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Origins and effects of oocyte quality in cattle

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Abstract

Oocyte quality is the resultant of multifactor interactions that should be carefully taken into account either for *in vitro* embryo production technologies or for studying follicle dynamics. Different approaches may be used to perform an analysis of variables related to oocyte quality which may focus on the ovary, the follicle, the cumulus-oocyte complex and, finally, the oocyte. The information obtained may answer key questions, such as what does the oocyte need to acquire meiotic competence and whether follicle activity can be manipulated to improve the *in vitro* embryo production efficiency. Although morphological evaluation represents the most common procedure used to discriminate the developmental potential of the oocyte and in spite of a good relationship that has been found between the quality of the oocyte and the atresia grade of the follicle that comprises it, there is not yet a clear correspondence between the visual criteria and the developmental competence of oocytes submitted to *in vitro* embryo production. New technologies have become available, including emerging 'omics' sciences that, through analysis of the cumulus cells, offer the opportunity, by a non-invasive method, to indirectly predict the developmental potential of the oocyte.

Keywords: bovine, cumulus-oocyte complex, *in vitro* embryo production, oocyte quality.

Introduction

Whatever productive procedure is performed, the quality of the raw materials involved strongly affects either the production efficiency or the characteristics of the end-product. A high oocyte quality is the prerequisite for *in vitro* embryo production. However, an extremely heterogeneous population of oocytes is commonly collected from ovaries to be used for *in vitro* technologies.

Sources of variability of the oocyte quality may be the age of these cells (Yamamoto *et al*., 2010), the stage of the estrous cycle (Tan *et al*., 1990; Wurth *et al*., 1994), hormonal patterns (Kruip and Dieleman, 1982) and biochemical characteristics of the follicular fluid (Wise, 1987; Kastrop *et al*., 1991b), diameter (Tan *et al*., 1990; Pavlok *et al*., 1992; Lonergan *et al*., 1994; Wurth *et al*., 1994) and atresia grade of the follicle (Wurth and Kruip, 1992; Wurth *et al*., 1994), and ovarian morphology (Gandolfi *et al*., 1997). Inevitably, this variability affects the efficiency of *in vitro* embryo production (IVEP).

First of all, it is necessary to fully define the meaning of oocyte quality. Under a functional point of view, oocyte quality may coincide with its developmental competence. Sirard *et al*. (2006) describes five levels of oocyte competence, i.e., the ability: (1) to resume meiosis; (2) to cleave following fertilization; (3) to develop to the blastocyst stage; (4) to induce a pregnancy and bring it to term; and (5) to develop to term in good health. These abilities may originate by separate events and succeeding in the first events does not ensure the success of the subsequent ones.

A functional evaluation is, however, difficult to be performed at the time of oocyte collection. Several strategies were, hence, employed in order to provide a predictive value of the *in vitro* embryo production potential of the collected oocytes. Most of these evaluations are based on morphological criteria which are assumed to be related to the physiological status of the follicle. The relationship between morphological and functional criteria, however, does not always provide results coming up to our expectations.

Besides the predictive value, the analysis of oocyte quality may provide useful information on the mechanisms underlying the oocyte maturation and developmental competence and suggest new possibilities to improve the low efficiency of IVEP. In order to define the effects of several variables on oocyte quality, we need to figure out the limits of a range of variability in which *in vivo* oocyte maturation may represent the highest level of efficiency (Leibfried-Rutledge *et al*., 1987) whereas *in vitro* maturation (IVM) in pre-pubertal oocytes may represent one possible opposite limit. The analysis of these extreme conditions should allow the elucidation of the mechanisms that underlie the acquisition of meiotic and developmental competence and provide useful information to address the *in vitro* conditions towards the best solution.

A detailed comparison of morphological features between *in vivo vs. in vitro* maturation of cattle oocytes has been proposed by de Loos *et al*. (1989, 1991a, 1992). These authors grouped the collected cumulus-oocyte complexes (COCs) in four classes which were related to their morphological characteristics. After *in vitro* maturation, the COCs formed a heterogeneous group either among or within classes. On the other hand, *in vivo* matured COCs formed a homogeneous group with respect to their morphological characteristics. In addition, compared to *in vivo* matured, the *in vitro* matured COC showed: (1) retraction of the cumulus cell process endings from the

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oocyte without the breaking down of these processes; (2) retardation of some aspects of cytoplasmic maturation; and (3) incomplete cumulus expansion. The same research group evaluated the protein synthesis and phosphorylation patterns of bovine oocytes which were matured either *in vivo* (Kastrop *et al*., 1991b) or *in vitro* (Kastrop *et al*., 1991a). Changes in protein synthesis were observed after germinal vesicle break-down (GVBD) coinciding in the case of *in vivo* maturation at approximately 8 h after the preovulatory LH peak and in *in vitro* maturation at the 8th h of culture. Also the protein phosphorylation patterns showed a significant change in relation to GVBD either *in vivo* or *in vitro*; this suggests that specific phosphoproteins accomplish an essential function during maturation and may be involved in GVBD. Rizos *et al*. (2002) examined the effect of oocyte maturation, fertilization and culture *in vivo vs. in vitro* on the proportion of oocytes reaching blastocyst stage and on blastocyst quality as measured by survival following vitrification. Significantly more blastocysts developed from oocytes matured *in vivo*; moreover, *in vitro* fertilization and culture depressed embryo survival rate following vitrification. Recently, Kats-Jaffe *et al*. (2009) carried out a transcriptomic analysis comparing *in vivo* and *in vitro* matured oocytes. Quantitative real-time PCR validated the microarray data and also revealed altered expression levels after IVM of specific putatively imprinted genes. Distinct transcription patterns reflected the response of the oocyte to its surrounding environment.

Juvenile oocytes represent an interesting experimental paradigm (Revel *et al*., 1995; Duby *et al*., 1996; Armstrong, 2001; Marchal *et al*., 2001; Kochhar *et al*., 2002). Studies in sheep and in cattle demonstrated a lower developmental potential for prepubertal oocytes (O'Brien *et al*., 1996, 1997; Ledda *et al*., 1997). This was attributed to the different morphological and physiological properties, such as smaller size (Gandolfi *et al*., 1998; Ledda *et al*., 1999), delayed organelle migration and their redistribution throughout the ooplasm during maturation (Damiani *et al*., 1996; O'Brien *et al*., 1996), and lower number of cumulus-oocyte communications (Ledda *et al*., 2001) of the prepubertal compared to the adult oocytes. Moreover, prepubertal oocytes showed lower metabolic activity than adult oocytes in relation to protein synthesis (Gandolfi *et al*., 1998; Ledda *et al*., 2001), glucose metabolism (Steeves and Gardner, 1999), glucose and pyruvate (Steeves *et al*., 1999) and glutamine (O'Brien *et al*., 1996) uptakes. By evaluating maturation promoting factor (MPF) and mitogen-activated protein kinase (MAPK) activities or performing reciprocal transfer of metaphase II chromosomes between cow and calf oocytes, Salamone *et al*. (2001) further supported the hypothesis that developmental competence of juvenile oocytes has a low efficiency due to a failure or inability to complete ooplasmic maturation. However, developmental competence of juvenile oocytes increases following hormonal treatment (Armstrong *et al*., 1997), and is positively related to follicle size (Kauffold *et al*., 2005). Recently, we compared several variables related to oocyte meiotic competence, i.e., oocyte plasma membrane electrical properties, IP3 sensitivity and calcium stores as well as the incidence of apoptosis in cumulus cells in juvenile and adult sheep oocytes (Boni *et al*., 2008). This analysis did not reveal significant differences in intracytoplasmic calcium stores and plasma membrane electrical properties between young and adult oocytes. IP3 sensitivity strongly increased after *in vitro* maturation following a dose-dependent pattern with a significant interaction between dose and maturational stage. The incidence of apoptosis in cumulus cells strongly increased after *in vitro* maturation and was greater in adult than in juvenile cumulus cells.

Different approaches may be used to perform an analysis of variables related to oocyte quality that may focus on the ovary, the follicle, the cumulus-oocyte complex (COC) and, finally, the oocyte. The information obtained may answer key questions, such as what the oocyte needs to acquire meiotic competence and whether follicle activity can be manipulated to improve IVEP efficiency.

Oocyte quality is related to ovarian features

Follicle dynamics varies in relation to several variables that affect both ovarian characteristics and oocyte quality. These variables may be attributed to the environment, such as seasonal variations, heat stress, or to physiological status, such as post-partum interval, hormonal status, milk production, energy balance, nutrition, estrous cycle activity, or to genetics. Consequently, a large variation of follicle/oocyte population may explain the size of the ovary, the number of follicles and the oocyte quality.

The effect of the stage of the estrous cycle on oocyte quality and on *in vitro* embryo production efficiency was evaluated by Wurth *et al.* (1994). By separating ovaries collected in pairs from individual cows in relation to the stage of the corpus luteum as follows: early luteal (0- to 7-day), late luteal (8- to 17-day), follicular phase (18- to 20-day) and non-cyclic ovaries, they did not find significant differences in relation to oocyte quality evaluated on the basis of morphological features; however, a significant increase in blastocyst production was found in oocytes collected from early luteal phase ovaries. These results were confirmed in a subsequent study of the same group (de Wit *et al*., 2000). Chohan and Hunter (2003), analyzing the effect of the reproductive status on *in vitro* developmental potential of bovine oocytes, also found the highest developmental competence in oocytes collected from metaestrus cows while the lowest competence was found in oocytes from pregnant and anoestrus cows in spite of the fact that these latter categories showed *in vitro* maturation and fertilization efficiencies which were not different from

those of the other reproductive categories.

Gandolfi *et al*. (1997) proposed an ovarian evaluation criterion on the basis of the number and size of the visible follicles. Ovaries were separated in 3 categories on the basis of (A) presence of a follicle >10 mm in diameter, (B) presence of more than 10 follicles from 2 to 5 mm in diameter and no follicles >10 mm and (C) presence of less than 10 follicles from 2 to 5 mm in diameter and no follicles >10 mm. The lowest number of COCs was collected from the category C ovaries. In addition, the COCs of this category showed the lowest *in vitro* embryo production efficiency and yielded blastocysts with the lowest number of cells. The authors did not have a solid explanation for this finding; either systemic factors as diet, environment, age, season or individual variations of growth factors, which mediate intra-ovarian stimuli, have been suggested as possible reasons. A further evaluation of ovaries collected in pairs from individual cows showed that only 16% of the cows had both ovaries of C category whereas in 39% of the cows the C category ovaries were coupled with A and B category ovaries.

Oocyte quality is related to follicle characteristics

A large proportion of the variability of COC morphology is explained by the atresia grade of the follicle that comprises it. The COCs collected from an ovary represent the cumulative result of follicles which are involved in growing or degenerative processes as regulated by follicular wave dynamics. If the growth stage can be evaluated on the basis of follicle size, the atresia grade may be evaluated by using different approaches, such as macroscopic and microscopic analysis, biochemical and hormonal procedures, etc.

Pavlok *et al*. (1992) compared the *in vitro* developmental capabilities of oocytes collected from follicles grouped on the basis of their size as follows: group A, 4-8 mm (large); group B, 2-4 mm (medium); and group C, 1-2 mm (small). A significantly lower fertilization rate was found in group C oocytes that completely lacked the capability to cleave beyond the 8-cell stage. The remaining 2 groups of oocytes did not show significant differences in relation to oocyte maturation and fertilization efficiency; however, the group A oocytes showed a higher 7-day blastocyst and expanded/hatched blastocyst rates that group B oocytes. Later on, Wurth *et al*. (1994), Rizos *et al*. (2002) and Machatkova *et al*. (2004) confirmed this finding and demonstrated a positive relationship between follicle size and developmental competence of the oocyte.

Kruip and Dieleman (1982) dissected bovine antral follicles free of stromal tissue and classified them under a stereomicroscope, as proposed by Moor *et al*. (1978) in sheep. In particular, follicles were classified as (1) *non-atretic* if they had uniformly bright appearance, an extensive and very fine vascularization, a regular granulosa layer and no free-floating particles in the follicular fluid; (2) *light-atretic* if they lost the translucent appearance, becoming slightly grayish, and might contain some free-floating particles in the follicular fluid; (3) *atretic* if they had a dull, gray appearance, blood vessels either irregularly filled with clotted blood or empty and many free-floating particles in the follicular fluid; (4) *heavy-atretic* if they showed a dark, often spotted, appearance and dark cumulus. These follicle classes were fixed, embedded, sectioned and stained for histological evaluation. The micromorphological criteria used were: (1) the presence of degenerated cells in the granulosa layer; (2) the presence of mitotic figures in the cumulus cells, granulosa cells and theca interna; (3) the organization and structure of the granulosa layer; (4) the continuity of the basal lamina. Finally, follicles of one cow were dissected and follicular fluid was individually collected. The follicular fluid was analyzed for 17β-estradiol content and related to the macroscopic and microscopic features of each analyzed follicle. A high correlation was found between the macroscopic and microscopic follicle evaluations. In addition, 17β-estradiol follicle content significantly increased along with follicle diameter and was inversely related to atresia grade. On the basis of this follicle evaluation methodology, Theo Kruip's teamwork proposed several COC classification criteria that related follicular atresia grade to the oocyte developmental potential (see below).

Oocyte quality is related to COC morphology

A first discriminatory analysis of COCs used for IVM was carried out by Leibfried and First (1979). This and several following studies were based on the compactness and quantity of cumulus cells surrounding the oocyte. So far, these investigations have led to the conclusion that 1) partial or total loss of the cumulus layer reduces developmental rates (Staigmiller and Moor, 1984; Hawk and Wall, 1994) and 2) the "best-looking" COCs (oocytes with a multilayered and compact cumulus layer) do not necessarily have the highest developmental competence (Hazeleger *et al*., 1995; Blondin and Sirard, 1995).

De Loos *et al*. (1989) distinguished four classes (i.e., category-1 to -4) of COCs based on the compactness and transparency of the cumulus layer and the homogeneity and transparency of the ooplasm. These COC classes were ultrastructurally analyzed and their capacity to mature *in vitro* was investigated. Although the morphological differences among these COC categories were confirmed at the ultrastructural level, they did not reflect different maturation capacities. Only the degenerating category-4 COCs exhibited a decreased capacity to mature *in vitro*.

The same research group used this morphological classification to associate the oocyte developmental potential to protein synthesis, which was evaluated as amino acid incorporation (Kastrop *et al*., 1991a, b). At the immature stage, similar incorporation rates and identical protein synthesis patterns were

observed between oocytes in categories 1-3. A lower incorporation was found in category-4 COCs which might be due to the lower number of penetrating junctional complexes (de Loos *et al*., 1989). After *in vitro* maturation, a lower incorporation of 35S-methionine was found in all COC categories; however, the patterns of category 4 were identical to the patterns of those in categories 1-3.

A refined evaluation aimed to better associate morphological characteristics and developmental competence of the COCs was proposed by Wurth and Kruip (1992). They distinguished three COC morphological grades in relation to the cumulus surrounding the oocytes and the ooplasm characteristics, designated as follows: A) presence of a clear and compact cumulus and translucent ooplasm, B) dark and compact cumulus and dark ooplasm, and C) dark and expanded cumulus and dark ooplasm. By using the follicle evaluation criteria proposed by Kruip and Dieleman (1982), they found that A-COCs showed the same IVEP efficiency irrespective of the follicular origin; B-COCs, originating from heavily-atretic follicles, showed a significantly lower IVEP efficiency in comparison to the B-COCs from the other follicle qualities.

Wurth *et al*. (1994) compared the COC classification as proposed by de Loos *et al*. (1989) with 1-4 COC categories to this new COC classification with A-C COC grades. They found that the A-C COC classification system was more precisely associated to the follicle quality than the 1-4 classification system. The A-COCs were mostly contained in non-atretic follicles and rarely found in heavy-atretic follicles. The C-COCs were mostly found in heavy-atretic follicles and never found in non atretic-follicles. The B-COCs progressively increased from non-atretic follicles to heavily-atretic follicles. This gross and simple classification avoided wasting time for follicle dissection and evaluation, and provided consistent information regarding the *in vitro* developmental potential of different COC grades. Surprisingly, the B-COC grade showed the highest IVEP potential, despite the fact that these originate mostly from atretic follicles. This was independent from cyclic activity and stage of the estrous cycle of the donor (Wurth *et al.*, 1994) and was confirmed by other authors with different classification criteria (Blondin and Sirard, 1995; Nagano *et al*., 2006; Li *et al*., 2009). Up to this date, there is no explanation for this finding; it may be due to the reduction of meiotic-arresting factor (cAMP) levels in the oocyte (Aktas *et al*., 1995) because of a decrease of cumulus-oocyte communications during atresia (de Loos *et al*., 1991b). It is, however, noteworthy to consider that there are significant differences in oocyte size among these classes, as referred by de Wit and Kruip (2001), who recorded in A-COC the smallest oocyte size.

Very recently, we used these morphological criteria to search for causes of variability in changes in Ca^{2+} intraooplasmic concentrations in COCs following gonadotropin exposure (Silvestre *et al*., 2012). Together with cAMP, Ca^{2+} is a candidate signal for resumption of meiosis. We evaluated the intracellular calcium ($[Ca^{2+}]i$) rise which was evoked by *in vitro* maturation promoters and we characterized the origin of this signal. This $[Ca^{2+}]i$ rise resulted from extra-, inter- and intra-cellular cumulative Ca^{2+} fluxes and was significantly affected by the COC quality grade as well as by follicle size. In particular, a significant decrease in responsiveness to gonadotropin stimuli was observed in C-COCs with respect to A- and B-COCs. The distribution of LH receptors at immunohistochemical localization in these three COC classes is shown (Fig.1). Follicular size was also important in determining the intensity of the COC responses to FSH; in fact, we observed an increase in the FSH-evoked $[Ca^{2+}]i$ surge when the follicular size increased from <2 mm to 2-4 mm diameter. Since we did not find any further improvement in $[Ca^{2+}]i$ responses in follicles larger than 4 mm, we argued that 2 mm diameter represents a threshold size of the bovine follicle that may mark different developmental competence of the related oocyte.

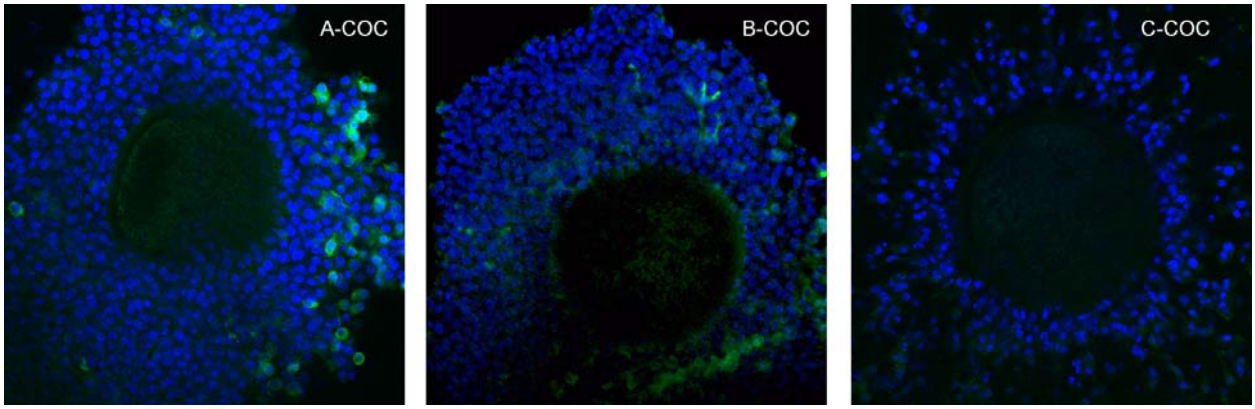

Figure 1. LH receptor immunohistochemical localization by confocal microscopy analysis in bovine immature cumulus–oocyte complexes divided in three categories (i.e., A-, B- and C-COC) on the basis of morphological criteria by Wurth and Kruip (1992). COCs were treated with goat anti-LHR antibodies, and then with FITC-marked anti-goat antibodies (green). Nuclei were stained with DAPI (blue).

Oocyte quality may be determined by cumulus cell examination

Oocyte quality evaluation based on morphological criteria may discriminate a group of collected oocytes with a higher developmental potential. Special needs, first required in human IVF and later on also in livestock animals, stimulated scientists to move towards more sophisticated technologies which have become available, including emerging 'omics' sciences, such as genomics, transcriptomics, proteomics and metabolomics. In this way, the study of the cumulus cell (CC) transcriptomic profile offers the opportunity, by a non-invasive method, to predict oocyte and embryo competence because bidirectional traffic of molecules between CCs and the oocyte is crucial for the acquisition of this competence. Using either RT-PCR or DNA microarrays, some studies have provided the basis for identifying genes expressed in CCs that could function as potential biomarkers to predict embryo quality and pregnancy outcomes.

A microarray analysis carried out in bovine cumulus cells (Assidi *et al*., 2008) revealed that the main candidates expressed in cumulus cells that could be valuable as indirect markers of oocyte competence are a set of genes, i.e., hyaluronan synthase 2 (HAS2), inhibin βA (INHBA), epidermal growth factor receptor (EGFR), gremlin 1 (GREM1), betacellulin (BTC), CD44, tumor necrosis factor-induced protein 6 (TNFAIP6), and prostaglandin-endoperoxide synthase 2 (PTGS2). These biomarkers could be potential candidates to predict oocyte competence and to select higher-quality embryos for transfer.

Oocyte quality may be evaluated on the basis of several oocyte characteristics

Several oocyte characteristics related to the ooplasm, the oolemma and the nuclear status have been analyzed in order to evaluate the quality of the collected oocytes. Unfortunately, most of these evaluation procedures require the removal of the cumulus layer or even more invasive procedures that are not compatible with the viability and the developmental competence of the oocyte. Hence, they cannot demonstrate the truthfulness of the analysis. The variability of these measurements was analyzed and related to non-invasive evaluation criteria resulting in correlations that may help to better define the oocyte quality. Careful investigations was conducted concerning the ultrastructural analysis of the oocyte, such as the evaluation of oocyte diameter, the distribution of organelles such as mitochondria and cortical granules, plasma membrane electrical properties, intracytoplasmic calcium stores, etc.

A detailed ultrastructural analysis of the cattle oocyte has been described by Hyttel and co-workers (reviewed by Hyttel, 2011).

Mitochondria play a vital role in the metabolism of energy-containing compounds in the oocyte cytoplasm to provide adenosine triphosphate (ATP) for fertilization and preimplantation embryo development. Confocal analysis of the oocytes labeled with the mitochondrion-specific membrane potential-sensitive fluorescent dye JC-1 allows to measure the activity of mitochondria in oocytes which are characterized by distinct localized aggregation patterns. In human, the activity of mitochondria in metaphase II oocytes was negatively correlated with either maternal age or the rate of embryo development on day 3 after fertilization (Wilding *et al*., 2001). Since maternal age is strongly related to oocyte developmental potential, mitochondrial activity may be considered a good marker for oocyte quality. In cattle, the mitochondrial activity has been used recently as marker of oocyte/embryo quality for evaluating the effect of *in vitro* culture in serum-free culture media (Abe and Hoshi, 2003) and in freezing/vitrification protocols (Abe *et al*., 2011).

The distributional changes of cortical granules **(**CG) within the ooplasm have been proposed as a marker of oocyte quality. Hosoe and Shiova (1997) classified bovine oocytes in relation to the morphology of their cumulus cell layers as follows: class A, compact and thick; class B, compact but thin; class C, naked; and class D, expanded. After the complete removal of cumulus cells, a part of these oocytes was stained for CG examination with *Lens culinaris* agglutinin before and after *in vitro* maturation and fertilization. The resulting distributional patterns of the CG were classified into four types: type I, CG distributed in clusters; type II, CG dispersed and partly clustered; type III, all CG dispersed; and type IV, no CG. Most of the oocytes before culture showed a type I pattern, but this pattern decreased after *in vitro* maturation, whereas type III increased in class A. The oocytes of class B showed similar changes to class A. In class C, many oocytes showed type I after culture, indicating that cytoplasmic maturation was not completed. In class D, 80.4% of the oocytes exhibited type III before *in vitro* maturation, indicating that their cytoplasmic maturation was different from classes A-C. Approximately 70% of class D oocytes were at the nuclear stage of GVBD before culture. The developmental rates to blastocysts in classes A-D were 28.7, 23.1, 0.5 and 3.4% respectively.
The electrical properties of the oocyte plasma membrane and intracytoplasmic calcium stores were analyzed by Boni *et al*. (2002) and related to the COC morphological evaluation proposed by Wurth and Kruip (1992). The oocyte is an electrogenic cell, i.e. capable of responding to electrical stimuli and modifying its electrical properties during the crucial periods of maturation and fertilization. Ion channels have been widely demonstrated on the plasma membrane of the oocyte in all animals studied, and electrical modifications in the oocyte plasma membrane are due to ion currents that are modulated via these ion channel

(Tosti *et al*., 2002; Tosti and Boni, 2004). The modification of intracellular calcium levels in gametes has been extensively studied, and these modifications are recognized to be a second messenger system for gamete maturation and fertilization (Boni *et al*., 2007; Malcuit and Fissore, 2011; Silvestre *et al*., 2011). Intracellular Ca^{2+} loading is largely expressed during early maturation and later decreases (Tosti *et al*., 2000). Our studies (Boni *et al*., 2002) confirmed the different developmental potential between the three COC classes proposed by Wurth and Kruip (1992) either following IVF or parthenogenic activation. In immature COCs, the amplitude of L-type Ca^{2+} channel activity was significantly higher in B- and C-COCs than in A-COCs ($P < 0.01$). Following Ca^{2+}-ionophore A23187 exposure, the resting potentials significantly hyperpolarized in the oocytes of all COC categories, reaching the highest values in B-COCs. The Ca^{2+} stores were significantly greater in A-COCs than in B- and C-COCs in the case of immature oocytes, and greater in B-COCs than in C- and A-COCs in the case of *in vitro* matured oocytes. These results demonstrated that, in the bovine, plasma membrane Ca^{2+} current activities are related to developmental potential in the immature oocyte, and calcium stores are related to morphological quality in immature oocytes and to developmental competence in *in vitro* matured oocytes.

Conclusions

Many approaches may be used for evaluating the quality of the collected oocytes. The method used and the arbitrary threshold for quality discrimination contribute to the variability of IVEP efficiency among laboratories. The awareness that the chosen standard of oocyte quality affects the quality and the development of the produced embryo to term lays the basis for improving IVEP efficiency in the future.

References

Abe H, Hoshi H. 2003. Evaluation of bovine embryos produced in high performance serum-free media. *J Reprod Dev*, 49:193-202.

Abe Y, Takakura K, Kaito K, Ogawa T, Yokoo M, Abe H. 2011. Effect of vitrification at germinal vesicle stage on the mitochondrial and cytoskeletal integrity in bovine oocytes. *Reprod Fertil Dev*, 24:134. (abstract).

Aktas H, Wheeler MB, First NL, Leibfried-Rutledge ML. 1995. Maintenance of meiotic arrest by increasing cAMP may have physiological relevance in bovine oocytes. *J Reprod Fertil*, 105:237-245.

Armstrong DT, Kotaras PJ, Earl CR. 1997. Advances in production of embryos in vitro from juvenile and prepubertal oocytes from the calf and lamb. *Reprod Fertil Dev*, 9:333-339.

Armstrong DT. 2001. Effects of maternal age on oocyte developmental competence. *Theriogenology*, 55:1303-1322.

Assidi M, Dufort I, Ali A, Hamel M, Algriany O, Dieleman S, Sirard MA. 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.

Blondin P, Sirard MA. 1995. Oocyte and follicular morphology as determining characteristics for developmental competence in bovine oocytes. *Mol Reprod Dev*, 41:54-62.

Boni R, Cuomo A, Tosti E. 2002. Developmental potential in bovine oocytes is related to cumulus-oocyte complex (COC) grade, calcium current activity and calcium stores. *Biol Reprod*, 66:836-842.

Boni R, Gualtieri R, Talevi R, Tosti E. 2007. Calcium and ion currents in relation to fertilization and oviduct-sperm interaction. *Theriogenology*, 68S:156-164.

Boni R, Cocchia N, Silvestre F, Tortora G, Lorizio R, Tosti E. 2008. Juvenile and adult immature and in vitro matured ovine oocytes evaluated in relation to membrane electrical properties, calcium stores, IP3 sensitivity and apoptosis occurrence in cumulus cells. *Mol Reprod Dev*, 75:1752-1760.

Chohan KR, Hunter AG. 2003. Effect of reproductive status on in vitro developmental competence of bovine oocytes. *J Vet Sci*, 4:67-72.

Damiani P, Fissore RA, Cibelli JB, Long CR, Balise JJ, Robl JM, Duby RT. 1996. Evaluation of developmental competence, nuclear and ooplasmic maturation of calf oocytes. *Mol Reprod Dev*, 45:521-534.

de Loos F, van Vliet C, van Maurik P, Kruip TAM. 1989. Morphology of immature bovine oocytes. *Gamete Res*, 24:197-204.

de Loos FA, Bevers MM, Dieleman SJ, Kruip TAM. 1991a. Morphology of preovulatory bovine follicles as related to oocyte maturation. *Theriogenology*, 35:527-535.

de Loos F, Kastrop P, van Maurik P, van Beneden ThH, Kruip TAM. 1991b. Heterologous cell contacts and metabolic coupling in bovine cumulus-oocyte complexes. *Mol Reprod Dev*, 28:255-259.

de Loos F, van Maurik P, van Beneden T, Kruip TAM. 1992. Structural aspects of bovine oocyte maturation in vitro. *Mol Reprod Dev*, 31:208-214.

de Wit AA, Wurth YA, Kruip TA. 2000. Effect of ovarian phase and follicle quality on morphology and developmental capacity of the bovine cumulus-oocyte complex. *J Anim Sci*, 78:1277-1283.

de Wit AA, Kruip TA. 2001. Bovine cumulus-oocyte-complex-quality is reflected in sensitivity for alpha-amanitin, oocyte-diameter and developmental capacity. *Anim Reprod Sci*, 65:51-65.

Duby RT, Damiani P, Looney CR, Fissore RA, Robl JM. 1996. Prepubertal calves as oocyte donors: promises and problems. *Theriogenology*, 45:121-130.

Gandolfi F, Luciano AM, Modina S, Ponzini A,

Pocar P, Armstrong DT, Lauria A. 1997. The in vitro developmental competence of bovine oocytes can be related to the morphology of the ovary. *Theriogenology*, 48:1153-1160.

Gandolfi F, Milanesi E, Pocar P, Luciano AM, Brevini TAL, Acocella F, Lauria A, Armstrong DT. 1998. Comparative analysis of calf and cow oocytes during in vitro maturation. *Mol Reprod Dev*, 49:168-175.

Hawk HW, Wall RJ. 1994. Improved yields of bovine blastocysts from in vitro-produced oocytes. I. Selection of oocytes and zygotes. *Theriogenology*, 41:1571-1583.

Hazeleger NL, Hill DJ, Stubbings RB, Walton JS. 1995. Relationship of morphology and follicular fluid environment of bovine oocytes to their developmental potential in vitro. *Theriogenology*, 43:509-522.

Hosoe M, Shioya Y. 1997. Distribution of cortical granules in bovine oocytes classified by cumulus complex. *Zygote*, 5:371-376.

Hyttel P. 2011. Electron microscopy of mammalian oocyte development, maturation and fertilization. *In*: Tosti E, Boni R (Ed.). *Oocyte Maturation and Fertilization*: *A long history for a short event*. Dubai: Bentham Science Publ. pp. 1-37.

Kastrop PM, Bevers MM, Destrée OH, Kruip TA. 1991a. Analysis of protein synthesis in morphologically classified bovine follicular oocytes before and after maturation in vitro. *Mol Reprod Dev*, 26:222-226.

Kastrop PM, Bevers MM, Destrée OH, Kruip TA. 1991b. Protein synthesis and phosphorylation patterns of bovine oocytes maturing in vivo. *Mol Reprod Dev*, 29:271-275.

Katz-Jaffe MG, McCallie BR, Preis KA, Filipovits J, Gardner DK. 2009. Transcriptome analysis of in vivo and in vitro matured bovine MII oocytes. *Theriogenology*, 71:939-946.

Kauffold J, Amer HA, Bergfeld U, Weber W, Sobiraj A. 2005. The in vitro developmental competence of oocytes from juvenile calves is related to follicular diameter. *J Reprod Dev*, 51:325-332.

Kochhar HP, Wu B, Morris LH, Buckrell BC, Pollard JW, Basrur PK, King WA. 2002. Maturation status, protein synthesis and developmental competence of oocytes derived from lambs and ewes. *Reprod Domest Anim*, 37:19-25.

Kruip TAM, Dieleman SJ. 1982. Macroscopic classification of bovine follicles and its validation by micromorphological and steroid biochemical procedures. *Reprod Nutr Dev*, 22:465-473.

Ledda S, Bogliolo L, Calvia P, Leoni G, Naitana S. 1997. Meiotic progression and developmental competence of oocytes collected from prepubertal and adult ewes. *J Reprod Fertil*, 109:73-78.

Ledda S, Bogliolo L, Leoni G, Naitana S. 1999. Follicular size affects the meiotic competence of in vitro matured prepubertal and adult oocytes in sheep. *Reprod Nutr Dev*, 39:503-508.

Ledda S, Bogliolo L, Leoni G, Naitana S. 2001. Cell coupling and maturation promoting factor activity in in vitro matured prepubertal and adult sheep oocytes. *Biol Reprod*, 65:247-252.

Leibfried-Rutledge ML, Critser ES, Eyestone WH, Northey DL, First NL. 1987. Developmental potential of bovine oocytes matured in vitro or in vivo. *Biol Reprod*, 36:376-383.

Li HJ, Liu DJ, Cang M, Wang LM, Jin MZ, Ma YZ, Shorgan B. 2009. Early apoptosis is associated with improved developmental potential in bovine oocytes. *Anim Reprod Sci*, 114:89-98.

Liebfried L, First NL. 1979. Characterization of bovine follicular oocytes and their ability to mature in vitro. *J Anim Sci*, 48:76-86.

Lonergan P, Monaghan P, Rizos D, Boland MP, Gordon I. 1994. Effect of follicle size on bovine oocyte quality and development competence following maturation, fertilization and culture in vitro. *Mol Reprod Dev*, 37:48-53.

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.

Malcuit C, Fissore RA. 2011. Recent advances in the understanding of the molecular effectors of mammalian egg activation. *In*: Tosti E, Boni R (Ed.). *Oocyte Maturation and Fertilization*: *A long history for a short event*. Dubai: Bentham Science Publ. pp. 121-134.

Marchal R, Feugang JM, Perreau C, Venturi E, Terqui M, Mermillod P. 2001. Meiotic and developmental competence of prepubertal and adult swine oocytes. *Theriogenology*, 56:17-29.

Moor RM, Hay MF, Dott HM, Cran DG. 1978. Macroscopic identification and steroidogenic function of atretic follicles in sheep. *J Endocrinol*, 77:309-318.

Nagano M, Katagiri S, Takahashi Y. 2006. Relationship between bovine oocyte morphology and in vitro developmental potential. *Zygote*, 14:53-61.

O'Brien JK, Dwarte D, Ryan JP, Maxwell WMC, Evans G. 1996. Developmental capacity, energy metabolism and ultrastructure of mature oocytes from prepubertal and adult sheep. *Reprod Fertil Dev*, 8:1029-1037.

O'Brien JK, Catt SL, Ireland KA, Maxwell WMC, Evans G. 1997. In vitro and in vivo developmental capacity of oocytes from prepubertal and adult sheep. *Theriogenology*, 47:1433-1443.

Pavlok A, Lucas-Hahn A, Niemann H. 1992. Fertilization and developmental competence of bovine oocytes derived from different categories of antral follicles. *Mol Reprod Dev*, 31:63-67.

Revel F, Mermillod P, Peynot N, Renard JP, Heyman Y. 1995. Low developmental capacity of in vitro matured and fertilized oocytes from calves compared with that of cows. *J Reprod Fertil*, 103:115-120.

Rizos D, Ward F, Duffy P, Boland MP, Lonergan P.

2002. Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: implications for blastocyst yield and blastocyst quality. *Mol Reprod Dev*, 61:234-248.

Salamone DF, Damiani P, Fissore RA, Robl JM, Duby RT. 2001. Biochemical and developmental evidence that ooplasmic maturation of prepubertal bovine oocytes is compromised. *Biol Reprod*, 64:1761-1768.

Silvestre F, Boni R, Fissore RA, Tosti E. 2011. Ca^{2+} signaling during maturation of cumulus-oocyte complex in mammals. *Mol Reprod Dev*, 78:744-756.

Silvestre F, Fissore RA, Tosti E, Boni R. 2012. $[Ca^{2+}]i$ rise at in vitro maturation in bovine cumulus-oocyte complexes. *Mol Reprod Dev*, 79:369-379.

Sirard MA, Richard F, Blondin P, Robert C. 2006. Contribution of the oocyte to embryo quality. *Theriogenology*, 65:126-136.

Staigmiller RB, Moor RM. 1984. Effect of follicle cells on the maturation and developmental competence of ovine oocytes matured outside the follicle. *Gamete Res*, 9:221-229.

Steeves TE, Gardner DK. 1999. Metabolism of glucose, pyruvate, and glutamine during the maturation of oocytes derived from prepubertal and adult cows. *Mol Reprod Dev*, 54:92-101.

Steeves TE, Gardner DK, Zuelke KA, Squires TS, Fry RC. 1999. In vitro development and nutrient uptake by embryos derived from oocytes of pre-pubertal and adult cows. *Mol Reprod Dev*, 54:49-56.

Tan SJ, Lu KH. 1990. Effect of different estrus stages of ovaries and size of follicles on generation of bovine embryos in vitro. *Theriogenology*, 33:335. (abstract).

Tosti E, Boni R, Cuomo A. 2000. Ca2+ current activity decreases during meiotic progression in bovine oocytes. *Am J Physiol Cell Physiol*, 279:C1795-C1800.

Tosti E, Boni R, Cuomo A. 2002. Fertilization and activation currents in bovine oocytes. *Reproduction*, 124:835-846.

Tosti E, Boni R. 2004. Electrical events during gamete maturation and fertilisation in animals and human. *Hum Reprod Update*, 10:1-13.

Wilding M, Dale B, Marino M, di Matteo L, Alviggi C, Pisaturo ML, Lombardi L, De Placido G. 2001. Mitochondrial aggregation patterns and activity in human oocytes and preimplantation embryos. *Hum Reprod*, 16:909-917.

Wise T. 1987. Biochemical analysis of bovine follicular fluid: albumin, total protein, lysosomal enzymes, ions, steroids and ascorbic acid content in relation to follicular size, rank, atresia classification and day of the estrous cycle. *J Anim Sci*, 64:1153-1169.

Wurth YA, Kruip TAM. 1992. Bovine embryo production in vitro after selection of the follicles and oocytes. *In*: Proceedings of the 12th International Congress of Animal Reproduction, 1992; The Hague, The Netherlands. The Hague: ICAR. v.1, pp. 387-389.

Wurth YA, Boni R, Hulshof SCJ, Kruip TAM. 1994. Bovine embryo production in vitro after selection of ovaries, follicles and oocytes. In: Wurth YA. *Bovine Embryo Production In Vitro*: *Influencing factors*. Utrecht, The Netherlands: Utrecht University Press. pp. 67-85.

Yamamoto T, Iwata H, Goto H, Shiratuki S, Tanaka H, Monji Y, Kuwayama T. 2010. Effect of maternal age on the developmental competence and progression of nuclear maturation in bovine oocytes. *Mol Reprod Dev*, 77:595-604.