragenomic conflict. In Levels of Selection (Keller, L., ed.), pp. 121–152, Princeton University Press

70. Leigh, E.G. (1999) Levels of selection, potential conflicts, and their resolution: the role of the ‘common good’. In Levels of Selection (Keller, L., ed.), pp. 15–30, Princeton University Press

71. Fisher, H.S. and Hoekstra, H.E. (2010) Competition drives cooperation among closely related sperm of deer mice. Nature 463, 801–803.

72. Holman, L. and Snook, R.R. (2008) A sterile sperm caste protects brother fertile sperm from female-mediated death in Drosophila pseudoobscura. Curr. Biol. 18, 292–296.

73. Higginson, D.M. and Pitnick, S. (2011) Evolution of intra-ejaculate sperm interactions: do sperm cooperate? Biol. Rev. 86, 249–270.

74. Immler, S. et al. (2007) By hook or by crook? Morphometry, competition and cooperation in rodent sperm. PLoS ONE 2, e170.

75. Moore, H.D. et al. (2002) Exceptional sperm cooperation in the wood mouse. Nature 418, 174–177.

76. Arnqvist, G. and Nilsson, T. (2000) The evolution of polyandry: multiple mating and female fitness in insects. Anim. Behav. 60, 164-145.

77. Jennions, M.D. and Petrie, M. (2000) Why do females mate multiply? A review of the genetic benefits. Biol. Rev. 75, 21–64.

78. Hosken, D.J. and Stockley, P. (2003) Benefits of polyandry: a life history perspective. Evol. Biol. 33, 173–194.

79. Parker, G.A. (1970) Sperm competition and its evolutionary consequences in insects. Biol Rev. 45, 525–567.

80. Birkhead, T.R. and Moller, A.P. (1998) Sperm Competition and Sexual Selection, Academic Press.

81. Simmons, L.W. (2001) Sperm Competition and its Evolutionary Consequences in the Insects, Princeton University Press.

82. Pessot CA, Brito M, Figueroa J, Concha II, Yanez A, Burzio LO. 1989 Presence of RNA in the sperm nucleus. Biochem Biophys Res Commun 1989; 158: 272–8.

83. Swain JL, Stewart TA, Leder P. Parental legacy determines methylation and expression of an autosomal transgene: a molecular mechanism for parental imprinting. Cell 1987;50: 719–27.

84. Cummins JM. Cytoplasmic inheritance and its implications for animal biotechnology. Theriogenology 2001; 55: 1381–99.

85. Ostermeier GC, Miller D, Huntriss JD, Diamond MP, Krawetz SA. Reproductive biology: delivering spermatozoan

RNA to the oocyte. Nature 2004; 429: 154.

86. Ziyyat A, Lefevre A. Differential gene expression in pre-implantation embryos from mouse oocytes injected with round spermatids or spermatozoa. Hum Reprod 2001; 16: 1449–56.

87. Ostermeier GC, Goodrich RJ, DiamondMP, Dix DJ, Krawetz SA. Toward using stable spermatozoal RNAs for prognostic assessment of male factor fertility. Fertil Steril 2005; 83: 1687–94.

88. Platts AE, Dix DJ, Chemes HE, Thompson KE, Goodrich R, Rockett JC, et al. Success and failure in human spermatogenesis as revealed by teratospermic RNAs. Hum Med Gen 2007; 16: 763–73.

89. Zhao Y, Li Q, Yao C, Wang Z, Zhou Y, Wang Y, et al. Characterization and quantification of mRNA transcripts in ejaculated spermatozoa of fertile men by serial analysis of gene expression. Hum Reprod 2006; 21: 1583–90.

90. Flesch FM, Gadella BM. Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochim Biophys Acta 2000; 1469: 197–235.

91. Miller D, Ostermeier GC. Towards a better understanding of RNA carriage by ejaculate spermatozoa. Hum Reprod Update 2006; 12: 757–67.

92. Miller* D, Ostermeier GC. Spermatozoal RNA: why is it there and what does it do? Gynecol Obstet Fertil 2006; 34: 840–6.

93. Gui LM, Joyce IM (2005): RNA interference evidence that growth differentiation factor-9 mediates oocyte regulation of cumulus expansion in mice. Biol Reprod 72, 195-199.Gur Y, Breitbart H. Mammalian sperm translate nuclear-encoded proteins by mitochondrial-type ribosomes. Genes Dev 2006; 20: 411–6.

94. Sutovsky P, Moreno RD, Ramalho-Santos J, Dominko T, Simerly C, Schatten G. Ubiquitinated sperm mitochondria, selective proteolysis, and the regulation of mitochondrial inheritance in mammalian embryos. Biol Reprod 2000; 63: 582–90.

95. Ebensperger C and Barros C: Changes at the hamster oocyte surface from the germinal vesicle stage to ovulation. Gamete Res 9: 387-397, 1984.

96. Johnson MH, Eager D, Muggleton-Harris A and Grave HM: Mosaicism in organisation concanavalin A receptors on surface membrane of mouse egg. Nature 257: 321-322, 1975.

97. Evans JP and Florman HM: The state of the union: the cell biology of fertilization. Nat Cell Biol 4: s57-s63, 2002.

98. Huang TTF Jr and Yanagimachi R: Inner acrosomal membrane of mammalian spermatozoa: Its properties and possible functions in fertilization. Am J Anat 174: 249-268, 1985.

99. Yanagimachi R and Noda YD: Physiological changes in the postnuclear cap region of mammalian spermatozoa: A necessary preliminary to the membrane fusion between sperm and egg cells. J Ultrastruct Res 31: 486-493, 1970.

100. Bedford JM, Moore HDM and Franklin LE: Significance of the equatorial segment of the acrosome of the spermatozoon in eutherian mammals. Exp Cell Res 119: 119-126, 1979.

101. Cho C, Bunch DO, Faure JE, Goulding EH, Eddy EM, Primakoff P and Myles DG: Fertilization defects in sperm from mice lacking fertilin β. Science 281: 1857-1859, 1998.

102. Nishimura H, Cho C, Branciforte DR, Myles DG and Primakoff P: Analysis of loss of adhesive function in sperm lacking cyritestin or fertilin beta. Dev Biol 233: 204-213, 2001.

103. Almeida EA, Huovila AP, Sutherland AE, Stephens LE, Calarco PG, Shaw LM, Mercurio AM, Sonnenberg A, Primakoff P, Myles DG and White JM: Mouse egg integrin α 6 β 1 functions as a sperm receptor. Cell 81: 1095-1104, 1995.

104. Chen H and Sampson NS: Mediation of sperm-egg fusion: Evidence that mouse egg α6β1 integrin is the receptor for sperm fertilin beta. Chem Biol 6: 1-10, 1999.

105. Eto K, Huet C, Tarui T, Kupriyanov S, Liu HZ, Puzon-McLaughlin W, Zhang XP, Sheppard D, Engvall E and Takada Y: Functional classification of ADAMs based on a conserved motif for binding to integrin α 9β 1: Implications for sperm-egg binding and other cell interactions. J Biol Chem 277: 17804-17810, 2002.

106. Zhu X and Evans JP: Analysis of the roles of RGD-binding integrins, α(4)/α(9) integrins, α(6) integrins, and CD9 in the interaction of the fertilin β (ADAM2) disintegrin domain with the mouse egg membrane. Biol Reprod 66: 1193-1202, 2002.

107. Mizuno M, Harris CL, Johnson PM and Morgan BP: Rat membrane cofactor protein (MCP; CD46) is expressed only in the acrosome of developing and mature spermatozoa and mediates binding to immobilized activated C3. Biol Reprod 71: 1374-1383, 2004.

108. Johnson PM, Clift LE, Andrlíková P, Jursová M, Flanagan BF, Cummerson JA, Stopka P and Dvorakova-Hortova K: Rapid sperm acrosome reaction in the absence of acrosomal CD46 expression in promiscuous field mice (Apodemus). Reproduction 134: 739-747, 2007.

109. Lozahic S, Christiansen D, Manié S, Gerlier D, Billard M, Boucheix C and Rubinstein E: CD46 (membrane cofactor protein) associates with multiple beta1 integrins and tetraspans. Eur J Immunol 30: 900-907, 2000.

110. Stein KK, Primakoff P and Myles D: Sperm-egg fusion: Events at the plasma membrane. J Cell Sci 117: 6269-6274, 2004.

111. Jégou A, Ziyyat A, Barraud-Lange V, Perez E, Wolf JP, Pincet F and Gourier C: CD9 tetraspanin generates fusion competent sites on the egg membrane for mammalian fertilization. Proc Natl Acad Sci USA 108: 10946-10951, 2011.

112. Kaji K, Oda S, Shikano T, Ohnuki T, Uematsu Y, Sakagami J, Tada N, Miyazaki S and Kudo A: The gamete fusion process is defective in eggs of Cd9-deficient mice. Nat Genet 24: 279-282, 2000.

113. Miyado K, Yamada G, Yamada S, Hasuwa H, Nakamura Y, Ryu F, Suzuki K, Kosai K, Inoue K, Ogura A, et al: Requirement of CD9 on the egg plasma membrane for fertilization. Science 287: 321-324, 2000.

114. Horváth G, Serru V, Clay D, Billard M, Boucheix C and Rubinstein E: CD19 is linked to the integrin-associated tetraspans CD9, CD81, and CD82. J Biol Chem 273: 30537-30543, 1998.

115. Takahashi Y, Bigler D, Ito Y and White JM: Sequence-specific interaction between the disintegrin domain of mouse ADAM 3 and murine eggs: Role of beta1 integrin-associated proteins CD9, CD81, and CD98. Mol Biol Cell 12: 809-820, 2001.

116. Rubinstein E, Ziyyat A, Prenant M, Wrobel E, Wolf JP, Levy S, Le Naour F and Boucheix C: Reduced fertility of female mice lacking CD81. Dev Biol 290: 351-358, 2006.

117. Ernesto JI, Weigel Muñoz M, Battistone MA, Vasen G, Martínez-López P, Orta G, Figueiras-Fierro D, De la Vega-Beltran JL, Moreno IA, Guidobaldi HA, et al: CRISP1 as a novel CatSper regulator that modulates sperm motility and orientation during fertilization. J Cell Biol 210: 1213-1224, 2015.

118. Inoue N, Ikawa M, Isotani A and Okabe M: The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. Nature 434: 234-238, 2005.

119. Inoue N, Hamada D, Kamikubo H, Hirata K, Kataoka M, Yamamoto M, Ikawa M, Okabe M and Hagihara Y: Molecular dissection of IZUMO1, a sperm protein essential for sperm-egg fusion. Development 140: 3221-3229, 2013.

120. Inoue N, Kasahara T, Ikawa M and Okabe M: Identification and disruption of sperm-specific angiotensin converting enzyme-3 (ACE3) in mouse. PLoS One 5: e10301, 2010. J Dairy Sci. 84, 1277-1293.

121. Bianchi E and Wright GJ: Izumo meets Juno: Preventing polyspermy in fertilization. Cell Cycle 13: 2019-2020, 2014.

122. Sawada H, Mino M and Akasaka M: Sperm proteases and extracellular ubiquitin-proteasome system involved in fertilization of ascidians and sea urchins. Adv Exp Med Biol 759: 1-11, 2014.

123. Zhou C, Kang W and Baba T: Functional characterization of double-knockout mouse sperm lacking SPAM1 and ACR or SPAM1 and PRSS21 in fertilization. J Reprod Dev 58: 330-337, 2012.

124. Besenfelder U, Havlicek V, Moesslacher G, Gilles M, Tesfaye D, Griese J, Hoelker M, Hyttel PM, Laurincik J, Brem G, Schellander K (2008): Endoscopic recovery of early preimplantation bovine embryos: effect of hormonal stimulation, embryo kinetics and repeated collection. Reprod Domest Anim 43, 566-572

125. Hendriksen PJ, Steenweg WN, Harkema JC, Merton JS, Bevers MM, Vos PL, Dieleman SJ (2004): Effect of different stages of the follicular wave on in vitro developmental competence of bovine oocytes. Theriogenology 61, 909-920.

126. Rizos D, Ward F, Duffy P, Boland MP, Lonergan P (2002): Consequences of bovine

127. Fair T, Carter F, Park S, Evans ACO, Lonergan P (2007): Global gene expression analysis during bovine oocyte in vitro maturation. Theriogenology.

128. Hagemann LJ (1999) Influence of the dominant follicle on oocytes from subordinate follicles. Theriogenology 51, 449-459.

129. Machatkova M, Jokesova E, Horky F, Krepelova A (2000): Utilization of the growth phase of the first follicular wave for bovine oocyte collection improves blastocyst production. Theriogenology 54, 543-550.

130. Machatkova M, Krausova K, Jokesova E, Tomanek M (2004): Developmental competence of bovine oocytes: effects of follicle size and the phase of follicular wave on in vitro embryo production. Theriogenology 61, 329-335.

131. Wood SC, Glencross RG, Bleach EC, Lovell R, Beard AJ, Knight PJ (1993): The ability of steroid-free bovine follicular fluid to suppress FSH secretion and delay ovulation persists in heifers actively immunized against inhibin. J Endocrinol 136, 137-148.

132. Wolfsdorf KE, Diaz T, Smitt EJP, Thatcher MJ, Drost M, Thatcher WW (1997): The dominant follicle exerts an interovarian inhibition on FSH-induced follicular development. Theriogenology 48, 435-447.

133. Nemcova L, Machatkova M, Hanzalova K, Horakovab J, Kankaa J (2006): Gene expression in bovine embryos derived from oocytes with different developmental competence collected at the defined follicular developmental stage. Theriogenology 65, 1254-1264.

134. Assidi M, Dufort I, Ali A, Hamel M, Algriany O, et al. (2008) Identification of potential markers of oocyte competence expressed in bovine cumulus cells matured with follicle-stimulating hormone and/or phorbol myristate acetate in vitro. Biol Reprod 79: 209-222.

135. Bettegowda A, Patel OV, Lee KB, Park KE, Salem M, et al. (2008) Identification of novel bovine cumulus cell molecular markers predictive of oocyte competence: functional and diagnostic implications. Biol Reprod 79: 301-309.

136. Tesfaye D, Ghanem N, Carter F, Fair T, Sirard MA, et al. (2009) Gene expression profile of cumulus cells derived from cumulus-oocyte complexes matured either in vivo or in vitro. Reprod Fertil Dev 21: 451-61.

137. Bilodeau-Goeseel S, Schultz GA (1997): Changes in ribosomal ribonucleic acid content within in vitro-produced bovine embryos. Biol Reprod 56, 1323-1329.

138. Lonergan P, Rizos D, Gutiérrez-Adán A, Moreira PM, Pintado B, de la Fuente J, Boland MP (2003a): Temporal divergence in the pattern of messenger RNA expression in bovine embryos cultured from the zygote to blastocyst stage

in vitro or in vivo. Biol Reprod 69, 1424-1431.

139. Tesfaye D, Ponsuksili S, Wimmers K, Gilles M Schellander K (2004): A comparative expression analysis of gene transcripts in post-fertilization developmental stages of bovine embryos produced in vitro or in vivo. Reprod Dom Anim 39, 396-404.

140. Wrenzycki C, Herrmann D, Lucas-Hahn A, Korsawe K, Lemme E, Niemann H (2005): Messenger RNA expression patterns in bovine embryos derived from in vitro procedures and their implications for development. Reprod Fertil Dev 17, 23–35.

141. Moore T (2001) Genetic conflict, genomic imprinting and establishment of the epigenotype in relation to growth. Reproduction 122, 185-193.

142. Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, Broadbent PJ, Robinson JJ, Wilmut I, Sinclair KD (2001): Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. Nat Genet 27, 153-154.

143. Blondin P, Farin PW, Crosier AE, Alexander JE, Farin CE (2000): In vitro production of embryos alters levels of insulin-like growth factor-II messenger ribonucleic acid in bovine fetuses 63 days after transfer. Biol Reprod 62, 384-389.

144. Lonergan P, Rizos D, Gutierrez-Adan A, Fair T, Boland MP (2003b): Oocyte and embryo quality: effect of origin, culture conditions and gene expression patterns. Reprod Domest Anim 38, 259-267.

145. Wrenzycki C, Herrmann D, Lucas-Hahn A, Lemme E, Korsawe K, Niemann H (2004): Gene expression patterns in in vitro-produced and somatic nuclear transfer-derived preimplantation bovine embryos: relationship to the large offspring syndrome?. Anim Reprod Sci 82-83, 593-603.

146. Somers J, Smith C, Donnison M, Wells DN, Henderson H, McLeay L, Pfeffer PL (2006): Gene expression profiling of individual bovine nuclear transfer blastocysts. Reproduction 131, 1073-1084.

147. Rizos D, Lonergan P, Boland MP, Arroyo-García R, Pintado B, de la Fuente J, Gutiérrez-Adán A (2002b): Analysis of differential messenger RNA expression between bovine blastocysts produced in different culture systems: implications for blastocyst quality. Biol Reprod 66, 589-595.

148. Khosla S, Dean W, Brown D, Reik W, Feil R (2001): Culture of preimplantation mouse embryos affect fetal development and the expression of imprinted genes. Biol Reprod 64, 918-926.

149. Lazzari G, Wrenzycki C, Herrmann D, Duchi R, Kruip T, Niemann H, Galli C (2002): Cellular and molecular deviations in bovine in vitro-produced embryos are related to the large offspring syndrome. Biol Reprod 67, 767-775.

150. El-Sayed A, Hoelker M, Rings F, Salilew S, Jennen D, Tholen E, Sirard MA, Schellander K, Tesfaye D (2006): Large-scale transcriptional analysis of bovine embryo biopsies in relation to pregnancy success after transfer to recipients. Physiol Genomics 28, 84-96.

151. Minami N, Aizawa A, Ihara R, Miyamoto M, Ohashi A, Imai H (2003): Oogenesin is a novel mouse protein expressed in oocytes and early cleavage-stage embryos. Biol Reprod 69, 1736-1742.