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Review

Developmental competence of *in vivo* and *in vitro* matured oocytes: A review

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The study of follicular dynamics has been particularly rapid in the last two decades. However, *in vitro* maturation and fertilization of oocytes results in reduced embryo production, which suggests that not all oocytes have the capacity to mature and to fertilize properly. The efficiency of bovine embryo production *in vitro*, measured as the blastocyst rate, obtained from oocytes matured and fertilized *in vitro*, is about 40%. Evidence has demonstrated that oocyte quality depends on the events that occur before germinal vesicle breakdown (GVBD) and has suggested that the oocyte must accumulate the appropriate information for meiotic resumption, fertilization and early embryonic development before chromosome condensation. The reduced blastocyst yield obtained *in vitro* seems to be related to oocyte source and quality, and the culture system applied. The oocyte quality and protocols of culture are the key factors that determine embryo development, establish a pregnancy and produce healthy offspring.

Key words: Oocyte, *in vitro* maturation (IVM), *vitro* fertilization (IVF), *in vitro* culture (IVC), embryo, bovine.

INTRODUCTION

In vitro embryo production (IVP) includes three major processes: *in vitro* maturation (IVM); *in vitro* fertilization (IVF) and *in vitro* culture (IVC). Maturation of mammalian oocytes is defined as the sequence of events occurring from the germinal vesicle stage to completion of the second meiotic division with formation of the second polar body.

Selection of good quality gametes (oocytes and spermatozoa) is the first bottleneck *in vivo* and involves a tremendous loss of unfit oocytes mostly by atresia in the ovarian and of sperm cells in the male and in the female reproductive tract before and after mating, respectively (Van Soom et al., 2007). The absence of such rigorous selection of gametes *in vitro* and suboptimal culture condition both decrease embryo quality, but there is no consensus on which factor is most important in the incidence of apoptosis during early embryo development.

The importance of oocyte quality, the second "intrinsic factor" in the developmental competence of embryos might be more apparent and is determined by the oocytes nuclear and cytoplasmic maturation which are attained during its growth in the follicle (Sirard, 2001). A competent oocyte is by definition able to sustain embryonic development to term (Brevini-Gandolfi and Gandolfi, 2001). Nuclear maturation is characterized by the oocyte's ability to resume meiotic division up to metaphase II during *in vitro* maturation. This is the case for the vast majority of *in vitro* matured oocytes punctured from antral follicles (Watson, 2007). Nuclear maturation can be visualized by the extrusion of the second polar body and the appearance of the second metaphase plate.

The efficiency of the processes of *in vitro* maturation, fertilization of oocytes and development of embryos is not sufficiently high. The number of pregnancies obtained under the same conditions is smaller when the transferred embryos are produced *in vitro* than when they are produced *in vivo*. Despite the efforts to improve bovine *in vitro* embryo production (IVP), its efficiency is still lower, since only 30 to 40% blastocyst development is obtained from oocytes after *in vitro* maturation, fertilization and embryo culture (Sirad et al., 2006).

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In both, in vivo and *in vitro* conditions, only the fully grown oocytes can resume meiosis and acquire the ability to be fertilized. Meiotic events are sensitive to various perturbations, such as pH; oxidative stress, toxins and *in vitro* culture conditions may have profound effects on the genetic competence of *in vitro*-matured oocytes (Carrell et al., 2005).

Embryo quality assessment is most commonly performed by non-invasive morphological evaluation of embryos based on embryo color, blastomere symmetry, cytoplasmic granulation and fragmentation, although this technique is prone to subjectivity (Merton, 2002).

OVARIAN MORPHOLOGY

Oocytes are female germ cells, and they are arrested at the early diplotene phase of meiotic prophase I. These cells are initially grouped within large, interconnected clusters known as oocytes nests. Two key developmental processes occur subsequent to the meiotic arrest of the oocytes: 1- the assembly of primordial follicles (follicular assembly) and 2- the recruitment of primordial follicles into a growing cohort identified as primary follicles (primordial to primary follicle transition). Both processes define the earliest stages of folliculogenesis and are believed to affect the duration of the female reproductive life span (Skinner, 2005).

These early events in folliculogenesis are mostly regulated by local growth factors and the predominant ovarian steroid hormones that is progesterone, estrogens and androgens). The component of folliculogenesis known as follicular transition is one of the most intriguing processes in ovarian biology, because little is known about its control mechanisms. This transition enables the growth of some primordial follicles, while others remain quiescent for moths, years, or decades depending on the species. Improper activation of follicular transition is proposed to be the cause of certain reproductive disorders (Skinner, 2005).

HORMONES - GROWTH FACTORS

Follicle stimulating hormone (FSH) is considered to have only a limited role in regulating follicular assembly (Roy and Albee, 2000; Balla et al., 2003), however, evidence does show that FSH exerts some degree of control over this process. A recent report (Roberts et al., 2005) evaluated IVM of cumulus-oocyte complexes and suggested that high levels of FSH may induce aneuploidy. In FSH receptor (FSHR) knockout mice, fewer naked oocytes are presented relative to wild-type control animals. Moreover, FSHR knockout mice possess a greater number of primary and secondary follicles (Balla et al., 2003). Other studies, in which gonadotropin-deficient mice, or gonadotropin receptor-deficient mice were used (Kumar, 2005), collectively indicated that follicular assembly occurs in the absence of gonadotropin support. In addition, several growth factors and cytokines, including tumor necrosis factor-α (TNFα), neurotrophins (NT), inhibins, and growth differentiation factor-9 (GDF-9), have been shown to control follicular assembly (Morrison and Marcinkiewicz, 2002; Wang et al., 2006).

Intraovarian growth factors appear to serve a more important function in the primordial to primary transition process; one example is kit ligand (KL) and its receptor, c- kit. The direct role of KL in mediating the primordial to primary follicle transition has been shown (Skinner, 2005). Granulosa cells expressed KL and theca cells expressed its receptor c-kit (Hutt et al., 2006). In response to stimulation by KL, oocytes produced factors such as basic fibroblast growth factor (bFGF), GDF-9, and bone morphometric factor 15(BMP-15), whereas theca cells secreted keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF). In turn, KGF and HGF exerted a number of regulatory effects within the growing follicles. In addition to KL and GDF-9, and other growth factors are potentially involved in the transition process, such as the neurotrophins and their receptors, bFGF, leukemia inhibitory factor (LIF) (Skinner, 2005).

Steroid hormones can also guide ovarian development, for example, *in vivo* and *in vitro* studies have shown that progesterone impairs follicular assembly in the rat and, a slight inhibitory effect of estradiol-17 β (E_2) on follicular assembly was also observed (Kezele and Skinner, 2003). When cultured with E_2 for 7 days, neonatal mice ovaries had a reduced oocytes nest breakdown, which resulted in a significantly lower number of primordial follicles (Chen and Pepling, 2006). Experiments also suggested that progesterone mediates early follicle development and inhibits both in rats (Kezele and Skinner, 2003). Estrogen bioactivity is mediated via the activation of estrogen receptors (ER), and two follicular ER isoforms: ERα and ERβ have been localized (Sar and Welsch, 1999). Experimental evidence has indicated that ERβ, but not ERα, mediates the estrogenic control of folliculogenesis (Emmen et al., 2005). Data has also shown that ERβ can facilitate mechanisms that promote follicle maturation from the early antral to the preovulatory stages (Emmen et al., 2005).

The LHR and FSHR are initially expressed in the ovary, when the secondary follicles first appear (Sokka and Huhtaniemi, 1990), suggesting that gonadotropins stimulate growth and differentiation of preantral follicles. Follicles can develop to the antral stage in absence of gonadotropins (Kumar, 2005). However, in secondary stages, FSH and LH are required for the maturation of preantral follicles. The cohorts of younger follicles undergo further growth and differentiation, which stop during the mid to late luteal phases. This stop-and-go pattern continues until the dominant follicle is ovulated and the non-selected sister follicles undergo atresia (McGee Hsueh, 2000).

In the selected follicles, both theca and granulosa cell, proliferate and the FSH-orchestated up-regulation of cAMP is the major mechanism that supports the expression of steroidogenic acute regulatory protein (StAR) (Logan et al., 2002). The orderly production of androgens (within theca cells), E_2, and progesterone are required for the maturation of healthy preovulatory follicles, ovulation and luteal development. The levels of StAR and biosynthetic enzymes are tightly regulated not only by cAMP-dependent signaling but also by interactions between intra-and-extra ovarian hormones, growth factors and cytokines (Terranova and Rice, 1997).

Growth differentiation factor-9 (GDF-9) protein and bone morphometric protein 15 (BMP15) were identified as oocyte-secreted paracrine factors involved in the regulation of cumulus and granulosa cells functions and considered necessary for the success of ovulation, fertilization and female reproduction (Gueripel et al. 2006). Cooperation between theca cells and granulosa cells increases E_2 production in the selected mature follicles. The selected follicle also secretes activin and stimulates FSH, but the secretion of FSH cannot rescue the dying (atretic) non-selected follicles (McGee Hsueh, 2000).

The vast majority of estrous cycles are composed of two or three follicular waves. The number of follicles recruited into each wave varies greatly among individuals, but is highly repeatable within each individual, and duration of dominance of the first wave is predictive of the wave pattern. Growing, static and regressing phases of dominant and subordinate follicles are morphologically distinct, and are reflective of functional status (steroid, protein metabolism and oocyte competence). Reproductive aging in cattle is characterized by an elevation in plasma FSH concentrations, a decrease in the number of follicles recruited into each wave, a lower superovulatory response, and a lower oocyte fertilization rate (Adams et al., 2008).

CYTOPLASM AND NUCLEAR MATURATION

Growing oocytes can be categorized as incompetent or competent to resume meiosis (Arlotto et al., 1996). Incompetent bovine oocytes remain at the stage because they do not have enough cycling B to progress beyond prophase I in sufficient quantities (Levesque and Sirard, 1996). Meiotic competence is closely correlated with oocyte size, which in turn is correlated with follicle size (Armstrong, 2001). Bovine oocytes acquire the ability to complete GVBD and meiosis by the time the antral follicle reaches 2-3 mm in diameter. Meiotic competence is also related to oocyte diameter, since bovine oocytes must have a diameter of 110 µm to complete nuclear maturation to the M-II stage.

Bovine oocytes with an inside-zona diameter smaller than 95 µm are unable to resume meiosis *in vitro*. A high proportion of bovine oocytes are able to resume it (Otoi et al., 1997), however, the oocyte must measure 110 µm or more to reach the MII stage. The ability to develop to the blastocyst stage *in vitro* increases with oocyte growth (Arlotto et al., 1996) to resume meiosis to the M-I stage once the oocyte diameter is at least 100 µm. In bovine, Varisanga et al. (1998) found that oocyte yield per ovary from ovaries bearing CL was significantly higher ($P<0.01$) than from ovaries without CL. A similar trend was reported by Dode et al. (2001), who found higher number of oocytes from pregnant than from non-pregnant cow ovaries.

The cytoplasm maturation includes a succession of transformations: essentially of mitochondria, cortical granules and smooth and rough endoplasmic reticulum (Hyttel et al., 1997); all of them necessary for the progress of the maturation and blockade of polispermea. It has also been indicated that proteins of new synthesis take place, like the “factor of the growth of masculine pronucleus” MPGF, and the maturation promoting factor MPF. Apart from these phenomena, the mucification and expansion of the cells that surround the oocyte and the reduction in the number of intercellular junctions between the granulose cells and the oocytes begins, which originates an interruption in the ionic transport between the cells of the cumulus and the oocytes (Hyttel et al., 1997).

Cytoskeleton proteins (Sun and Schatten, 2006) and organelle distribution (Brevini et al., 2007) are important for oocyte maturation and may affect oocyte competence. Kim et al. (1996, 2000) demonstrated the close relationship between nuclear maturation and cytoskeleton dynamics in pig and cattle oocytes. Cytoskeleton dynamics are also related to oocyte developmental competence (Brevini et al., 2007). Correct organelle positioning is also important, because lesser quality bovine oocytes fail to translocation mitochondria from the cytoplasm periphery to the center (Stojkovic et al., 2001).

Bilodeau-Goeseels (2006) reported that glucose, piruvate, lactate and glutamine were stimulatory to nuclear maturation in bovine oocytes. Insufficient cytoplasm maturation of M II oocytes may be one reason for the low rate of embryo production *in vitro*. A two-step procedure of IVM using defined culture system MEMα supplemented with 0.1% Polyvinyl Alcohol was able to maintain meiosis arrest while the cytoplasm undergoes maturation (Oliveira et al., 2010).

Oocyte diameter

The ability of bovine oocytes of different sizes to mature *in vitro* was also investigated by Fair et al. (1995). Oocytes were placed into four treatment groups based on diameter (<100 µm, 100 to 110 µm, 110 to 120 µm and >120 µm). After IVM, a significantly larger number of oocytes were at M-II when oocyte diameter was 110 to

120 µm and >120 µm when compared with oocytes having a diameter of <100 µm and 100 to 110 µm. Most oocytes with a diameter of <100 µm remained at the germinal vesicle (GV) stage of nuclear maturation. It appeared that bovine oocytes acquired the ability to complete nuclear maturation at a diameter of ~110 µm. Hyttel et al. (1997) and Sirard et al. (1998) concluded that oocytes gradually acquire competence to undergo meiotic maturation and sustain embryonic development after reaching a diameter between 110 and 120 µm.

In vitro oocyte maturation (IVM)

In bovine, Mingoti et al. (2002) demonstrated that cumulus cells (COCs) are able to secrete oestradiol and progesterone in culture systems for *in vitro* maturation, and how steroidogenesis is modulated by the steroids progesterone, testosterone and oestradiol. Expansion of cumulus cells surrounding bovine oocytes was altered in response to FSH and/or LH in semi-defined medium, while cumulus-cell expansion was not related to the rates of cleavage or subsequent embryonic development *in vitro*. The effects of LH on cumulus-cells expansion can be caused by as little as 1 part per 10,000 contaminations with FSH (Choi et al., 2001). Also, Beker et al. (2002) found that culture of cow COCs for 22 h in the presence of 1 µg/ml oestradiol significantly ($P<0.0001$) decreased the percentage of metaphase II-stage oocytes as compared to the control group (56.3 and 74.0%, respectively). Moreover, the proportion of oocytes presenting nuclear aberrations was also significantly ($P<0.0001$) higher in the presence of oestradiol (2.1 vs.13.3%).

Another study in bovine (Machatkova et al., 2004) showed that the mean number of all counted follicles 2-5 mm, 6-10 mm and all usable oocytes collected from growing or dominant phase of the first follicular wave per donor were similar. In this respect, Hendriksen et al. (2004) indicated that the dominant bovine follicle reduced the developmental competence of oocytes from subordinate follicles at a relatively late stage of dominance. Hagemann et al. (1999) reported what does it means stage of the estrous cycle significantly affected both oocyte competence and levels of follicular atresia in bovine.

The oocyte ovulated during the normal estrous cycle of the cow originates from the dominant follicle. The dominant follicle grows from 2 to 15 mm in approximately 5 days. Most oocytes collected for *in vitro* maturation originate from subordinate or growing follicles that are, at least, 4 to 10 days away from any possible ovulation. Even though most of these oocytes complete their nuclear maturation, few develop to the blastocyst-stage. Oocyte maturation *in vitro* is independent of the estrous cycle (Arlotto et al., 1996). Germinal vesicle breakdown occurs earlier and meiotic maturation proceeds more rapidly during *in vitro* than *in vivo* maturation (Hyttel et al., 1997). Different patterns of protein synthesis have been reported for oocytes matured *in vivo* versus those matured *in vitro* (Kastrop et al., 1991). Differences can also be found at the ultra-structural levels such as the localization of cortical granules. Finally, cumulus cell expansion is more extensive *in vivo* than *in vitro* (Hendriksen et al., 2000).

Knowledge of apoptosis in oocytes and follicles and particularly on how early follicle atresia affects oocyte viability is seriously lacking. Presumably, apoptosis begins in the follicle and then affects the oocyte. There is some evidence that even good quality cumulus oocyte complexes isolated from bovine follicles with a high degree of apoptosis in the follicle wall lose their developmental capacity. On the other hand, it has been suggested recently that a limited degree of atresia or apoptosis in the cumulus may be associated with improved oocyte competence (Hendriksen et al., 2000). Basic information on the control of apoptosis in the ovary and in cells and tissues is beginning to emerge (Markström et al., 2002). The application of this knowledge may eventually allow us to detect apoptotic oocytes by using a simple vital stain and, in cases of incipient apoptosis, allow us to reverse the process. Glucose-6-phosphate dehydrogenase is synthesized by oocytes during their growth phase in the follicle, but once the oocyte is fully grown and developmentally competent, G6PDH disappears (Tian et al., 1998). The non-toxic dye brilliant cresyl blue (BCB) can be degraded by glucose-6-phosphate dehydrogenase (G6PDH) and is a marker for disappearance of G6PD (Tian et al., 1998). After completion of the growth phase, developmentally competent immature oocytes show decreased G6PDH levels and stain blue after BCB staining (BCB+), whereas incompetent growing oocytes with high levels of G6PDH become colorless after staining (BCB-). Staining of immature oocytes has already successfully been used in many species to select the developmentally most competent oocytes (Alm et al., 2005; Opiela et al., 2008). The use of BCB staining has mostly been applied as a positive marker, to select for better developmental competence (BCB+ oocytes). Hypothetically, it is also possible to use it as a marker for negative qualities, such as an increased tendency for apoptosis (BCB-oocytes).

For instance, there is evidence in some cells that activation of protein kinase C inhibits apoptosis (Zhuang et al., 1998) and it is possible that activation of protein kinase C may be used to inhibit incipient apoptosis in oocytes and thus improve rates of oocyte maturation *in vitro*. Studies on apoptosis do not constitute the only approach to improving oocyte maturation and therefore IVP. There is a considerable amount of information now available on the role of various signal transduction systems in oocyte maturation and activation (Conti et al., 1998), calcium (Homa, 1995), inositol trisphosphate (Stricker, 1999). There is also a vast body of information

on the molecular control of the cell cycle, particularly on the role of cycling dependent kinase, much of it based on work with oocytes (Taieb et al., 1997; Winston, 2001). Another developing research area with the potential for improving *in vitro* maturation of oocytes is that of follicular fluid meiosis-activating sterols**.** Even though as yet there has been little or no investigation of the role of these compounds in the oocytes of farm animals, the meiosis-inducing properties of these compounds have been studied extensively in mouse, rat and human; there is evidence that they may have a role in mediating the action of the gonadotropic surge on oocyte maturation (Byskov et al., 2002; Tsafriri et al., 2002).

It has been reported (Wu et al., 2001) that oocytes from *in vitro*-grown pig preantral follicles can acquire meiotic competence and undergo fertilization and *in vitro* culture to blastocyst. During the period between the LH surge and ovulation, the oocyte is thought to be able to respond to follicular factors that determine its subsequent developmental capacity. Furthermore, under the influence of yet unknown factors, the oocytes undergo a series of profound changes involving both the nucleus and cytoplasm during this time (Eppig and O'Brien, 1996). These changes are essential for the formation of an egg having the capacity for fertilization and development. Many factors act together to prepare the immature oocyte for successful development to a competent embryo after fertilization. Carvalhais et al. (2010) evaluated the oocyte quality after 3 and 7 days after fertilization and reported oocytes classified as A or B categories presented more cleavage and a faster development to the morulae /blastocyst stage.

Cow oocytes contain more microvilli on their cell surface and more endocytic vesicles than calf oocytes (De Paz et al., 2001). Numerous reports have indicated that fewer calf oocytes developed to blastocyst as compared with cow oocytes in IVF (Majerus et al., 2000; Salamone et al., 2001). Rizos et al. (2005) found no differences in the number of oocytes collected per ovary, however, following IVF significantly more cow oocytes developed to blastocyst on day 8 as compared to heifer's oocytes.

In vitro meiosis resumption of oocyte occurs by absence of meiosis inhibition factor(s) from the follicle (Kotsuji et al., 1994). However, *in vitro* maturation results show reduced embryo production, suggesting that not all oocytes present cytoplasmic and nuclear maturation.

The FSH hormone activates cumulus cells expansion *in vitro* in a similar manner as the LH surge acts *in vivo*. The cumulus cell mucification provides the optimal environment for ovulation and supports fertilization. Through cumulus cells expansion, FSH induces the expression of many essential factors, mainly hyaluronic acid (HA), which has hydrophilic capacity to retain water and promote cumulus cells expansion. It is important to note that the action of FSH on cumulus or granulose cells is influenced by some oocyte-secreted factors that are members of the TGF-β family (Gilchrits et al., 2007). The action of FSH is achieved through secondary messengers such as cAMP (adenosine 3'-5' monophosphate), protein kinase A (PKA) (Izadyar et al., 1998) and/or protein kinase C (Fan et al., 2004). The addition of E_2 to a serum free maturation medium negatively affected bovine oocyte nuclear maturation and subsequent embryo development. Although these effects are attenuated in the presence of FSH, the authors strongly suggest omission of E_2 in routine maturation protocols of bovine oocytes (Beker et al., 2002).

In another study, Morales-Pliego et al. (2008) reports that while 23 h maturation was optimal for oocytes with compact or partially expanded cumulus, similar blastocyst rates were obtained with oocytes with expanded cumulus if maturation time was shortened to 1 h.

CULTURE MEDIA

When immature bovine oocytes are released from their follicles and cultured in standard maturation medium, they resume the first meiotic division. The culture media play an important role in the development of *in vitro* fertilized embryos. It is necessary to indicate that the selection of culture media is of extreme importance, since it can condition the success of the oocyte maturation, fertilization and, later, the development of the embryos (Gliedt et al., 1996).

The limited developmental competence of bovine oocytes after IVM can be used to understand the factors involved in the acquisition of such ability. For this reason, and to understand the requirements for the development of immature oocytes through IVM, all products with undefined components should be eliminated from culture conditions.

The maturation media are supplemented habitually with diverse types of serum, such as bovine fetal serum: FCS; serum of cow in estrous: ECS, or albumine fraction V of bovine serum: BSA (Lee and Fujii, 1996; Blanco and Simonetti, 2002). Another study investigated the use of synthetic serum substitute (SSS) instead of fetal calf serum in maturation medium to stimulate IVM, IVF and the subsequent development of bovine oocytes. The results show that serum supplementation of the IVM medium is necessary to obtain a higher cleavage rate and development rate to the blastocyst stage of immature bovine oocytes (Sagirkaya et al., 2004). Other supplements play an important role in the process of maturation, sodium pyruvate (Arlotto et al. 1996), sodium lactate, glutamine (Fukui, 1990), glucose and sodium bicarbonate (Younis et al., 1989) and EGTA (Blanco and Simonetti, 2000). They determine whether maturation and subsequent blastocyst development of *in vitro* matured oocytes can be improved by *in vivo* follicle stimulating hormone (FSH) or human chorionic gonadotrophin (hCG), but FSH or hCG priming does not improve development to the blastocyst stage.

Atef and Sirard (2002) observed that the addition of BSA-V during IVM retarded nuclear maturation when compared with the addition of PVP-40 or with the use of SOF alone. The inclusion of different concentrations of BSA-V, fetal calf serum (FCS), or PVA during IVM had no positive effect on the developmental capacity of the oocytes compared with the use of SOF alone with no supplement but it significantly decreased the percentage of embryos reaching the morula and blastocyst stages. The presence of PVP-40 during IVM significantly increased morula and blastocyst production.

The serum-free culture systems have proven to be beneficial for the production of good quality embryos from IVM-IVF bovine oocytes. Furthermore, recent studies have shown a correlation between mitochondrial function (oxygen consumption) and embryo quality (Abe and Hoshi, 2003). Serum-free media: IVD101 and IVMD101, produced better results than serum-containing medium, including increased rates of blastocyst formation, post-thaw embryo viability and pregnancy after transfer (Hochi, 2003).

Follicular fluid from small and large bovine follicles contains large amounts of progesterone and, during the preovulatory period, progesterone concentration increases by 18 h after LH surge. Furthermore, cumulus cells express membrane progestin receptor beta (Liu el al., 2008). The results suggest that progesterone supplementation of *in vitro* maturation medium affects the competence of the oocytes to develop into blastocyst *in vitro*, and 5 µg mL^{-1}P4 supplementation has increasing embryo production (Miyata et al., 2010). Carrell et al. (2005) reports improved survival, maturation and meiotic competence in oocytes isolated without enzymatic treatment and cultured in individual microdrops.

In vitro fertilization

It is essential that the IVF media are supplemented with diverse factors: glucosaminoglicans, caffeine, factors of spermatic motility, granulosa cells, bovine cumulus cells, etc (Birler, 1996). The association between heparin and a second messenger such as calcium and cAMP has been studied by different authors (Uguz et al., 1994; Mahmoud and Parrish, 1996). Another study proposed a capacitation mechanism based on a calcium-mediated cAMP-dependent pathway for capacitation. The increase in intracellular calcium leads to adenylate cyclase activation and, consequently, to a cAMP increase. The use of millimolar amounts of caffeine increases the recovery and quality of bovine sperm (Correa and Zavos, 1996).

Calcium and caffeine may affect sperm function during swim-up procedures, resulting in a more homogeneous sperm population. Coscioni et al. (2001) reported that the greatest frequency of capacitated sperm (53%) was observed with 7.5 uM caffeine, and different calcium and caffeine concentrations in swim-up resulted in no significant differences in the cleavage rate and embryo development.

Another study has evaluated an *in vitro* fertilization procedure that avoids the washing-selection centrifugation of spermatozoa prior to co incubation with oocytes, and the results indicated that this method yielded similar results but the “easy-IVF” reduced the manipulation of the sperm with eventual good effects in the long run toward improving *in vitro* reproduction procedures (Urrego et al., 2008).

Culture of bovine embryos

Current techniques of farm animal embryo culture from 1-cell to early blastocyst seem to fall into one or other of two categories. The first category involves a co-culture system using a complex tissue culture medium with various cell types such as oviductal epithelial cells, granulosa cells and even cells of a tissue culture cell line such as BRL cells (Reed et al., 1996). Much effort at improving these media has been concentrated on the type of co-culture cells. The second category uses a cell-free system based very often on a simple medium such as synthetic oviduct fluid (SOF) supplemented, to varying degrees, with amino acids, vitamins, serum and other components (Holm et al., 1999). In recent years, co-culture systems appear to have been largely abandoned by commercial operators and replaced by culture in SOF-BSA-AA under a low oxygen concentration (Galli et al., 2001); this emphasizes the importance of amino acid supplementation of the medium. Efforts to improve such media consist mainly of varying concentrations of the standard medium constituents and trying out new constituents, so far with only limited success. For instance, two nutrients, inositol and citrate, first shown by laboratory (Gray et al., 1992) to be essential for rabbit blastocyst growth, appear to improve cattle blastocyst development (Vajta et al., 2000), but the effect does not appear to be very substantial, except in the case of inositol in protein-free medium (Holm et al., 1999).

Sun and Schatten, (2006) found a beneficial effect of sodium citrate and BSA on the *in vitro* development of bovine IVF embryos during co-culture, and also determined that differential embryotrophic factor(s) contained in BSA and serum, probably not sodium citrate, is necessary for promoting competent morula and blastocyst development in cattle.

There is also no clear evidence that peptide growth factors added to the medium improve culture of embryos to any marked degree (Kane et al., 1997); most effects seem to be minor and do not result in an improvement that is considerable enough to be used commercially on a routine basis. However, it is possible that in the absence of serum or bovine serum albumin in the medium, there may be beneficial effects of growth factors. Also some

unusual growth factors may be beneficial, for example, parathyroid hormone-related protein (Nowak et al., 1999), vasoactive intestinal peptide (Servoss et al., 2001).

Shoko et al. (1999) developed serum-free culture systems for *in vitro* development of bovine embryos with or without somatic cell co-cultures. The survival rates of blastocysts derived in IVMD101 medium (73.3%) and IVD101 medium (60.0%) based on hatching after 72 h of post-thaw culture were superior to that of blastocysts derived in the serum-supplemented medium (48.1%). These serum-free culture systems can offer several advantages over the culture in serum-containing medium, including increased rates of blastocysts formation, cell numbers, and embryo viability after freezing and thawing. In particular, IVD101 medium without somatic cell co-cultures is useful, not only to obtain a large number of good quality embryos for practical uses such as embryo transfer, cloning and transgenesis, but also to study the mechanisms of early embryogenesis.

The use of oviductins in embryo culture is an area which might be worth exploring. The oviductins are a family of glycoproteins synthesized and secreted by oviduct cells, which bind to the pellucid zone of the oocyte after ovulation (Malette and Bleau, 1993). It is possible that their only function is merely to change the properties of the zone so as to provide increased protection to the embryo but one cannot exclude the possibility that they may affect the embryo directly.

One factor that needs examination is the volume of medium in which embryos are cultured. Currently, embryos are cultured in relatively large volumes of medium in spite of the fact that a mammalian embryo of just over 150 µ diameter has a volume of only 1.5–2.0 µl and in the oviduct it is probably surrounded by mere picoliters of fluid at any one time. Kane (2003) suggested "the requirements for nutrients for cleavage *in vitro* could well be an artefact caused by the fact that the embryo is swimming in an ocean of fluid into which it is leaking its own internal nutrients". Thus, it is possible that culture of cleavage stage embryos in volumes of medium 1 µl or less might markedly improve development in culture. This is at least partially supported by the findings of the group (Schini and Bavister, 1988a) whose culture of hamster embryos at high embryo density in 0.75 _l microdrops also overcame the incidence of the 2-cell block to some degree. Unfortunately, this observation has not been supported to any great extent. Galli et al. (2001) found that increasing cattle embryo density in 20 µl drops did not improve embryo development. However, a new method of culturing cattle embryos in miniature wells (0.04 and 0.15 µ) within larger wells (the well of the well or WOW system) has been reported to give improved development of cattle embryos in culture (Vajta et al., 2000).

Other possible changes to culture conditions might usefully be examined, for example, very mild agitation could be used to mimic the movement of the female reproductive tract although there is evidence that, in some circumstances at least, agitation is harmful (Hickman et al., 2002).

Little if any current work has been reported on *the in vitro* culture of cattle, sheep or pig early blastocyst to elongated blastocyst, almost certainly because of the difficulty of making progress in this area and because its commercial usefulness seems to be limited. One approach to improve blastocyst expansion might be to use co-culture on polarized uterine epithelial monolayers; such monolayers are produced by growing the cells on collagen-impregnated filters with culture medium above and below the filter (Dickens et al., 1993). It is possible that such monolayers might constitute an environment analogous to the uterine environment. Thus, when adding new constituents to a medium, consideration should always be given to possible negative as well as beneficial effects. Toxic contaminants in culture medium ingredients, including water, are always a major concern.

It has been suggested that free oxygen radicals produced in culture media block embryo development (Legge and Sellens, 1991). Various methods of remedying this problem have been used. Other approaches have included the use of antioxidants, for example thioredoxin and superoxide dismutase (Nonogaki et al., 1991), EDTA**,** catalase (Nasr-Esfahani et al., 1992) and vitamins C and E (Vermeiden et al., 1995). Recently, Seidel's laboratory examined the effects of EDTA and vitamins E and C on culture of IVP cattle embryos; vitamin E markedly improved blastocyst development but EDTA and vitamin C had no obvious beneficial effect (Olson and Seidel Jr., 2000).

There is evidence that *in vitro* embryo culture and manipulation causes abnormal DNA methylation which, in turn, causes abnormal gene expression both in preimplantation and fetal stages resulting in fetal overgrowth and large offspring syndrome (Niemann and Wrenzycki, 2000; Young et al., 2001).

Undefined cultured media have been used for embryo culture because protein supplementation is critical for embryo development and because better embryo development was frequently observed in undefined media compared to defined media. Chemically defined culture media without bovine serum albumin (BSA) or fetal bovine serum (FBS) improved developmental competence of *in vitro* cultured bovine embryos and delivery of viable calves after embryo transfer. Another study (Lim et al., 2007) reported there was higher developmental ability of embryos cultured in chemically defined medium with myo-inositol. However, supplementation of myo-inositol in undefined media containing BSA did not increase embryo developmental competence.

Growth factors such as growth hormone (GH) and epidermal growth factor (EGF) may be viewed as local regulators involved in the subtle coordination of cellular proliferation and differentiation. EGF stimulates distinct

cellular functions, and suggests a possible effect on early development of mammalian embryos (Teruel et al., 2000). Mtango et al. (2003) reported that addition of EGF to the culture medium resulted in higher developmental capacities than did other growth factors used. In any culture medium, energy substrate is one of the important ingredients for optimum *in vitro* development of embryos. Although piruvate and lactate in the absence of glucose are able to support *in vitro* development of bovine embryos, glucose is widely supplemented as major energy substrate in most of the culture media. Contrasting to the metabolism of piruvate and lactate, glucose metabolism in bovine IVF embryos differs from their in vivo counterparts (Khurana and Niemann, 2000). Like glucose, fructose can be metabolized through the glycolytic pathway. It has been reported that fructose is present in the reproductive tract of cattle and is utilized by bovine embryos *in vitro* (Guyader-Joly et al., 1996). Consequently, it was hypothesized that replacement of fructose with glucose in culture medium may improve the *in vitro* development of bovine IVF embryos. Another study indicated that fructose up to 5.6 mM concentration in protein-free potassium simplex optimization medium (KSOM-PVA) can be used as an alternative for energy substrate in culture media without any detrimental effect on pre-implantation development in bovine IVF embryos (Bhuiyan et al., 2007).

Gómez et al. (2008) showed higher day 6 blastocyst rates in embryos development with FCS (Groups Vero and FCS than in the group SOF- BSA). One potential limitation on the conventional culture systems is the disruption of follicle architecture that can occur when follicles are cultured on a two-dimensional substrate (Smitz and Cortvrindt, 2002). The change in follicle morphology may alter the paracrine signaling that is critical to follicle maturation because the altered cell-cell orientation could result in diffusion of paracrine signals away from the target cells. It was found that FSH and insulin (Louhio et al., 2000) promoted the growth and survival of the follicles as well as growth differentiation factor-9: GDF-9) Growth factors such as cGMP also showed improvement in follicle survival (Scott et al., 2004). The recent application of tissue engineering technologies to follicle culture has opened up new insights into follicle physiology *in vitro* and yielded favorable results (Xu el al., 2006). The novel three-dimensional cultured system, in which individual immature mouse granulose-oocyte complexes or intact follicles are encapsulated within alginate beads, is one such system. In this system, the alginate matrix provides a mechanical support for the follicle as it increases in size, allowing examination of the role of various factors in follicle growth while maintaining an *in vivo*-like morphology (Pangas et al., 2003; Kreeger et al., 2006).

Zygotes were cultured in groups or individually, either in drops or in the modified ‘‘well of the well’ (mWOW) system to improve the potential and quality of *in vitro* produced bovine embryos. Cleavage and blastocyst rates at Days 6, 7 and 8 and total cell number of Day 6 blastocyst were similar for all treatments. However, in Day 7 blastocyst, total cell number was lower in embryos cultured individually in a small drop than in those cultured in the mWOW. In conclusion, embryos cultured in groups in the mWOW system had the same blastocyst rates but better quality (measured by their survival after vitrification) than those cultured individually in the mWOW system (Pereira et al., 2005).

CONCLUSION

Evidence suggests that the intrinsic quality of the oocyte is the key factor determining the proportion of oocytes developing to the blastocyst stage. The oocyte maturation is a complicated process and the selection of oocytes for culture *in vitro* only by morphology is not enough to obtain better IVM and IVF. There is considerable evidence that the medium and conditions of culture also impact on the development of bovine *embryos in vitro*. These processes involve numerous levels of checks, balances and are sensitive to regulation of endogenous and exogenous factors.

Defects in oocyte maturation and further development could be caused by the oocyte quality or an inadequate nuclear maturation or even by a failure of both. Therefore, the type of selection of oocytes for IVM/ IVF and embryo culture is very important, and other studies to observe the nuclear and cyoplasmatic maturation and chromosome abnormalities so as to evaluate the positive or negative effect of each substance, media or condition of culture should be added to selection by morphology. Many studies have demonstrated the difficulties for comparing protocols that vary in multiple factors. These factors affect the oocyte competence and embryo development, thus, it is necessary to carry out a systematic and wider evaluation of the effects that take place when changes in the protocols of maturation, fertilization and *in vitro* development are introduced.

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REFERENCES

Abe H, Hoshi H (2003). Evaluation of bovine embryos produced in high performance serum-free media. J. Reprod. Dev., 49(3): 193-202.

Adams GP, Jaiswal R, Singh J, Malhi P (2008). Progress understands ovarian follicular dynamics in cattle. Theriogenol., 69: 72-80.

Alm H, Torner H, Löhrke B, Viergutz T, Ghoneim I, Kanitz W (2005). Bovine blastocyst development rate in vitro is influenced by selection

of oocytes by brillan cresyl blue stainig before IVM as indicator for glucose-6-phosphate dehydrogenase activity. Theriogenology, 63: 2194-2205.
Arlotto T, Schwartz JL, First NL, Leibfried ML (1996). Aspects of follicle and oocyte stage that affect *in vitro* maturation and development of bovine oocytes. Theriogenology, 45: 943-956.
Armstrong DT (2001). Effect of maternal age on oocyte developmental competence. Theriogenology, 55: 1303-1322.
Atef A, Sirard MA (2002). Effect of absence or presence of various proteins supplements on further development of bovine oocytes during *in vitro* maturation. Biol. Reprod., 66(4): 901-905.
Balla A, Danilovich N, Yang Y, Sairam MK (2003). Dynamics of ovarian implications for ovarian reserve. Biol. Reprod., 69: 1281-1293.
Beker AC, Colenbrander B, Bevers MM (2002). Effect of 17 β-estradiol on the *in vitro* maturation of bovine oocytes. Theriogenology, 58: 1663-1711.
Bhuiyan MU, Kang SK, Lee BC (2007). Effect of fructose supplementation in chemically defined protein-free medium on development of bovine *in vitro* fertilized embryos. Anim. Reprod. Sci ., 102: 137-144.
Bilodeau-Goeseels S (2006). Effect of culture media and energy sources on the inhibition of nuclear maturation in bovine oocytes. Theriogenology, 66: 297-306.
Birler S (1996). Sigir Oositlerinin *in vitro* fertilizasyonu uzerine kumulus hucrelerinin etkileri. Tr. J. Vet. Anim. Sci., 20: 163-167.
Blanco MR, Simonetti L, Palermo P (2000) Morphological survival and *in vitro* maturation of immature bovine oocytes exposed to EGTA with or without cryopreservation. *In vitro* Cell Dev. Biol., 36(3): Part. II, VT 2042.
Blanco MR, Simonetti L (2002). Morphological survival of immature and *in vitro* matured bovine oocytes after cryopreservation in Solutions containing different protein concentrations. *In vitro* Cell Dev. Biol., 38: 19A, V-1004.
Brevini-Gandolfi T, Gandolfi F (2001). The maternal legacy to the embryo: Cytoplasmic components and their effects on early development. Theriogenology, 55: 1255–1276.
Brevini TL, Cillo F, Antonini A, Gandolfi F (2007). Cytoplasmic remodeling and the acquisition of developmental competence in pig oocytes. Anim. Reprod. Sci., 98: 23-38.
Byskov AG, Andersen CY, Leonardsen L (2002). Role of meiosis activating sterols, MAS, in induced oocyte maturation. Mol. Cell. Endocrinol., 187: 189–196.
Carrell DT, Liu L, Huang I, Peterson M (2005). Comparison of maturation, meiotic competence, and chromosome aneuploidy of oocytes derived from two protocols for *in vitro* culture of mouse secondary follicles. J. Assist. Reprod. Gen., 22: 347-354.
Carvalhais I, Faheem M, Habibi A, Geraldo A, Agricola M, Chaveiro A, Moreira da Silva F (2010). Effects of bovine oocyte quality on kinetics of nuclear maturation and embryonic development after *in vitro* fertilization. Reprod. Fertil. Dev., 22: 322-323.
Chen Y, Pepling M (2006). Estradiol inhibits cyst breakdown in neonatal Mouse ovary organ culture. Biol. Reprod., 95(Special issue).
Choi YH, Carnevale EM, Seidel GE Jr., Squires EL (2001). Effects of gonadotropins on bovine oocyte matured in TCM-199. Theriogenology, 56: 661-670.
Conti M, Andersen CB, Richard FJ, Shitsukawa K, Tsafriri A (1998). Role of cyclic- nucleotide phosphodiesterases in resumption of meiosis. Mol. Cell. Endocrinol., 145: 9-14.
Correa JR, Zavos PM (1996). Preparation and recovery of frozen-thawed bovine spermatozoa via various sperm selection techniques employed in assisted reproductive technologies. Theriogenology, 46: 1225-1232.
Coscioni AC, Reichenbach HD, Schawartz J, LaFalci VSN, Rodriguez JL, Brandelli A (2001). Sperm function and production of bovine embryos *in vitro* alter swim-up with different calcium and caffeine concentration. Anim. Reprod. Sci., 67: 59-67.
De Paz P, Sánchez AJ, De la Fuente J, Chamorro CA, Álvarez M, Anel E, Anel L (2001). Ultrastuctural and cytochemical comparison between calf and cow oocytes. Theriogenology, 55(5): 1107-1116.
Dickens CJ, Southgate J, Leese HJ (1993).Use of primary cultures of rabbit oviduct epithelial cells to study the ionic basis of tubal fluid formation. J. Reprod. Fertil., 98: 603-610.
Dode MN, Rodovalho NC, Ueno VG, Alves RO (2001). Oocyte recovering from Bos indicus females. Arch. Zootechnol., 50: 415-418.
Emmen JM, Couse JF, Elmore SA, Yates MM, Kissling GE, Korach KS (2005). *In vitro* growth and ovulation of follicles from ovaries of estrogen receptor ER (alpha) and ER (beta) null mice indicate a role for ER (beta) in follicular maturation. Endocrinal., 146: 2817-26.
Eppig JJ, O'Brien MJ (1996).Development *in vitro* of mouse oocytes from primordial follicles. Biol. Reprod., 54: 197–207.
Fan HY, Huo LJ, Chen DY, Schatten H, Sun QY (2004). Protein kinase C and mitogen-activated protein kinase cascade in mouse cumulus cells: Cross talk and effect on meiotic resumption of oocyte. Biol. Reprod., 70: 1178-87.
Fukui Y (1990). Effect of follicle cells on the acrosome reaction fertilization and developmental competence of bovine oocytes matured *in vitro*. Mol. Reprod. Dev., 26: 40-46.
Galli C, Crotti G, Notari C, Turini P, Duchi R, Lazzari G (2001). Embryo production by ovum pick up from live donors. Theriogenology, 55: 1341–1357.
Gilchrist RB, Thompsom JG (2007). Oocyte maturation: Emerging concepts and technologies to improve developmental potential *in vitro*. Theriogenology, 67: 6-15.
Gliedt DW, Rosenkraus CF, Rorie RW, Munyon AL, Pierson JN, Miller GF, Rakes JM (1996). Effects of media, serum, oviduct cells and hormones during maturation of bovine embryo development *in vitro*. J. Dairy Sci., 79: 536-542.
Gómez E, Rodriguez A, Muñoz M, Caamaño JN, Hidalgo CO, Morán E, Facal N, Díaz C (2008). Serum free embryo culture medium improves *in vitro* survival of bovine blastocyst to vitrification. Theriogenology, 69: 1013-1021.
Gray CW, Morgan PM, Kane MT (1992). Purification of embryotrophic factor from commercial bovine serum albumin and its identification as citrate. J. Reprod. Fertil., 94: 471–480.
Gueripel X, Brun V, Gougeon A (2006). Oocyte bone morphogenetic protein 15, but not growth differentiation factor 9, is increased during gonadotropin-induced follicular development in the immature mouse and is associated with cumulus oophorus expansion. Biol. Reprod., 75: 836-843.
Guyader-Joly C, Khatchadourian C, Menezo Y (1996). Comparative glucose and fructose incorporation by *in vitro* produced bovine embryos. Zygote, 4: 85-91.
Hagemann LJ, Beaumont SE, Berg M, Donnison, MJ, Ledgard A, Peterson JA, Schurmann A, Tervit HR (1999). Development during single IVP of bovine oocytes from dissected follicles: Interactive effects of estrous cycle stage, follicle size and atresia. Mol. Reprod. Dev., 50: 451- 458.
Hendriksen PJM, Vos PALM, Steenweng MM, Bevers MM, Dieleman SJ (2000). Bovine follicular development and it effect on the *in vitro* competence of oocytes. Theriogenology, 53: 11-20.
Hendriksen PJM, Steenweg WNM, Harkema SC, Merton JS, Bevers MM, Vos PLA. M, Dieleman SJ (2004). Effect of different stages of the follicular wave on *in vitro* developmental competence of bovine oocytes. Theriogenology, 61: 909-920
Hickman DL, Beebe DJ, Rodriguez-Zas SL, Wheeler MB (2002). Comparison of static and dynamic medium environments for culturing of pre-implantation mouse embryos. Comp. Med., 52: 122–126.
Hochi H (2003). *In vitro* production of bovine embryos and their application for embryo transfer. Theriogenology, 59(2): 675-685.
Holm P, Booth PJ, Schmidt MH, Greve T, Callesen H (1999). High bovine blastocyst development in a static *in vitro* production system using SOF a medium supplemented with sodium citrate and myo-inositol with or without serum-proteins. Theriogenology, 52: 683–700.
Homa ST (1995). Calcium and meiotic maturation of the mammalian oocyte. Mol. Reprod. Dev., 40: 122–134.
Hutt KJ, McLaughlin EA, Holland MK (2006). Kit ligand and c-kit have diverse roles during mammalian oogenesis and folliculogenesis. Mol. Hum. Reprod., 12: 61-69
Hyttel P, Fair T, Callensen H, Greve T (1997). Oocyte growth capacitation and final maturation in cattle. Theriogenology, 47: 23-32.
Kane MT (2003). A review of *in vitro* gamete maturation, embryo culture and potential impact on future animal biotechnology. Anim. Reprod. Sci., 79: 171-190.
Kane MT, Morgan PM, Coonan C (1997). Peptide growth factors and

preimplantation development. Hum. Reprod. Update, 3: 137–157.
Kastrop PM, Bevers M, Destree OH, Kruip TAM (1991). Protein synthesis and phosphorylation patterns of bovine oocytes maturing *in vivo*. Mol. Reprod. Dev., 29: 271-275.
Kezele P, Skinner MK (2003). Regulation of ovarian primordial follicle assembly and development by estrogen and progesterone, endocrine model of follicle assembly. Endocrinology, 144: 3329-3337.
Khurana NK, Niemann H (2000). Energy metabolism in preimplantation bovine embryos derived *in vitro* or *in vivo*. Biol. Reprod., 62: 847-856.
Kim NH, Funahashi H, Prather RS, Schatten H, Day BD (1996) Microtubule and microfilament dynamics in porcine oocytes during meiotic maturation. Mol. Reprod. Dev., 43: 248-255.
Kim NH, Cho SK, Choi SH, Kim EY, Park S, Lim JH (2000). The distribution and requirements of microtubules and microfilaments in bovine oocytes *in vitro* maturation. Zygote, 8: 25-32.
Kotsuji F, Kubo M, Tominaga T (1994). Effects of interactions between granulosa and theca cells on meiotic arrest in bovine oocytes. J. Reprod. Fertil., 100: 151-156.
Kreeger PK, Deck J, Woodruff TK, Shea LD (2006). The *in vitro* regulation of ovarian follicle development using alginate-extracellular matrix gels. Biomater., 27: 714-723.
Kumar TR (2005). What have we learned about gonadotropin subunit and receptor knockout mice? Reproduction, 130: 209-302.
Lee ES, Fujii Y (1996). A comparative study on developmental capacity to blastocyst derived from 1-and 2-cell bovine embryos after *in vitro* maturation and fertilization. Theriogenology, 45: 1151-1162.
Legge M, Sellens MH (1991). Free radical scavengers ameliorate the 2-cell block in mouse embryo culture. Hum. Reprod., 6: 867–871.
Levesque JT, Sirard MA (1996). Resumption of meiosis is initiated by the accumulation of cyclin B in bovine oocytes. Biol. Reprod., 55: 1427-1436.
Lim KT, Jang G, Ko KH, Lee WW, Park HJ, Kim JJ, Lee SH, Hwang WS, Lee BC, Kang SK (2007). Improved *in vitro* bovine embryo development and increased efficiency in producing viable claves using defined media. Theriogenology, 67: 293-302.
Liu L, Ju JC, Yang X (2008). Parthenogenetic development and protein patterns of newly matured bovine oocytes after chemical activation. Mol. Reprod. Dev., 49: 298-307.
Logan KA, Juengel JL, McNatty KP (2002). Onset of steroidogenic enzyme gene expression during ovarian follicular development in sheep. Biol. Reprod., 66: 906-916.
Louhio H, Hovatta O, Sjoberg J, Tuuri T (2000). The effects of insulin and insulin-like growth factors I and II on human ovarian follicles in long term culture. Mol. Hum. Reprod., 6: 694-698.
Machatkova M, Krausova K, Jokesova, E, Tomanek, M (2004). Developmental competence of bovine oocytes: Effects of follicle size and the phase of follicular wave *in vitro* embryo production. Theriogeology, 61: 329-335.
Mahmoud AI, Parrish JJ (1996). The role of extracellular calcium during capacitation of bovine sperm by heparin. Theriogenology, 45: 315.
Majerus V, Lequarre AS, Ferguson M, Kaidi S, Massip A, Dessy F, Donnay I (2000). Characterization of embryos derived from calf oocytes: Kinetics of cleavage, cell allocation to inner cell mass, and trophoectoderm and lipid metabolism. Mol. Reprod. Dev., 57(4): 346-352.
Malette B, Bleau G (1993). Biochemical characterization of hamster oviductin as a sulphated zone pellucid-binding glycoprotein. Biochem. J., 295: 437-445.
Markström E, Svensson EC, Shao RJ, Svanberg B, Billig H (2002). Survival factors regulating ovarian apoptosis dependence on follicle differentiation. Reproduction, 123: 23-30.
McGee EA, Hsueh AJ (2000). Initial and cyclic recruitment of ovarian follicles. Endocrine Rev., 21: 200-214.
Merton S (2002). Morphological evaluation of embryos in domestic animals. In: Van Soom A, Boerjan M (Eds). Assessment of mammalian embryo quality: invasive and non-invasive techniques. Dordrecht, The Netherlands: Kluwer Academic Publishers, pp. 31-55.
Mingoti GZ, Garcia JM, Rosa-e-Silva AM (2002). Steroidogenesis in cumulus cells of bovine cumulus-oocytecomplexes matured in vitro with BSA and different concentrations of steroids. Anim. Reprod. Sci., 69: 175-186.
Miyata K, Koyama H, Lessard C, Singh J, Dochi O (2010). Effects of progesterone supplementation maturation medium on development of IVM-IVF-IVC bovine embryos. Reprod. Fertil. Dev., 22: 331-331.
Morales-Pliego F, Barcel-Fimbres M, Amorin LS, Seidel GE (2008). Optimal *in vitro* maturation time depends on morphological characteristics of bovine cumulus-oocyte complexes. Reprod. Fertil. Dev., 20(1): 203-204.
Morrison LJ, Marcinkiewicz JL (2002). Tumor necrosis factor alpha enhances oocyte/follicle apoptosis in the neonatal rat ovary. Biol. Reprod., 66: 450-457.
Mtango NR, Varisanga MD, Dong YJ, Rajamahendran R, Suzuki T (2003). Growth factors and growth hormone enhance *in vitro* embryo production and post-thaw survival of vitrified bovine blastocyst. Theriogenology, 59: 1393-1402.
Nasr-Esfahani MH, Winston NJ, Johnson MH (1992). Effects of glucose, glutamine, ethylenediaminetetraacetic acid and oxygen tension on the concentration of reactive oxygen species and on development of the mouse preimplantation embryo *in vitro*. J. Reprod. Fertil., 96: 219–231.
Niemann H, Wrenzycki C (2000). Alterations of expression of developmentally important genes in preimplantation bovine embryos by *in vitro* culture conditions: Implications for subsequent development. Theriogenology, 53: 21–34.
Nonogaki T, Noda Y, Narimoto K, Umaoka Y, Mori T (1991). Protection from oxidative stress by thioredoxin and superoxide dismutase of mouse embryos fertilized *in vitro*. Hum. Reprod., 6: 1305-1310.
Nowak RA, Haimovici F, Biggers JD, Erbach GT (1999). Transforming growth factor stimulates mouse blastocyst outgrowth through a mechanism involving parathyroid hormone-related protein. Biol. Reprod., 60: 85–93.
Oliveira e Silva I, Vasconcelos RB, Caetano JVO, Gulart LVM, Camargo LS, Báo SN, Rosa e Silva AA (2010). Dissociation of nuclear and cytoplasmatic maturation of bovine oocytes in defined culture system. Reprod. Fertil. Dev., 22(1): 332-332.
Olson SE, Seidel Jr. GE (2000). Culture of *in vitro*-produced bovine embryos with vitamin E improves development *in vitro* and after transfer to recipients. Biol. Reprod., 62: 248–252.
Opiela J, Katska-Ksiakiewicz L, Lipinski D, Slomski R, Bzowska A, Rynska B (2008). Interaction among activity of glucose-6-phosphate dehydrogenase in immature oocytes, expression of apoptosis-related genes Bcl-2 and Bax, and developmental competence following IVP in cattle. Theriogenology, 69: 546-555.
Otoi T, Yamamoto K, Koyama N, Tachikawa S, Suzuki T (1997). Bovine oocyte diameter in relation to developmental competence. Theriogenology, 48: 769-774.
Pangas SA, Saudye H, Shea LD, Woodruff TK (2003). Novel approach for three-dimensional culture of granulose cell-oocyte complexes. Tissue Eng., 9: 1013-1021.
Pereyra DC, Dode M, Rumpf R (2005). Evaluation of different culture systems on the *in vitro* production of bovine embryos. Theriogenology, 63: 1131-1141.
Reed WA, Suh T, Bunch TD, White KL (1996). Culture of *in vitro* fertilized bovine embryos with bovine oviductal epithelial cells, buffalo rat liver (BRL) cells, or BRL-cell conditioned medium. Theriogenology, 45: 439–449.
Rizos D, Burke L, Duffy P, Wade M, Mee JF, Farrell KJ, Macsiurtain M, Boland MP, Lonergan P (2005). Comparison between nulliparous heifers, cows as oocyte donors for embryo production *in vitro*. Theriogenology, 638(3): 939-949.
Roberts R, Iatropoulou A, Ciantar D, Stark J, Becker DL, Franks Hardy K (2005). Follicle stimulating hormone affects metaphase I chromosome alignment and increases aneuploidy in mouse oocytes matured *in vitro*. Biol. Reprod., 72(1): 107-118.
Roy CK, Albee L (2000) Requirement for follicle-stimulating hormone action in the formation of primordial follicles during perinatal ovarian development in the hamster. Endocrinology, 141: 4449-4456.
Sagirkaya H, Yagmur M, Nur Z, Soylu MK (2004). Replacement of fetal calf serum with synthetic serum substitute in the *in vitro* maturation medium: Effects on maturation, fertilization and subsequent development of cattle oocytes *in vitro*. Turk. J. Vet. Anim. Sci., 28: 779-784.
Salamone DF, Damiani P, Fissore RA, Robl JM, Duby RT (2001). Biochemical and developmental evidence ooplasmic maturation of

prepuberal bovine oocytes is compromised. Biol. Reprod., 64: 1761-1768.
Sar M, Welsch F (1999). Differential expression of estrogen receptor-beta and estrogen receptor-alpha in the rat ovary. Endocrinology, 140: 963-971.
Schini SA, Bavister BD (1988a). Two-cell block to development of cultured hamster embryos is caused by phosphate and glucose. Biol. Reprod., 39: 1183-1192.
Scott JE, Zhang P, Hovatta O (2004). Benefits of 8-bromo-guanosine 3'5'cyclic monophosphate (8-br-cGMP) in human ovarian cortical tissue culture. Reprod. Biomed. Online, 8: 319-324.
Servoss SJ, Lee SJ, Gibney G, Gozes I, Brenneman DE, Hill JM (2001). IGF-I as a mediator of VIP activity-dependent neurotrophic factor-stimulated embryonic growth. Endocrinology, 142: 3348-3353.
Sirard MA (2001). Resumption of meiosis: Mechanism involved in meiotic progression and its relation with developmental competence. Theriogenology, 55: 1241-1254.
Sirard MA, Richard F, Mayes M (1998). Controlling meiotic resumption in bovine oocytes: Review. Theriogenology, 49: 483-497.
Sirard MA, Richard F, Blondin P, Robert C (2006) Contribution of the oocyte to embryo quality. Theriogenology, 65: 126-136.
Skinner MK (2005). Regulation of primordial follicle assembly and development. Hum. Reprod., 11: 461-471.
Smitz JEJ, Cortvrindt RG (2002). The earliest stages of folliculogenesis *in vitro*. Reproduction, 123: 185-202.
Sokka T, Huhtaniemi I (1990). Ontogeny of gonadotropin receptors and gonadotropin stimulated cyclic AMP production in the neonatal rat ovary. J. Endocrinol., 127: 297-303.
Shoko Y, Hiroyuki A, Takehiro I, Takeshi S, Hiroyoshi H (1999) A serum-free culture system for efficient *in vitro* production of bovine blastocysts with improved viability after freezing and thawing. Cytotechnology, 31: 121-129.
Stojkovic M, Machado A, Sotojkovic P, Zakhartchenko V, Hutzler P, Gonçalves PB, Wolf E (2001). Mitochondrial distribution and adenosine triphosphate content of bovine oocytes before and after *in vitro* maturation, correlation with morphological criteria and developmental capacity after *in vitro* fertilization and culture. Biol. Reprod., 64: 904-909.
Stricker SA (1999). Comparative biology of calcium signaling during fertilization and egg activation in animals. Dev. Biol., 211: 157–176.
Sun QY, Schatten H (2006) Regulation of dynamic events by microfilaments during maturation and fertilization. Reproduction, 131: 193-203.
Taieb F, Thibier C, Jessus C (1997). On cycling oocytes and eggs. Mol. Reprod. Dev., 48: 397-411.
Terranova PF, Rice VM (1997). Review: cytokine involvement in ovarian processes. Am. J. Reprod. Immunol., 37: 50-63.
Teruel M, Smith R, Catalano R (2000). Growth factor: An embryo development. Biocell, 24: 107-122.
Tian WN, Braunstein LD, Prang J, Stuhlmeir KM, Xiq C, Tian X, Stanton RC (1998). Importance of G6PDH activity for cell growth. J. Biol. Chem., 273: 10609-10617.
Tsafriri A, Cao X, Vaknin KM, Popliker M (2002). Is meiosis activating sterol (MAS) an obligatory mediator of meiotic resumption in mammals. Mol. Cell. Endocrinol., 187: 197-204.
Uguz C, Vrekenburg WL, Parrish JJ (1994). Heparin-induced capacitation but not intracellular alkalinization of bovine sperm is inhibited by rp-adenosine 3'5'-cyclic monophosphorothioate. Biol. Reprod., 51: 1031-1039.
Urrego R, Tarazona A, Olivera M, Camargo O (2008). Simplificación de la fertilización de ovocitos durante la producción *in vitro* de embriones bovinos. Rev. Colom. Cs. Pec, 21: 3: 1-7.
Vajta G, Peura TT, Holm P, Páldi K, Greve T, Trounson AO, Callesen H (2000). New method for culture of zone-included or zone-free embryos: The well of the well (WOW) system. Mol. Reprod. Dev., 55: 256-264.
Van Soom A, Vandaele L, Goossens K, de Kruif A, Peelman L (2007). Gamete original in relation to early embryo development. Theriogenology, 68(1): S131-S137.
Varisanga MD, Sumantri, C, Murakami M, Fahrudin M, Suzuki T (1998). Morphological classification of the ovaries in relation to the subsequent oocyte quality for IVF produced bovine embryos. Theriogenology, 50: 1015-1023.
Vermeiden JW, Bast A (1995). Antioxidants in IVF culture media. Hum. Reprod., 10: 697-698.
Wang C, Roy SK (2006). Expression of growth differentiation factor 9 in the oocytes is essential for the development of primordial follicles in the hamster ovary. Endocrinology, 147: 1725-34.
Watson AJ (2007). Oocyte cytoplasmic maturation: A key mediator of oocyte and embryo developmental competence. J. Anim. Sci., 85 (13): E1-E3.
Winston N (2001). Regulation of early embryo development: functional redundancy between cycling subtypes. Reprod. Fertil. Dev., 13: 59-67.
Wu J, Emery BR, Carrell DT (2001). *In vitro* growth, maturation, fertilization and embryonic development of oocytes from porcine preantral follicles. Biol. Reprod., 64: 375-381.
Xu M, Kreeger PK, Shea LD, Woodruff TK (2006). Tissue-engineered follicles produce live, fertile offspring. Tiss. Eng., 12: 2739-2746.
Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, Broadbent PJ, Robinson JJ, Wilmut I, Sinclair KD (2001). Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. Nat. Genet., 27: 153-154.
Younis AI, Brackett BG, Fayrer-Hosken RA (1989). Influence of serum and hormone on bovine oocyte maturation and fertilization *in vitro*. Gamete Res., 23: 189-201.
Zhuang SG, Lynch MC, Kochevar IE (1998). Activation of protein kinase C is required for protection of cells against apoptosis induced by singlet oxygen. FEBS Lett., 437: 158-162.