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Developmental competence of mammalian oocytes-insights into molecular research and the promise of microfluidic technology: a review

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ABSTRACT: Developmental competence of female gametes determines their maturation ability, successful fertilisation, and proper zygote formation. Oocyte quality may be assessed by expression profiling of several gene markers such as *Cx43, TGFB, GDF9, BMP, Lox* and *Pdia5* that determine the biological features of oocytes. Conversely, several other extrinsic factors, including follicular size or heat shock may significantly influence oocyte quality and ability to grow and develop during folliculo- and oogenesis. However, using molecular methods for evaluation of oocyte quality often leads to destruction of an analysed cell. Therefore, there is an increased requirement to seek new non-invasive methods of oocyte-embryo quality assessment. Here we describe the Lab-on-Chip system based on microfluidic technology, which is the first parametric and objective device for evaluation of oocyte developmental competence using spectral images. In this review several extrinsic factors and molecular markers of oocyte developmental competence are discussed. Furthermore, based on our previous studies, we discuss the possibility of applying the spectrophotometric system (Lab-on-Chip) in both biomedical and reproductive research in domestic animals.

Keywords: pig; oocyte; developmental capacity; Lab-on-Chip

Contents

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1. Introduction

Oocyte quality has an impact on several important processes and mechanisms, the proper course of which allows the achievement of fully competent gametes, ready for maturation and fertilisation, as well as the reaching of the blastocyst stage, proper implantation and delivery of healthy offspring (Kempisty et al. 2011b). This stage is achieved following a long course of morphological, cellular, biochemical and molecular changes and is reached during follicular differentiation and oogenesis. Such a prolonged process is regulated by several external and internal signals which lead to differentiation of cells that surround the oocytes as well as to molecular and subcellular changes inside the oocyte cytoplasm. Therefore, oocyte quality may be tested using several tools capable of recognising the molecular and cellular changes of a gamete (Kempisty et al. 2012). Then, after several analyses every oocyte may be classified as 'competent' or 'incompetent'. Taking into consideration a number of methods, which seek to define 'ideal' oocytes, it is clear that there is no single adequate method of oocyte quality assessment. Several of them aim to detect cellular or subcellular changes in oocytes while others reveal molecular changes (expression of marker genes dependent on mRNA and protein storage specific for each gamete) of the gametes (Kempisty et al. 2011a). In the last decade many new markers defining oocyte quality were discovered. However, these markers characterising subtle differences between individual gametes, are related only to some aspects of cell quality, and fail to represent the complete characteristics of an 'ideal' oocyte. Therefore, based on the knowledge accumulated in the last 10 years we can state that an 'ideal' oocyte should be characterised by several traits, associated with markers, which can be assessed using techniques available in the 21st century, and which could add new information to fully define oocyte quality in the future (Kempisty et al. 2011d).

2. Mammalian oocyte developmental capacity

Oocyte developmental competence is defined as the ability of a gamete to mature, become fertilised and develop into an embryo capable of reaching the blastocyst stage. Therefore, the selected molecular markers of oocyte maturation, fertilisation or embryo developmental ability are mostly related to the expression of specific genes encoding proteins responsible for facilitating the achievement of the proper growth and developmental potential of oocytes (Jackowska et al. 2013; Kempisty et al. 2013a). The achievement of developmental potential involves several biochemical and molecular changes which finally lead to formation of a fully competent cell. Therefore, any disturbances in these pathways may result in decreased activity of several proteins or enzymes and improper catalysis of reactions important for each stage of oocyte growth and development during folliculogenesis and oogenesis. Similarly to oocyte quality, developmental competence may be assessed by several markers which reflect processes and mechanisms, the proper course of which results in developmental competence. The main cellular predictor of a competent gamete is glucose-6-phosphate dehydrogenase activity, detected using the BCB staining test (Wongsrikeao et al. 2006; Kempisty et al. 2011a; Kempisty et al. 2013b). In this test the fully competent gamete is stained blue while an incompetent oocyte remains unstained. This test is applied as the main method to determine developmental competence of oocytes following *in vitro* fertilisation (IVF) and *in vitro* maturation (IVM) programs in several species of mammals, including cows, pigs, goats and even dogs.

Morphology of cumulus-oocyte complexes (COC) or oocytes is still one of the most important as well as most widely used factors in determination of gamete quality. The morphology of the female gamete is highly associated with its ability to grow and to undergo development, maturation, fertilisation, formation of the zygote or to reach blastocyst stage. The most important predictors of adequate oocyte morphology include characteristics of somatic cells which surround the oocyte, the number of cumulus cell layers, the protein ultrastructure of the zona pellucida (composition and expression of zona glycoproteins), the structure of the oocyte cytoplasm, related to its granularity and colour, the structure of the polar body and formation of the meiotic spindle. The development of new molecular biology techniques allows the characterisation of oocyte morphology by means of molecular markers (expression of specific genes and proteins), related to each aspect of oocyte morphological classification (Jackowska et al. 2009; Kempisty et al. 2009).

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Oocyte developmental competence is defined by several molecular and biochemical markers, which can be detected using different techniques. However, the majority of methods used in the assessment of oocyte developmental potential are invasive and, when applied, lead to destruction of the analysed cell. Therefore, the search for new, non-invasive methods of oocyte quality and developmental competence assessment continues. In this review we present our newly developed Lab-on-Chip system, based on microfluidic technology. The Lab-on-Chip system analyses are related to oocyte/embryo spectral specificity and, therefore, represent their physical characterisation. Through several experiments presented here we find that this system can be applied for the evaluation of several reproductive features of oocytes/embryos. Further, the implementation of this system reveals a correlation between the biological (reproductive) and physical specificity of the analysed biological material.

In this article, selected aspects of oocyte developmental competence assessment are discussed. Moreover, the influence of selected factors, e.g. follicular size, on expression of selected genes encoding proteins responsible for maturation or fertilisation ability of the female gamete is reviewed. We also present the basic technology of the Lab-on-Chip system, its application in reproductive biology and in several experimental set-ups involving porcine and bovine oocytes and embryos, used as experimental models.

3. Role of selected factors in developmental competence of oocytes

In parallel to the role of molecular factors in the attainment of developmental competence by oocytes, there are many factors which may influence oocyte maturation and embryogenesis on a macro scale. The negative role of heat stress in oocyte development and fertilisation is well documented (Dutt 1963; Tompkins et al. 1967; Dunlap and Vincent 1971; Badinga et al. 1985). Morula-stage embryos from heat-stressed oocytes are more susceptible to heat stress than control, non-heat-stressed oocytes (Edwards et al. 2009). Additionally, dairy cows that were exposed to heat stress during artificial insemination had a higher ratio of miscarriages than non-heat-stressed cows (Rutigliano et al. 2008). Induction of heat leads to reduction in *de novo* synthesis of proteins (Edwards and Hansen 1996). Although the mechanism of this reduction remains unclear, Payton and Edwards (2005) discovered abnormal fluctuations in the concentration of polyA mRNA after 12 h of *in vitro* maturation culture. However, after 24 h of culturing these changes could not be detected. This approach has led to the hypothesis that heat stress may influence the stability of specific mRNA subpopulations. Twelve hours of heat stress exposure had a minimum impact on mRNA synthesis during oocyte maturation, in cumulus cells and in resultant 4- to 8-cell embryos (Payton et al. 2011). However, at the blastocyst stage a higher abundance of mRNA of less than 3200 nt in length was detected, which means that perturbations at early stages of oocyte development can be carried over to the later, embryo stage. Additionally, a higher abundance of mRNA in the blastocyst stage can be associated with higher metabolic activity, induced by heat stress during meiotic maturation of the oocyte, the activity of which can be measured as an increase in intracellular ATP content. This assumption is in agreement with results obtained by Nilsson et al. (2002), in whose hands higher total mRNA content was related to higher content of ATP in cold-starved *Helicobacter pylori*. mRNA sizes in mammalian species range from 300 to 6500 nt, with an average of 1400 nt. At the blastocyst stage mRNA species of 3200 nt in length increase (Payton et al. 2011). It is still unclear which mechanism is responsible for these changes. However, equine blastocysts obtained from heat-stressed oocytes had increased levels of HSP70 mRNA in comparison to non-heat-stressed oocytes. Additionally, the overall mRNA population did not change during meiotic maturation, which is in agreement with results reported by Payton et al. (2010). During meiotic maturation, a significant 30% to 50% decrease was observed in the poly (A) mRNA subpopulation (Lequarre et al. 2004). Oocyte exposure to heat stress conditions prevented a reduction in the number of poly (A) mRNA species, in contrast to previous findings (Payton and Edwards 2005). The difference in the results might be a consequence of several factors which include, e.g. disease or the nutritional status (Fray et al. 2000; Armstrong et al. 2003).

In vitro developmental competence and fertilisation ability of porcine oocytes was associated with seasonal changes in atmospheric temperature (Suzuki et al. 2010). These results suggested that the best tem-

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perature for oocyte maturation up to the blastocyst stage was found in winter. Interestingly, maturation efficiency did not depend on the season but blastocyst formation was variable among seasons.

Several other factors like feeding, humidity and photoperiod are also associated with seasonal effects on reproduction (Peltoniemi et al. 2000). It is proposed that after beneficial progesterone stimulation of embryonic survival, restricted post-mating feeding may have a negative effect on maintenance of early pregnancy. This can be linked to a reduction in the LH concentration after a summer diet.

Insulin-like growth factor-1 is a stimulator of oocyte maturation and embryonic development as well as a suppressor of apoptosis, activated by heat stress (Palma et al. 1997). It has been also established that IGF-1 plays a role in nuclear maturation of the oocyte (Xia et al. 1994). However, Zhandi et al. (2009) stated that during heat stress, supplementation of IGF-1 led to activation of some unknown pathway, which resulted in an increase in apoptosis. Supplementation of IGF-1 into IVM medium failed to increase the number of oocytes that reached the blastocyst stage. These results contrasted with observations from Markkula and Makarevich (2001), in whose hands the addition of IGF-1 increased the blastocyst yield. These differences might reflect different culture conditions and also might support the idea that IGF-1 is more effective in blastocyst development when culture conditions are not optimal (Block et al. 2008). Heat stress induces several forms of damage in oocytes and these insults are carried over to the blastocyst stage. It is well established that heat stress alters the *zona pelucida* surface and cytoplasm, which leads to impaired sperm penetration (Edwards and Hansen 1996). Moreover, increased production of reactive oxygen species due to the high glucose consumption leads to apoptosis even in the presence of IGF-1 (Zhandi et al. 2009).

Taken together, it is accepted that heat stress conditions have a highly negative influence on oocyte developmental competence and quality, affecting the oocyte diameter. Moreover, they have a pronounced impact on the molecular level by modification of fatty acids and proteins in the oocyte plasma membrane (Zeron et al. 2001; Berger and Roberts 2009). High temperatures may also lead to damage to cytoskeleton organisation and meiotic processes (Ju and Tseng 2004; Barati et al. 2008).

A negative energy balance (NEB) is believed to decrease fertility in cows (Walters et al. 2002). During periods of metabolic stress there is an increased concentration of free fatty acids (fatty acid complexed to albumin) in blood and follicular fluid (Leroy et al. 2005). Under normal conditions fatty acids are esterified into triacylglycerols and cholesterol-esters, and stored in lipid droplets as neutral lipids. It is suggested that neutral lipids are important for early embryonic development as they represent an energy reservoir and fulfil an important function in the biosynthesis of membranes (Kruip et al. 1983). The droplet stores also protect the oocyte from lipotoxicity induced by fatty acids. Depending on the species involved, the main fractions of lipids are comprised of palmitic, oleic and stearic acids, along with linoleic and arachidonic acids (Kim et al. 2001; Leroy et al. 2005) and the levels reflect their composition in blood and follicular fluid. Kim et al. (2001) observed that the composition of specific fatty acids may reflect the developmental competence of an oocyte. A good quality oocyte contains more oleic, linoleic and arachidonic acids (Kim et al. 2001). Additionally, to supplement these results Aardema et al. (2011) analysed the influence of fatty acid saturation to evaluate if saturated fatty acids were toxic for oocytes. The results indicated that oocytes can effectively incorporate and metabolise external fatty acids as a response to changes in follicular fluid concentrations which can damage oocytes, influencing their lipid metabolism (Roche 2006). Additionally, exposure to a higher level of saturated palmitic or stearic acid negatively affected oocyte quality and reduced the number of lipid droplets. This resulted in a severely impaired development of oocytes. Conversely, exposure to a higher concentration of oleic acid resulted in an increased number and volume of droplets. Interestingly, oleic acid blocked the effect of palmitic acid when both were added to cultures. The detrimental effect of palmitic acid was due to activation of the apoptosis cascade. Mono-unsaturated fatty acids were reported to prevent the effect of palmitic acid by induction of Bcl-2 protein expression, prevention of mitochondrial release of cytochrome C or by competition with fatty acids for transport into the cell or in cell metabolism (Cnop et al. 2001; Listenberger et al. 2003). It has been proposed that the ratio between saturated and unsaturated fatty acids be used as a marker of oocyte developmental competence.

Glucose as a source of energy is necessary for oocyte cumulus complex (COC) development. Sutton et al. (2003) observed that mature bovine COCs con-

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sumed two-fold more glucose, oxygen and pyruvate than immature COCs. A well-established aspect of COC metabolism is that the oocyte has an only weak ability to convert glucose into energy and that cumulus cells themselves are the main suppliers of energy via glucose metabolism. Under physiological conditions cumulus cells are exposed to 31% to 81% glucose dissolved in follicular fluid (Stokes et al. 2008). Although the oocyte harbours the glucose transporters SLC2A1, SLC2A3 and SLC2A8, its ability to convert glucose into energy is limited (Pisani et al. 2008). Conversely, cumulus cells express an additional glucose transporter, SLC2A4, which has high affinity to glucose and is more reliant on insulin and insulin-like growth factors than on glucose concentration. Glucose concentration positively corresponds with ovarian follicle size (Nandi et al. 2007). However, fluctuations in the concentration of glucose in *in vitro* culture may lead to dysfunction in oocyte development. A high concentration of glucose during *in vitro* maturation (IVM) can result in the increased production of reactive oxygen species, increased O-linked glycosylation and a reduction in reduced glutathione (GSH) concentrations. When COC-metabolised glucose pyruvate and lactate are transported into the oocyte, they go through the tricarboxylic acid cycle, followed by oxidative phosphorylation, rather than the glycolytic pathway. Results by Herrick et al. (2006) suggested that despite the low glycolytic activity of oocytes, glycolysis was necessary for proper oocyte maturation. Interestingly, the level of glycolysis remained constant and higher consumption of glucose by COCs caused activation of other metabolic pathways. This observation may be similar to the Crabtree effect (pC).

The age of females is one of the major causes of infertility-dependent decreases in oocyte quality. A fully mature oocyte is a result of a six month-long maturational process in the developing follicle, culminating in either ovulation or atresia. The developmental process is usually divided into nuclear and cytoplasmic maturation. Nuclear maturation is strongly related to age: in oocytes from older donors a higher rate of aneuploidy occurs. Cytoplasmatic maturity is related to the arrest of mRNA and proteins in the inactivated form (selective cytoplasmatic polyadenylation of mRNA) and proper activation in a tightly scheduled manner (Gandolfi and Gandolfi 2001). Proper activation of transcripts and resumption of translation define oocyte developmental competence, and includes: (1) timely translation of transcripts which provide new proteins, (2) post-transcriptional modifications of transcripts and (3) degradation of transcripts and proteins (7 EM). Transcriptional activity of oocytes progresses in an age-dependent manner. Using microarrays to compare oocytes from younger and older donors, Grondahl et al. (2010) found differential expression in more than 300 genes. Interestingly, genes encoding proteins involved in spindle assembly checkpoint regulation, DNA stability and chromosome segregation were influenced by age. A global overview of biological processes indicated changes in the areas of: (1) cellular processes, (2) biological regulation, (3) metabolic processes and (4) developmental processes. Most of those groups included genes involved in mitosis and meiosis. Additionally, catalytic activity of the oocyte was highly enriched which confirmed previous findings: degradation of mRNA and protein was important for oocyte maturation (Stitzel and Seydoux 2007).

Laboratory preparation of the material may also exert a negative influence on oocyte competence and quality, which can lead to false-positive or false-negative results. Formalin fixation of material reduced the total RNA yield and altered RNA integrity (Goldsworthy et al. 1999; Cox et al. 2006). Additionally, formalin fixation chemically modified RNA, so that it could be no longer used in qPCR studies (Masuda et al. 1999).

4. Molecular markers of oocyte developmental competence

Phosphorylation cascades represent important processes that drive many aspects of cellular metabolism. In the case of oocytes, phosphorylation supports both nucleus and cytoplasm maturation and is required for developmental competence following fertilisation (Besterman and Schultz 1990). Disruption of kinase signalling leads to defects in chromosome condensation, which results in aneuploidy (Swain and Smith 2007). Moreover, it is well established that in somatic cells global changes in phosphorylation induce activation of integrins and growth factors, and stimulate entry into the cell cycle. Culture conditions supporting tyrosine kinase activity have a positive influence on oocyte quality (McGinnis and Albertini 2010). Additionally, tyros-

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ine phosphorylated proteins increased in both the cell cortex and cytoplasm after oocytes fully developed in disagreement with previous findings (Ben-Yosef et al. 1998). However, an increasing number of phosphorylated proteins during the G–MI stage corresponded with their decreasing number in the transition to the MI–MII stage (Pelech et al. 2008).

The number of mitochondrial DNA copies was described to play a significant role during embryonic development (Wai et al. 2010). The mitochondrial genome is small and maternally inherited, containing 13 essential genes involved in the function of the respiratory chain. Studies in several species proved that mtDNA replication did not occur during the cleavage of the embryo. Moreover, female gametes had a high level of mtDNA synthesis, in contrast to males, in whose sperm the number of mtDNA copies was decreasing (Larsson et al. 1997). This reduction could be a consequence of a preventive mechanism that protects sperm against ROS, which could damage sperm function. It has been proposed that accumulation of maternal mtDNA copies is a kind of a programmed mechanism providing the proper amount of material necessary for embryo development when transcriptional activation of mtDNA restarts (Shoubridge and Wai 2007). TFAM is a basic protein of the HMG box family, responsible for packing mtDNA (Kaufman et al. 2007). Additionally, it also has a role in controlling mtDNA copy number (Ekstrand et al. 2004). Knockout mice (TFAM–/–), (responsible for mtDNA replication and expression of, e.g., polymerase gamma or TFAM), died at embryonic Day 8.5 and 10.5 (Hance et al. 2005). It has been established that for proper embryo development in humans, 100 000 copies of mtDNA are required (Reynier et al. 2001). However, the transfer of TFAM knockout (–/–) embryos to pseudopregnant females resulted in normal preimplantation development but subsequent death during organogenesis. Conversely, reductions in mtDNA copy numbers in male gametes did not negatively influence fertility, sperm motility and

Table 1. Marker genes of oocyte/embryo quality

Gene	Protein	Function	Reference
Gdf9	GDF9	granulose cell proliferation factor	Juengel et al. (2002)
Bmp15	BMP15	inhibitor of FSH receptor	Otsuka et al. (2001)
lox	lysyl oxidase	oxidizes peptidyl lysine to peptidyl aldehyde	Smith Mungo and Kagan (1998)
Dnmt	methylotransferases	addition of CH_3 group	Chen and Lee (2006)
Cx37	connexin 37	oocyte – cumulus communication	Kidder and Mhawi (2002)
Cx43	connexin 43	granulose cell communication	Sommersberg et al. (2000)
Tfam	TFAM	packing of mtDNA, controlling number of mtDNA copies	Kaufman et al. (2007)
Rxfp1	RXFP1	relaxin receptor	Bathgate et al. (2005)
Zar1	ZAR1	arrest of zygote	Wu et al. (2003)
Mater	mater	maternal antigen	Tong et al. (2000)
Bcl2	B-cell CLL/lymphoma 2	apoptosis regulator	Boumela et al. (2011)
Nrg1	neuregulin	development stimulator	Noma et al. (2011)
Syt11	synaptotagmin XI	calcium sensor	Sudhof (2002)
Chgb	chromogranin B	co-released with neurotransmitters following appropriate stimuli	Taupenot et al. (2003)
Nrp1	neuropilin 1	coreceptor to tyrosine kinase	Pellet-Many et al. (2008)
Ar	androgen receptor	intercellular signal transducer, steroidogenesis	Freedman (1998)
Calm1	calmoduiln 1	calcium-binding protein	Zhang et al. (2012)
Calu	calumenin	calcium-binding protein	Yabe et al. (1997)
Rpl9	ribosomal protein L9	component of 60S ribosomal subunit	Wool et al. (1996)
Hist1h4c	histone cluster 1, H4c	encodes member of histone H4 family	Marzluff et al. (2002)
Tom1	target of myb1	ubiquintinated protein sorting	Katoh et al. (2004)

The table presents marker genes and the function of the encoded proteins in the acquisition developmental capacity by mammalian oocytes and embryos. Moreover, the described proteins determine gamete/embryo quality and growth potential

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sperm count (Wai et al. 2010). These results suggest a substantial role of mtDNA copy number in the success of embryo development and reproduction.

Some maternal transcripts are known to be oocyte-specific and are required for early post fertilisation cleavage (Dean 2002). This group includes maternal antigens that embryos require (*Mater*), zygote arrest 1 (*Zar1*) and nucleoplasmin 2 (*Npm2*) (Tong et al. 2000; Burns et al. 2003; Wu et al. 2003). After fertilisation both maternal and paternal genomes must undergo remodelling to give rise to the functional diploid embryo genome. Reorganisation of the parental genome is mediated by nucleoplasmin (NPM). Additionally, NPM is responsible for sperm DNA remodelling in *Xenopus laevis* (Philpott and Leno 1992). The injection of NPM into the bovine oocyte after fertilisation results in a significant improvement in the rate of pregnancy (Betthauser et al. 2006). This suggests that the role of NPM is extended to reprogramming the somatic nucleus (Lingenfelter et al. 2011). Conversely, the common regulation system that down-regulates gene expression is associated with microRNAs (miRNA) and pre-microRNAs (miR). MicroRNAs are a specific subgroup of RNA that are up to 23 nt in length and bind to the 3' UTR region of target mRNAs. The miR-181a is a highly conserved miRNA that has been detected in many species (Lingenfelter et al. 2011). It is responsible for tumour suppression in cancer cells, modulating cisplatin-induced cell death and down-regulating zinc finger protein expression (Shi et al. 2008; Galluzzi et al. 2010; Huang et al. 2010). Additionally, quantification of NPM2 using densitometry confirmed that miRNA-181a repressed translation of NPM2.

Taken together, all the mentioned genetic factors play an important role during oocyte maturation, communication between the oocyte and surrounding granulosa cells or in development of cumulus cells. The most important developmental competence markers (genes and proteins) are presented in Table 1.

5. Microfluidic research

Flow phenomena and experiments on droplet dynamics in the last ten years have facilitated the development of the new field of microfluidics. Fluidic devices usually involve thin wafers, containing etched or printed interconnecting channels through which fluids are pumped and can react with each other. Usually, microfluidic devices have several inlet nodes and outlets that crosslink each other, and a structural layout characteristic for an expected reaction product. Previous results are fundamental for understanding the dynamics of reagents in the channels, the relation to pressure conditions and the choice of a critical path in channel branches. A mathematical model of fluid channels that is similar to electrical circuits of the Wheatstone bridge and which can be described by Kirchhoff's law has been developed. A matrix containing flow rates in columns and pressure values in rows allows the representation of every state in the model. As a new and rapidly growing field of science, microfluidics will find application in pharmacy, biotechnology, the life sciences, defence, public health, agriculture and in miniaturised laboratories, called Lab-on-Chips.

Microfluidic technology is based on the transport and/transfer of a small amount of fluid through the electronic system composed of a computer station and one or more integrated electronic devices. In the past few years these microfluidic devices were successfully used in several fields of industry, including husbandry, energetic economy, pharmacological industry, environmental protection and the monitoring of human health. Moreover, microfluidic technology was also applied in medicine, biotechnology and bioengineering. Although the microfluidic research in medicine and biotechnology is currently of high interest, there is a paucity of data regarding the application of microfluidics in reproductive biology research. The Lab-on-Chip system dedicated to reproductive research is mostly composed of microfluidic chips, which are formed of two or more optical fibres and a channel through which the oocyte/embryo is passed „in" and/or „out" of the chip. The most recent research indicated the possibility to apply these Lab-on-Chip systems in spectral characterisation of the analysed cell. The final results described the correlation between cell morphology and/or the physiological status with their spectral (based on absorbance) specificity. However, until now the application of these microfluidic systems in mammalian oocytes/embryos quality assessment is not widespread. The possibility of using Lab-on-Chip systems in reproductive medicine and biotechnology may allow a fuller characterisation of mammalian gametes and/or embryos developmental capacity and plasticity.

Furthermore, recent experiments indicated the opportunity of applying microfluidic technology

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in reproductive biology (Kempisty et al. 2011c; Kempisty et al. 2011e). Szczepanska et al. (2009, 2010) used the spectral images of oocytes to assess the quality of gametes isolated form different follicles. After a series of experiments applying spectral images of recovered porcine oocytes and fertilised bovine embryos, they presented the first non-invasive and objective methods to evaluate gamete and embryo quality.

6. Conclusions

Progress over several years has led to the hope that molecular research may lead to a full, comprehensive description of oocyte quality. However, all molecular assays are invasive and often lead to destruction of the investigated cell. The application of new microfluidic methods represents a novel non-invasive method for the assessment of gamete and embryos quality. The 21st century may come to be described as the 'nanomedicine century', in which nano- and/or micro-devices are used in health protection, disease diagnosis and medical micro-engineering. Moreover, microfluidic devices are also used in reproductive biology as a new method for assisted reproduction.

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