New molecular markers for the evaluation of gamete quality

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Abstract

Purpose Only 30 % of IVF cycles result in a pregnancy, so that multiple embryos need to be replaced, per treatment cycle, to increase pregnancy rates, resulting in a multiple gestation rate of 25 %. The use of new markers in the gamete selection, could reduce the number of the oocytes to be fertilized and embryos to be produced, but the tools to evidence the gamete competence remain unavailable and more studies are needed to identify bio-markers to select the best oocyte and sperm to produce embryos with higher implantation potentiality.

Methods To define oocyte competence, the apoptosis of the surrounding cumulus cells and the oxygen consumption rates for individual oocytes before fertilization seems to provide a non-invasive marker of oocyte competence and hence a quantitative assessment of the reproductive potential for the oocyte. The chromatin integrity seems to be used also as biological marker of sperm competence, together with the morphological evaluation of large vacuoles in the head.

Results The apoptosis rate of cumulus cells lower than 25 % and an higher oxygen consumption could be an evidence of an overall metabolic activity, related to a better fertilization ability and embryo cleavage quality. The apoptosis rate of the sperm chromatin, evaluated by direct Tunel in situ analysis, seems to be, also for the male gamete, a marker of competence and implantation potentiality, in particular when it is lower than 20 %. The evaluation of the presence of large vacuoles in the sperm head prior to perform ICSI seems to increase the implantation rate, but it is not associated to chromatin integrity.

Conclusions The biological concept of competence appears unrelated to any morphological parameters, so that it is necessary to investigate new molecular markers in the gamete selection. Apoptosis of cumulus cells in the oocytes and spermatozoa, revealing the presence of large vacuoles, could help to determine the competence of the gamete to be fertilize.

Keywords Apoptosis · Oocyte · Sperm · Competence

Capsule To identify bio-markers to select the best oocyte and sperm to produce embryos with higher implantation potentiality, is one of the main interests of the researchers. Cumulus cells apoptosis, oocyte oxygen consumption, vacuolization of sperm head and chromatin integrity seems to be a promising tools.

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Introduction

In the last years, new knowledge in the field of mammalian gamete biology has allowed to define the new concept of oocyte and sperm competence. The gamete competence is the ability to undergo successful fertilization, reach the blastocyst stage and implant in the uterus. Many researchers are working to identify markers to select the best oocyte and sperm to produce embryos with higher implantation potentiality. Only 32 % of IVF cycles result in a pregnancy [1], so that multiple embryos are replaced, per treatment cycle, to increase pregnancy rates, resulting in a multiple gestation rate of 25 %. Most of clinicians and embryologists, in the

world, are changing strategies in the number of embryos to be produced and transferred to avoid the risk of multiple pregnancies. The single embryo transfer (SET) is a new effective strategy to reduce multiple gestation rates, but its widespread acceptance is limited because it lacks of an accurate method of embryo assessment and selection for transfer during IVF cycles.

The use of new markers in the gamete selection could reduce the number of the oocytes to be fertilized and embryos to be produced, in particular in the Countries where the law limits the number of oocytes to be fertilized.

The oocyte competence

The oocyte is unique and highly specialized cell responsible for creating, activating and controlling the embryonic genome, as well as supporting basic processes, such as cellular homeostasis, metabolism and cell cycle progression in the early embryo. Oocyte quality is also related to early embryonic survival, and the establishment and maintenance of pregnancy, fetal development and adult disease. The biological competence for the oocytes is defined as the intrinsic ability to undergo meiotic maturation, fertilization, embryonic development and successful pregnancy. The developmental competence is gradually acquired during the long-lasting period of oogenesis. But it is important the final stage for optimal development, prior to ovulation, because the synchronization between nuclear and cytoplasmic maturation in the oocytes is completed [2].

Usually, in the IVF/ICSI clinical routine, the oocyte selection is based on the morphological parameters of the cytoplasm, polar body and cumulus cells [3, 4]. However, all the morphological criteria for grading and screening of oocytes are subjective and controversial, and seem to not be related to the intrinsic competence of the oocyte [5, 6]. Lot of research projects are designed to define objective and non-invasive molecular markers predictive of oocyte competence. What it is known is that the relationship between the oocyte and the surrounding somatic cells is more complex than previously thought, and represents a determining factor of later developmental competence [7]. Acquisition of oocyte competence is closely associated with normal follicular development, whereby the oocyte plays an active role in regulating the functions of surrounding somatic cells. It is currently established that the communication between cumulus cells and the oocyte is essential for the competence acquisition process. Therefore, identification of key molecules and signaling pathways within the oocyte–cumulus cell regulatory loop will be instrumental in gaining deep insights into the intricate mechanisms underlying the development of oocyte competence and uncovering novel regulators and reliable molecular predictors of oocyte quality.

These efforts will ultimately lead to improve efficiency and health outcomes of ART (i.e. reduced prematurity/perinatal mortality rate and maternal and pediatric complications) [2, 8, 9]. Oocytes clearly depend on the presence of follicle cells to generate specific cellular signals that coordinate their growth and maturation [10]. The fate of each follicle, in the ovary, can evolve towards the ovulation through specific pathways of follicular survival, or to the atresia in which predominate the apoptosis pathway.

From follicular apoptosis new tools in oocyte selection?

It has been demonstrated that the percentage of apoptotic cumulus cells in women who achieved pregnancy was considerably fewer compared with women who did not become pregnant. Moreover, DNA fragmentation in oocytes associated with apoptotic evidence might be one of the reasons for poor oocyte quality and lower fertility in aged mice [11]. A study on apoptosis in human oocytes collected in ICSI cycles, showed that apoptosis occurs also in human oocytes retrieved after hormonal stimulation and it is associated to the oocyte quality [12].

Apoptosis of the oocyte and of the surrounding cumulus cells can be used as a biological marker of competence? A recent study has demonstrated that in vitro-matured oocytes showed a higher rate of DNA fragmentation compared to in vivo-matured oocytes. The cleavage and blastocyst formation rates of in vitro-matured oocytes were significantly lower than those of in vivo-matured oocytes, evidence of a lower competence. A possible cause of the reduced developmental competence of in vitro-matured oocytes may be due to DNA fragmentation that was significantly higher than in vivo-matured oocytes based on the presence and length of the comet tail [13].

The affected developmental competence of in vitro matured oocytes could explain the lower implantation rate of IVM treatment cycles compared to conventional gonadotropin stimulated IVF cycles [14]. The use of DNA fragmentation as a biological marker of oocyte competence cannot be used to select the oocyte to be fertilized by ART. It is important to underline that oocyte apoptosis could be evidence of a non-adequate folliculogenesis in which are involved also the cumulus and granulosa cells. The presence of apoptosis in a pool of cumulus cells from immature oocytes compared with cumulus cells from mature oocytes in metaphase II was studied by Host et al. [15]. The study demonstrated a significant increase in the number of apoptotic cumulus cells in the pool of cells derived from immature oocytes (germinal vesicles and MI) compared with cumulus cells derived from mature oocytes (metaphase II). Although, during reproductive life, apoptosis is strictly related to the reduction of the number of follicles, we can

speculate that apoptotic events in follicles selected after hormonal stimulation, could be related to a reduced oocyte developmental competence. In physiology, a dominant and ovulating follicle should be a cellular compartment (theca, granulosa, cumulus cells an oocyte) in which the surviving pathway prevail over apoptosis. Thus, the chromatin integrity of the granulosa/cumulus cells should be considered as an important marker in defining adequate biological activity that would allow optimum synchronization of cytoplasmic and nuclear maturation of the oocyte. Different papers have evaluated the apoptosis rate in the cumulus cells to verify the relationship with clinical outcomes, in terms of pregnancy and implantation rate, demonstrating that apoptotic rate of cumulus cells could be used as a molecular marker in selecting oocytes with higher implantation potentiality. Recently, our study, focused on verifying whether the administration of LH could increase pregnancy and implantation rates by improving the quality of oocytes collected after multiple follicular growth therapy, has demonstrated that the administration of r-LH during the late follicular phase improves clinical outcomes, with an increased pregnancy and implantation rates, that may be correlated with the reduction of apoptosis evaluated in the cumulus cells of patients treated with r-LH compared with patients treated with r-FSH alone [16].

Apoptotic pathways

Close molecular communication between oocytes and granulosa cells exists during folliculogenesis (hypothesis of an oocyte-granulosa cell regulatory loop), essential for inducing and coordinating differentiation in the oocyte and in the somatic compartment. Several molecules are involved in specific pathways: oocyte growth differentiation factor 9 [17, 18], for example, seems to control the physiological synchronization of cytoplasmic and nuclear maturation [19, 20]. The mRNA of epidermal growth factor (EGF) was also found in granulosa, cumulus, and oocyte cells, and it acts on granulosa and cumulus cells with a strong anti-apoptotic effect [21]. Growth differentiation factor-9 synthesis is activated by EGF in the oocyte, with a positive effect on cumulus cells. EGF has been identified in the follicular fluid and shown to have a positive effect during the in vitro maturation (IVM) of immature oocytes in a variety of species, including cattle [22], pigs [23], rodents [24] and humans [25], and its receptor has been demonstrated on the surfaces of oocytes. The expression of EGF seems to be induced by LH in the theca cells, and as a molecular cascade, it involves granulosa, cumulus, and oocyte synthesis of EGF [26], preserving these cells from apoptotic destiny. New researches have confirmed the pivotal role of different molecules of the EGF family in supporting the survival pathways, playing an anti-apoptotic action [27, 28]. Other studies demonstrated that several molecules are involved in the control of apoptosis, in particular the extracellular signal-regulated kinase (ERK 1/2) pathway and the phosphorylation and activation of Akt [29]. Another important factor seems to be the Neuregulin-1(NRG-1) that activates PI3K/Akt signaling and inhibits apoptotic events [30].

New strategies in investigating oocyte competence

Oxygen consumption has often been regarded as a good indicator of overall metabolic activity. The measurement of Oxygen consumption rates for individual oocytes before fertilization seems to provide, also, a noninvasive marker of oocyte competence and hence a quantitative assessment of the reproductive potential for the oocyte [31]. It has been demonstrated that Oxygen consumption is related to fertilization ability, which is consistent with previous data regarding higher ATP turnover in mature oocytes. Lower ATP production (lower oxygen uptake) could compromise fertilization and subsequent embryo cleavage, and it could be used as an effective and non-invasive tool for selecting competent oocyte.

Oocyte quality and subsequent embryo development can be affected by factors such as nutrition, hormonal regulation, and environmental influence. Several studies are trying to identify genes expressed in oocytes and/or cumulus cells, across a diverse range of species, which may be linked to the ability of the oocyte to develop following fertilization. Some of these seem to drive to the identification of 56 candidate genes associated with oocyte quality across several species, 4 of which were identified in the cumulus cells that surround the oocyte. Twenty-one potential biomarkers were associated with increased competence and 35 potential biomarkers were associated with decreased competence [32]. These genes could potentially act as biomarkers of oocyte competence or as pharmacological targets for manipulation in order to improve oocyte developmental potential, but more research is needed to apply in the routine of IVF clinic.

The sperm competence

In the last decade new knowledge on sperm biology, allowed us to speculate that the concept of biological competence could be considered also for the male gamete. The traditional sperm parameters, concentration, progressive motility and morphology, seem to be good marker to predict clinical outcome in natural cycle [33], in intra-uterine insemination cycles [34] and in conventional IVF [35], but not for ICSI outcome. The selection of a single sperm, according to traditional parameters, does not allow embryologists

to select the sperm with the highest developmental potential. Routinely, the selection of the sperm before ICSI is performed according to motility and morphology evaluation, avoiding to use immotile and abnormal sperm, preferring sperm with normal head (normal shape, normal size, having an acrosome), no mid-piece and tail defects.

The chromatin integrity as biological marker of sperm competence

During recent years, in assisted reproductive technology (ART), in the clinical setting, the nuclear deterioration of human sperm, in particular DNA fragmentation, as a consequence of double-strand breaks, attracts attention, considering DNA fragmentation index (DFI) as an independent predictor of fertility in couples undergoing intrauterine insemination [36]. In contrast to the standard semen parameters, which do not act as powerful discriminators between fertile and infertile men, sperm DNA damage assessment seems to yield better prognostic value. It has been shown that fecundity starts to decrease when sperm DFI exceeds 15 % [37]. Above a threshold of 30 %, chances for pregnancy are close to zero, either by means of natural conception or intrauterine insemination. This shows that the DFI possesses a high predictive value for male infertility in vivo [36]. Several authors have demonstrated a relationship between high DFI value and clinical outcomes in ART cycles. If a sperm with damaged DNA is incorporated into the embryonic genome, it may lead to sperm derived chromosomal aberrations [38] which may in turn result in higher miscarriage rates [39] and an increased risk of pregnancy loss [40]. The resultant aberrations can also be potentially inherited through the germ line for generations.

Higher percentage of fragmented chromatin in a sperm seminal sample compared with that of low chromatin fragmentation, is associated with a reduced pregnancy and implantation rate after conventional IVF [41] and ICSI [42]. The DNA fragmentation could be related to different modification of gene expression and epigenome [43]. The high magnification morphological selection of motile spermatozoa (MSOME) in performing IMSI, was proposed [44] to increase ICSI clinical outcomes, emphasizing that sperm morphology may have a significant impact on pregnancy outcome in ICSI treatments. In particular, the authors focused on the role of large vacuoles in the sperm head probably evidencing chromosomal or DNA defect. Other authors have demonstrated that the presences of sperm nuclear large vacuoles are related to an abnormal sperm chromatin packaging [45].

A recent publication demonstrated that there is a close relationship between nuclear vacuoles and clinical outcomes after ICSI, confirming that the nuclear morphology could be considered a morphological marker of developmental competence, but without any relationship with the DNA fragmentation [46, 47]. To date, does not exit any tools to select sperm with chromatin integrity. The IMSI, birefringence of sperm head [48], Hyaluronic acid- sperm selection [49] seem to increase the clinical outcome after ICSI, but none of these techniques is associated with the quality and integrity of the sperm chromatin.

Conclusions

The developmental fate of the embryo is principally dictated by the oocyte and sperm competence. The biological concept of competence seems to be not related to any morphological parameters, so that it is necessary to investigate new molecular markers in the gamete selection. In the last years an increased attention has been given to the gamete DNA integrity and the epigenetic effects on the embryo development. The embryologists know more about the molecular communication between somatic and germinal cells in the follicle, and about the molecules involved in the control of surviving pathways, in particular in the dominant follicle. Therefore, apoptosis of cumulus cells may be a marker of oocyte competence and it could be an objective and non-invasive molecular markers predictive of oocyte competence, to be used in selecting the best oocytes to be fertilized in ART cycles. On the sperm side, only the morphological evaluation, at higher magnification, of the sperm head nucleus, that reveals the presence of large vacuoles, could help to determine the competence of the sperm population provided for injection and is likely to play an important role in ensuring the safety of clinical ICSI. It is necessary to improve researches in better defining the biological conditions that lead to the competence acquisition in the human gametes, so to give effective tools to select gametes with higher development potential, to reduce the number of the oocytes to be fertilized and embryos to be produced.

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