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In vitro Maturation of Oocytes with Special Reference to Goat: A Review

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Abstracts: Oocyte *in vitro* maturation (IVM) is an Assisted Reproductive Technology (ART) that enables mature oocytes to be generated *ex vivo*. *In vitro* production (IVP) of embryos is currently the central focus in livestock industry including goat industry. For any successful IVP program in goat and other livestock, artificial removal of cumulus-oocyte complexes (COCs) from antral follicles and culturing them in essentially standard cell culture conditions or IVM until the maturity is a primary requirement. Therefore, it is very important two know what changes really occur to goat oocytes during IVM. As with *in vivo*, goat oocytes must undergo both nuclear and ooplasmic maturation for normal fertilization and embryonic development when cultured *in vitro*. Various locally produced factors work as co-regulators of folliculogenesis and oocyte nuclear and ooplasmic maturation in addition to extrinsic regulation by gonadotrophins and metabolic hormones. Cumulus cells (CCs) surrounding the oocyte play an important role in IVM. The morphology of the cumulus investment is commonly used as selection criteria prior to IVM which greatly influence to the maturity of goat oocytes. Embryo development is also influenced by the events occurring during oocyte maturation. Therefore, it is essential to know those events occurring during goat oocyte maturation *in vitro*. Various factors such as follicle size; follicular fluid or cells; hormones, serum, growth factors or vitamins in the IVM medium, age of the donor goat and the culture conditions are involved for successful IVM of goat oocytes. The current review describes the criteria and factors affecting maturation of goat oocytes *in vitro*.

Key words: Goat, *in vitro* maturation, nuclear maturation, ooplasmic maturation, cumulus-oocyte complexes, cumulus cells

INTRODUCTION

Oocyte *in vitro* maturation (IVM) is an Assisted Reproductive Technology (ART) that enables mature oocytes to be generated *ex vivo*. This involves artificial removal of cumulus-oocyte complexes (COCs) from antral follicles and culturing them in essentially standard cell culture conditions until they reach maturity or metaphase II (MII) stage (Gilchrist and Thompson, 2007). In mammals, embryos produced *in vitro* - in sequential steps of IVM, conventional *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) and *in vitro* culture (IVC) - display marked differences from their *in vivo* counterparts with regard to morphology, timing of development, resistance to low temperature, metabolism and gene expression (Lazzari *et al*., 2002). Thus, their clinical applications remain suboptimal (Hendriksen *et al*., 2000; Trounson *et al*., 2001). Therefore, the availability of viable, developmentally competent oocytes is crucial for the progress of *in vitro* maturation, fertilization and culture (IVMFC), ICSI and related ARTs in goat. Like other mammals, the primary oocytes of goat become arrested at the diplotene stage of meiosis at birth *in vivo*. However, they are capable of resuming meiosis spontaneously when removed from their follicles and cultured *in vitro* (Gilchrist and Thompson, 2007). Before any oocyte can be expected to be able to mature *in vitro*, it must be visualized as being normal. Normal oocytes should have cumulus cell (CC) investment surrounding the zona pellucida (ZP), absence of cracked ZP and absence of vesicles in the ooplasm. The presence of more and compact layers of CCs is considered better. A good goat oocyte will appear golden, golden-yellow or brownish in color and has granulated appearance in the

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ooplasm (Rajikin *et al*., 1994). The size of an oocyte is also important for the attainment of maturation. De Smedt *et al*. (1992) showed that 86% of goat oocytes from follicles 2 to 6 mm in diameter progressed to MII, whereas only 24% of oocytes from follicles 1-1.8 mm attained that stage. A good oocyte also has ooplasm which fills the entire part of vitelline space (Rajikin *et al*., 1994). The current study will discuss IVM of goat oocytes with special reference to IVM criteria including nuclear and ooplasmic maturation and various factors affecting oocyte maturation.

CRITERIA FOR *IN VITRO* MATURED OOCYTES

Goat oocytes must undergo both nuclear and ooplasmic maturation for normal fertilization and embryonic development. Various locally produced factors work as co-regulators of folliculogenesis and oocyte nuclear and ooplasmic maturation in addition to extrinsic regulation by pituitary gonadotrophins and metabolic hormones (Knight and Glister, 2001). Optimal expansion of the cumulus mass or CCs appears to be essential for ooplasmic maturation (Chen *et al*., 1993). Like *in vivo*, CCs also play a very important role in the maturation of oocytes *in vitro*.

Cumulus cell expansion and role of CCs in IVM: The cumulus oophorus is unique to the oocytes of eutherian mammals. It consists of a mass of granulosa cells (GCs) that surround the oocyte. *In vivo*, the cumulus oophorus expands after ovulation due to the deposition of proteoglycan matrix. The major carbohydrate in this muco-elastic matrix is hyaluronic acid (Salustri *et al*., 1999). The roles of CCs in the acquisition of full developmental competence of the oocytes have been investigated earlier (Sato *et al*., 1977; Xu *et al*., 1986). The CCs are known to supply nutrients (Haghight and Van Winkle, 1990), energy substrates (Sutton *et al*., 2003) and/or messenger molecules for the development of oocyte (Buccione *et al*., 1990) and to mediate the effects of hormones on the COC (Zuelke and Brackett, 1990). No maturation or low maturation rate was obtained from bovine oocytes when CCs were removed before maturation *in vitro* (Fukui and Sakuma, 1980; Zhang *et al*., 1995). The presence of cumulus investment increases fertilization and embryo developmental rates *in vitro* compared with that of denuded or corona-enclosed oocytes (Stojkovic *et al*., 2001; Tange *et al*., 2003). Similar developmental patterns were also observed in goat (Rajikin *et al*., 1994). Significantly ($p<0.001$) higher percentages of goat oocytes were matured when they were surrounded by more than five layers of CCs than those with less than five CC layers and denuded oocytes (Rahman *et al*., 2006).

The morphology of the cumulus investment surrounding an oocyte is commonly used as selection criteria prior to IVM (Shioya *et al*., 1988; Lonergan *et al*., 1994) and the degree of CC expansion can be used as a morphological indicator of oocyte quality following IVM. It has been suggested that an expanded CC indicates mature and good quality oocytes, while a compact CC characterizes immature oocytes (Veeck, 1988). It is contentious as to whether CC expansion is directly related to developmental capacity of the oocyte (Ali and Sirard, 2002; Luciano *et al*., 2004), although culture conditions that promote improved IVM generally also promote CC expansion, such as Follicle Stimulating Hormone (FSH) and macromolecule supplementation (Choi *et al*., 2001). The morphology of COC and oocyte developmental competence change during the growing, static and regressing phases of subordinate follicle development (Salamone *et al*., 1999). The developmental ability of goat oocyte is associated with CC expansion that increases with follicle size and decreases with increasing granulosa cell (GC) atresia (Rajikin *et al*., 1994; Han *et al*., 2006b). The process of CC expansion is accompanied by modifications of gap junctions, which contain transmembrane channels formed by hexamers of proteins belonging to the connexion family. Horse, cattle, sheep and mouse CCs express connexion 43 proteins (Sutovsky *et al*., 1993; Valdimarsson *et al*., 1993; Nuttinck *et al*., 2000; Marchal *et al*., 2003). Initiation of meiotic resumption is associated with connexion 43 protein levels in horse, pig and rat (Grazul-Bilska *et al*., 1997; Shimada *et al*., 2001; Marchal *et al*., 2003). In the same way, during IVM of cattle COCs, the connexion 43-positive gap junctions disappeared (Sutovsky *et al*., 1993). Prostaglandin E_2 is involved in CC expansion *in vitro* in mouse (Eppig, 1981), rat (Phillips and Dekel, 1982) and cattle (Calder *et al*., 2001). Till now, these phenomena were not reported in goat. In goat oocytes, the effect of an inhibitory agent roscovitine was tested (Han *et al*., 2006a). It was found from the study that a) CCs alleviated the toxicity of roscovitine on goat oocytes, b) eCG released goat oocytes from roscovitine block through the mediation of CCs and c) oocyte nuclear maturation and activation were not depended on CC expansion, but the embryo development occurred in association with CC expansion (Han *et al*., 2006a). Goat CCs were found to express Epidermal Growth Factor (EGF) receptors (Gall *et al*., 2004), one of the regulators of oocyte maturation. It is also found that EGF triggers signaling through the Mitogen-Activated Protein Kinase (MAPK) pathway during IVM in goat CCs (Gall *et al*., 2005). Although not reported in goat, CCs in cattle oocytes started to expand after 12 h of incubation *in vitro*

when all the oocytes are in metaphase I (MI) stage (Shamsuddin *et al*., 1993). The degree of expansion increased up to 18 hours of incubation and remained steady thereafter (Shamsuddin *et al*., 1993).

Nuclear and ooplasmic maturation: Like *in vivo*, both nuclear and ooplasmic maturation is required to ensure normal fertilization and embryo development *in vitro*. However, ooplasmic changes during oocyte maturation are still difficult to evaluate. As a result, maturation is judged indirectly by nuclear and chromatin structure and/or by the ability of the oocyte to be fertilized.

Nuclear maturation: Like other mammalian oocytes, goat oocytes are arrested at the dictyate or Germinal Vesicle (GV) stage of meiosis during their growth and maturation *in vivo*. Fully grown oocytes are able to resume meiosis *in vivo* after the Luteinizing Hormone (LH) surge, or spontaneously after their release from the follicle and subsequent IVC (Edwards, 1965). *In vitro*, when fully grown oocytes are removed from their follicles to the culture medium they can resume meiosis spontaneously despite ooplasmic maturity. Two major events are involved in this process. First, the COC is removed from the influence of follicular environment or Follicular Fluid (FF) and second, physical contact with mural GCs is ruptured, terminating intercellular communications via the gap junctions. This chemico-physical stimulation of the oocyte causes condensation of the chromatin and breakdown of the GV leading to MII and a second artificial arrest in the cycle (Edwards, 1965). In contrast, growing oocytes are not able to resume or complete meiosis. Oocyte competence is acquired during the growth phase, when the synthesis and storage of proteins and ribosomal and heterogeneous RNA take place (Crozet *et al*., 1981).

In goat oocytes, a number of studies were conducted to understand the mechanism of meiotic resumption, meiotic progression and its control effects of inhibitory substances. The regulation of meiotic events from prophase I to MII was studied by inhibiting protein synthesis at different times of the transition and by analyzing the changes in the protein synthesis pattern during maturation (Le Gal *et al*., 1992; Gall *et al*., 1993). The synthesis of a 67 kDa polypeptide increased during maturation and became predominant at the end of the maturation process; the synthesis of actin decreased after 18 h of culture from a very high to a low level of synthesis (Le Gal *et al*., 1992). In their study, De Smedt *et al*. (1994) found that the acquisition of meiotic competence was accompanied by nucleolar compaction and a dramatic decrease in RNA synthesis. Using cycloheximide (CHX), a protein synthesis inhibitor, Pawshe and Totey (2003) concluded that protein synthesis is required for the maintenance and transition of goat oocytes from GV to MII during IVM. At the GV stage, meiotically incompetent and competent goat oocytes display different patterns of protein phosphorylation and once oocytes are able to resume meiosis they undergo specific phosphorylation changes (Gall *et al*., 1996). Using hypoxanthine (HX), it was found that the decline of HX inhibitory effect was not due to HX depletion but rather due to the negative feedback of the metabolites on its further uptake by oocytes (Ma *et al*., 2003). Goat oocytes were capable of normal nuclear maturation and activation after temporal arrest by HX, but prolonged exposure to HX induced spontaneous activation (Ma *et al*., 2003). The inhibitory effect on meiotic resumption in goat oocytes was also studied with roscovitine that inhibits maturation or M-phase Promoting Factor (MPF) and MAPK activity and maintains the oocyte at GV stage (Han *et al*., 2006a; Jiménez-Macedo *et al*., 2006b). In their study using adult goat oocytes, (Han *et al*., 2006a) found that a) the efficiency and reversibility of roscovitine block was both drug concentration and exposure-time dependent and b) roscovitine block quickened the nuclear maturation and improved the developmental competence of meiosis-incompetent oocytes, possibly due to a sustained nuclear activity during inhibition culture. Jiménez-Macedo *et al*. (2006b) found a significantly higher number of adult goat oocytes (64.5%) blocked at GV stage compared with prepubertal goat oocytes. Low percentage of prepubertal goat oocytes block at GV stage after roscovitine incubation may be due to the fact that most of the oocytes had reinitiated the meiosis inside the follicle.

Configuration of GV chromatin has been studied and found associated with the developmental competence of oocytes in several mammalian species. In their study, Sui *et al*. (2005) found that the configurations of GV chromatin in the goat differ from those of other species in that the chromatin did not condense into a perinucleolar ring. Based on both the size of nucleoli and the degree of chromatin condensation, they classified GV chromatin of goat oocytes into a) GV1: characterized by large nucleoli and diffuse chromatin, b) GV2: with medium-sized nucleoli and condensed net-like (GV2n) or clumped (GV2c) chromatin, c) GV3: with small nucleoli and net-like (GV3n) or clumped (GV3c) chromatin and d) GV4: with no nucleolus but clumped chromatin. They stated that the GVn pattern might represent a healthy state, but the GVc an atretic state. It was found from their study that the nucleolar size decreased significantly with oocyte growth and maturation both *in vivo* and *in vitro*.

Ooplasmic maturation: Oocyte ooplasmic maturation includes those events that instill upon the oocyte a capacity to complete nuclear maturation, fertilization and

early embryogenesis and thus provides a foundation for implantation, initiation of pregnancy and normal fetal development (Bravini-Gandolfi and Gandolfi, 2001; Sirard *et al*., 2006). The ooplasmic maturation involves: a) accumulation of protein and mRNA, b) development of calcium regulatory mechanisms, c) changes in the activity of MPF (Masui and Markert, 1971) and MAPK and d) redistribution of cellular organelles. These are required to achieve oocyte developmental competence that fosters embryonic developmental competence (Bravini-Gandolfi and Gandolfi, 2001; Krisher, 2004; Sirard *et al*., 2006; Watson, 2007). The regulation of ooplasmic maturation is not as well known as nuclear maturation regulation, therefore, is one of the primary limiting factors in the production of viable embryos from immature oocytes *in vitro* (Eppig, 1996; Krisher and Bavister, 1998; Abeydeera, 2002). A number of criteria have been suggested to assess ooplasmic maturation. These include cytoskeletal organisation of oocytes such as migration of cortical granules (CGs) to the oolemma, increased number of mitochondria and lipid droplets, changes in the arrangement of Golgi apparatus and the presence of only granular endoplasmic reticulum; MPF activity and oocyte metabolism.

It is known that CG distribution is species specific. Migration of CGs to the cortex of the oocyte is a common phenomenon in mammalian oocytes (Yanagimachi, 1994). In their study, Rajikin *et al.* (1994) found that at the start of IVM culture (0 h), CGs in goat COC were numerous but dispersed randomly; whereas at the later stage of maturation (20-40 h), they were not only numerous but also distributed at the periphery, just under the oolemma. On the other hand, in cumulus-free oocytes or CFOs very few CGs were observed without any definite pattern of distribution (Rajikin *et al*., 1994). Using prepubertal goat oocytes, Velilla *et al.* (2004) reported a similar observation. At GV stage CGs were distributed homogeneously in the ooplasm, whereas CGs were located in the cortex with the formation of a monolayer beneath the oolemma in IVM-oocytes at MII and ovulated oocytes. Distribution of microfilaments, microtubules and mitochondria in goat oocytes have also been studied (Velilla *et al*., 2005; Velilla *et al*., 2006). At GV stage microfilaments were distributed in the cortex of the oocytes in both adult and prepubertal goat. After IVM, 91.7% of MII oocytes from adult goats displayed microfilaments in the cortex and within the first polar body (PB-1) and were characterized by the presence of a microfilament thickening at the cortical region over the meiotic spindle. On the other hand, only 5.7% of prepubertal goat oocytes displayed microfilaments in the cortex and within the PB-1. An undefined microtubular network was observed in adult and prepubertal goat oocytes at GV stage. After IVM, 100% of MII oocytes from adult goats displayed microtubules on the meiotic spindle and within the PB-1. This pattern of distribution was observed in 71.6% of prepubertal goat oocytes (Velilla *et al*., 2005). While looking on the distribution of mitochondria in prepubertal goat, oocytes at GV stage presented mitochondria localized in the cortical and perinuclear region (Velilla *et al*., 2006). IVM-oocytes at MII presented mitochondria peripherally polarized to the region opposite to the MII spindle and within the PB-1. Ovulated oocytes presented peripheral mitochondria distribution and mitochondrial aggregation around the MII spindle (Velilla *et al*., 2006).

Many researchers have hypothesized that if oocytes are cultured *in vitro*, before maturation, under conditions that maintain oocytes arrested at GV stage, they may have more chance of completing the process of ooplasmic maturation (Mermillod *et al*., 2000; Ponderato *et al*., 2001; Ponderato *et al*., 2002; Franz *et al*., 2003). The oocyte growth phase is characterized by an increase in the synthesis and storage of proteins and RNA (Crozet *et al*., 1981). Mobilization of these stored products will be used for meiotic and early embryo developmental events. The major changes that occur during oocyte maturation are related to protein phosphorylation. Correlated with this burst of phosphorylation is the activation of 2 major M-phases kinases: MPF and MAPK. The MPF is the main meiotic regulator and a possible regulator of ooplasmic maturation (Naito *et al*., 1992); therefore, it could be a key factor in understanding the differences between competent and incompetent oocytes. MPF is a heterodimer composed of a p34cdc2 catalytic subunit, with serine-threonine kinase activity and a cyclin B1 regulatory subunit (Labbe *et al*., 1989). MPF activity has been described in many mammalian oocytes including goat (De Smedt *et al*., 1994; Dedieu *et al*., 1998). In competent oocytes MPF appears just before germinal vesicle break down or GVBD and increases until MI, its activity decreases in anaphase-telophase and increases again, reaching its maximum level at MII (De Smedt *et al*., 1994; Dedieu *et al*., 1998). However, p34cdc2 accumulated in partially competent and incompetent oocytes within 27 h of culture, but the level of p34cdc2 in incompetent oocytes remained very low and was not sufficient to allow spontaneous resumption of meiosis (Dedieu *et al*., 1998). Recently, the relationship between oocyte diameter, meiotic and embryo developmental competence and the expression of p34cdc2, at mRNA, RNA and protein level, as well as its kinase activity, in prepubertal (1-2 mo old) goat oocytes were studied (Anguita *et al*., 2007). Oocytes

were classified according to oocyte diameter in four categories: <110, 110 to 125, 125 to 135 and >135 μm. The oocyte diameter was positively related to the percentage of oocytes at MII after IVM (0, 20.7, 58 and 78%, respectively). The expression of RNA and mRNA p34cdc2 did not vary between oocyte diameters at 0 and 27 h. Protein expression of p34cdc2 increased in each oocyte category after 27 h of maturation. MPF activity among diameter groups did not vary at 0 h but after IVM there was a clear and statistically significant increase of MPF activity in the biggest oocytes. In a recent study, the relationship between the developmental competence of goat oocytes and their total RNA and protein contents and the level of Cyclin B1 transcription was evaluated (Anguita *et al*., 2008). Their results revealed that the RNA content and the Cyclin B1 RNA expression of prepubertal goat oocytes and their development to embryos varied among oocyte size categories.

Oocyte metabolism also plays a prominent role in ooplasmic maturation and acquisition of developmental competence and the oocyte environment is one of the numerous factors controlling this important process. Carbohydrates are among the most influential of the numerous components of IVM medium that affect metabolism and developmental potential (Herrick *et al*., 2004). Concentrations of glucose and lactate in the IVM medium play a very important role for developmental competence of goat oocytes (Herrick *et al*., 2006).

FACTORS AFFECTING IVM OF GOAT OOCYTES

Embryo development is influenced by events occurring during oocyte maturation (Rajikin *et al*., 1994; Teotia *et al*., 2001). A number of maturation media have been developed in different laboratory for IVM of goat oocytes. In general, goat oocytes are matured in buffered TCM-199 supplemented with pyruvate, heat-inactivated serum and hormones (FSH, LH, estradiol) (Ongeri *et al*., 2001; Izquierdo *et al*., 2002; Wang *et al*., 2003). Low level of efficiency, developmental arrest and losses of viability are often observed when goat oocytes are cultured *in vitro* compared with those of *in vivo* studies, which is almost certainly related to the quality of the oocytes at the outset of IVM (Cognié *et al*., 2003). Therefore, many studies have been done in an attempt to determine what conditions are needed during the IVM, fertilisation and culture processes to maximize embryo production. Because of advanced research in goat IVP, higher maturation rates (70-90% or even 100%) were achieved with pre-selected oocytes under specific conditions (Samaké *et al*., 2000; Bormann *et al*., 2003). A number of factors are attributed to the IVM of goat oocytes, for example, follicle size, hormones, serum and different growth factors in the IVM medium and culture condition.

Effect of follicle size: Follicle size has been reported to influence the oocyte's ability to resume meiosis and reach maturation (Gall *et al*., 1993; Martino *et al*., 1994; Gall *et al*., 2002). In a study by Crozet *et al*. (1995), it was observed that oocytes from larger follicles (>5 mm in diameter) gave better yield of blastocysts compared to follicles measuring less than 5 mm diameter. Martino *et al*. (1994) reported that follicles measuring more than 3 mm diameter contained more CC layers and gave better IVM results. These researchers concluded that as follicle size increased, oocytes completed their growth and achieved meiotic competence, thus giving better *in vitro* embryo production yield. In addition, there was no difference in embryo developmental capacity between oocytes collected from prepubertal and adult goats (Mogas *et al*., 1997). It has also been reported that goat oocytes from early antral follicles grew and acquired the ability to resume meiosis when cultured for 9 days on GC monolayers (Crozet *et al*., 2000).

Effect of hormones in the IVM medium: Typically, most caprine IVM culture media are supplemented with gonadotrophins (FSH and LH) and estradiol-17β which are reported to improve maturation rates significantly (Keskintepe *et al*., 1994; Izquierdo *et al*., 1998, 1999). The final concentration of FSH, LH and estradiol-17β used varied among researchers. For example, FSH final concentration ranged from 0.1 μg mL^{-1} (Cognié *et al*., 2003) to 10 μg mL^{-1} (Jiménez-Macedo *et al*., 2005, 2006a, 2007), LH final concentration ranging from 3 μg mL^{-1} (Ongeri *et al*., 2001) to 100 μg mL^{-1} (Keskintepe *et al*., 1994) and estradiol-17β final concentration from none (Ongeri *et al*., 2001) to 1 μg mL^{-1} (Keefer *et al*., 2002; Jiménez-Macedo *et al*., 2005, 2006a, 2007). Higher maturation rates (up to 95%) were achieved using 10 μg mL^{-1} human chorionic gonadotrophin (hCG) in combination with 10 μg mL^{-1} FSH and 1 μg mL^{-1} estradiol-17β from both normal and dysmorphic goat oocytes (Rahman *et al*., 2006, 2007a, b). The inclusion of gonadotrophins in IVM medium was reported to enhance oocyte quality and developmental potential by possible alteration of metabolic processes (Brackett and Zuelke, 1993). Gonadotrophins are the primary regulators of *in vitro* nuclear maturation in mammalian oocytes. The beneficial effect of gonadotrophins in the IVM medium was more pronounced for oocytes from juvenile or prepubertal females (Ledda *et al*., 1997). Estradiol may be involved in

ooplasmic maturation by stimulating DNA polymerase β and enhancing the synthesis of presumed male pronucleus growth factors. Blastocyst production was significantly increased for oocytes matured in the presence of estradiol-17β (Pawshe and Totey, 2003). However, investigations had shown that inadequate priming of sheep oocytes with estradiol-17β exacerbated the possibility of embryo cleavage anomalies and thus led to failure of blastocysts formation (Oussaid *et al*., 1999).

Effect of serum in the medium: Semi-defined protein preparation such as serum is usually included in IVM media because it contains unidentified growth factors, hormones and peptides that may support growth and development of oocytes. Caprine IVM media are generally supplemented with 10 to 20% heat-inactivated serum. It is found that serum provides nutrition to cells in the COCs and prevents ZP hardening in sheep oocytes (Wani, 2002). In goats, sera used in IVM media include fetal bovine serum or FBS (Crozet *et al*., 2000; Samaké *et al*., 2000; Mayor *et al*., 2001; Velilla *et al*., 2002), fetal calf serum or FCS (Crozet *et al*., 1995; Gall *et al*., 1996; Rho *et al*., 2001), steer serum or SS (Rodriguez-Gonzalez *et al*., 2003b; Urdaneta *et al*., 2003; Jiménez-Macedo *et al*., 2005, 2006a, 2007) and homologous or heterologous Estrus Goat Serum (EGS) (Mogas *et al*., 1997; Malik *et al*., 1999; Rahman *et al*., 2006, 2007a). The EGS was used alone (Mogas *et al*., 1997; Rahman *et al*., 2006, 2007a) or in combination with BSA (Rajikin *et al*., 1994). The effect of type and concentration of serum on maturation rates has been investigated (Pawshe *et al*., 1996; Tajik and Esfandabadi, 2003). Although one study showed that the presence of EGS in the maturation medium was not essential (Pawshe *et al*., 1996), high maturation rates of caprine oocytes were obtained after 24-25 h of culture in IVM medium supplemented with 10% FBS (83%), 10% EGS (86%), or 10% ESS (94%), without addition of gonadotrophins (Tajik and Esfandabadi, 2003). Fatty acids contained in serum also served as energy substrates for embryonic growth (Kane, 1979).

Supplementation of follicular fluid or co-culture with follicular cells: In addition to the supplementation of IVM medium with gonadotrophins, the effects of FF have also been examined. Supplementation of IVM medium with FF from non-atretic or gonadotrophin-stimulated large follicles (>4 mm) had some beneficial effect in goat oocytes (Martino *et al*., 1995; Cognié *et al*., 2004). This beneficial effect on goat oocyte maturation may be due to the presence of growth factors, hormones and intra-ovarian peptides in more physiological proportions in FF (Cognié *et al*., 2004).

Like FF, follicular cells are also being used in the IVM medium to culture goat oocytes (Teotia *et al*., 2001; Jiménez-Macedo *et al*., 2005). In sheep, it has been reported that follicular cells support oocyte maturation by providing nutrition and signals for the synthesis of specific structural and maturation proteins (Wani, 2002). Culture of goat oocytes over GC monolayer delayed maturation but significantly increased the maturation rate (Tyagi *et al*., 1997). When goat oocytes were matured over GC monolayer, higher fertilization and cleavage rates were achieved than the oocytes those matured with GC co-culture (Teotia *et al*., 2001) which suggested that the GC monolayer improved ooplasmic maturation. GCs from small and large follicles were used for IVM and IVC, with approximately the same efficiency after conditioning with IVM and IVC media 18-24 h before culture (Teotia *et al*., 2001).

Effect of cysteamine in the IVM medium: The effect of cysteamine supplementation was also extensively investigated. Cysteamine is a low molecular weight thiol that, when present during IVM of oocytes and IVC of embryos, increases the intracytoplasmic oocyte glutathione (GSH) concentration and improves embryo development rates (De Matos *et al*., 1995; Luvoni *et al*., 1996; De Matos and Furnus, 2000). GSH participates in various mechanisms such as amino acid transport, protein synthesis, reduction of disulphides and protection against oxidative damage. The glutathione content of goat oocytes seemed to be a good indicator for ooplasmic competence and that the addition of cysteamine to a defined IVM medium improved caprine IVP (Cognié *et al*., 2003). Subsequent investigations revealed that supplementing a defined IVM medium with 50, 100 or 400 μM cysteamine significantly increased intracellular glutathione levels in goat oocytes, improved maturation rate as well as blastocyst yield compared with a control medium without cysteamine supplementation (Cognié *et al*., 2003; Rodriguez-Gonzalez *et al*., 2003a, b; Urdaneta *et al*., 2003, 2004). Supplementation of cysteamine (100 μM) was reported to modify the kinetics of oocyte nuclear maturation and increased blastocyst yield on day 8 post-IVF (Cognié *et al*., 2003).

Effect of growth factors, vitamins and use of defined media: Efficacy of different growth promoting factors, vitamins or other substances alone or with supplementation in defined medium have been tested. Epidermal Growth Factor (EGF) influenced oocyte maturation and blastocyst production rates in a number of mammals. Goat CCs express EGF receptors (Gall *et al*., 2004) and EGF triggers signaling through the MAPK

pathway during IVM in goat CCs (Gall *et al*., 2005). EGF involved in the regulation of follicular growth and oocyte maturation in goats. Recently, it is reported that EGF and its receptor are also expressed in goat ovarian follicles at all stages of follicle development, in corpus luteum and in ovarian surface epithelium (Silva *et al*., 2006). Goat oocytes matured *in vitro* in the presence of EGF had greater CC expansion, higher maturation and fertilization rates than the control oocytes (Nagar and Purohit, 2005). Although not reported in goat, nerve growth factor is produced *in vitro* by GCs in response to gonadotrophin stimulation and may be involved in the control of sheep oocyte maturation as well as in resumption of meiosis (>70% of the oocytes) (Barboni *et al*., 2002). Insulin-like growth factor-I did not seem to affect oocyte IVM (Guler *et al*., 2000). The beneficial effect of vitamin supplementation in goat IVM media was also studied. According to Bormann *et al.* (2003), inclusion of vitamins significantly increased overall blastocyst development, percentage of cleaved embryos and mean blastocyst cell number.

Recent studies also demonstrated that goat oocytes could be matured successfully under Synthetic Oviduct Fluid (SOF)-based defined conditions medium supplemented with EGF, amino acids, gonadotrophins and BSA (Ongeri *et al*., 2001). A modified SOF maturation medium or mSOFmat containing polyvinylalcohol or hyaluronate with citrate as a macromolecular supplement resulted in better maturation and development rates for goat oocytes than commonly used TCM-199 supplemented with EGS. In addition, the concentrations of pyruvate and lactate in the medium closely mimicked the concentrations normally present in FF (Herrick *et al*., 2004). Addition of Minimum Essential Media (MEM) vitamins to SOFmat medium was reported to be beneficial for subsequent blastocyst development and viability (Bormann *et al*., 2003). However, maturation competence of goat oocytes was suppressed by streptomycin when compared with penicillin or gentamicin (Kang *et al*., 2004).

Effect of culture conditions: Incubation time and temperature in CO_2 incubator have a great influence on goat oocyte maturation. Although, sheep oocytes required 23 to 26 h of incubation in the desired medium at 38 to 39°C in humidified atmosphere of 5% CO_2 in air (or 7% O_2 and 88% N_2) (Baldassarre *et al*., 1996; Al-Aghbari and Menino, 2002), goat oocytes required 24 to 27 h in the desired medium in the same culture conditions (Samaké *et al*., 2000). Incubation time necessary for maturation of goat oocytes seemed to be longer than that needed for sheep/cattle oocytes. A higher proportion of goat oocytes reach MII stage after 27 h than after 24 h of culture (Rho *et al*., 2001; Rahman *et al*., 2007a). Other studies suggested that culture for 32 h in TCM-199 with 20% OGS was the best alternative for IVM of goat oocytes (Sharma *et al*., 1996). Currently most of the laboratory engaged with goat ICSI studies culturing goat oocytes for 27 h derived from laparoscopic ovum pick-up (LOPU) (Wang *et al*., 2003; Rahman *et al*., 2007a; Abdullah *et al*., 2008) or abattoir (Jiménez-Macedo *et al*., 2005, 2006a, 2007) sources in a humidified atmosphere (5% CO_2) of the CO_2 incubator at 38 to 39°C.

Age of the donor goat: Donor age has been reported to affect developmental competence of oocytes from juvenile or prepubertal does (Izquierdo *et al*., 2002). Oocytes derived from prepubertal does had high rates of polyspermy (Palomo *et al*., 1999), failure of sperm head decondensation and formation of male pronucleus or MPN (Mogas *et al*., 1997), low blastocyst production rate (Izquierdo *et al*., 2002) and high percentage of haploid embryos (Villamediana *et al*., 2001). The lower developmental competence of prepubertal doe oocytes may be due to a deficiency in ooplasmic maturation leading to reduced sperm penetration, lack of MPN formation, failure to block polyspermy, cleavage failure, failure to reach or survive the transition from maternal to embryonic genomic expression and developmental problems leading to pregnancy loss during the preimplantation and postimplantation stages (Armstrong, 2001; Velilla *et al*., 2004). The high rate of polyspermy may have been due to abnormal distribution of cortical granules and a failure in the cortical reaction (Velilla *et al*., 2004). Prepubertal goat oocytes displayed the same maturation, but lower fertilization rate, compared with adult oocytes when cultured with adult goat GCs (Martino *et al*., 1994, 1995). Gonadotrophin stimulation of prepubertal goats resulted in high oocyte yield, with similar IVM and developmental rates than oocytes from adult goats (Koeman *et al*., 2003). It was also reported that addition of glutathione in the IVM medium was not associated with a higher normal fertilization rate of prepubertal goat oocytes (Mayor *et al*., 2001).

CONCLUSION

The present state of IVM of goat oocytes shows a great deal of promise. A number of studies have been conducted to investigate IVM competency of goat oocytes, from both adult and prepubertal goat. Studies were carried out with oocytes derived from abattoir ovaries, LOPU, laparotomy or ovariectomy using

untreated or hormonally treated goats. Generally, oocytes recovered from >2 to 6 mm follicles and COCs with more than three complete CC layers provide higher maturation rates. A number of IVM media was tested depending on the design of experiments or the choice of the researchers. However, TCM-199 is the main base medium which is supplemented with hormones, serum, carbohydrates and other components as required depending on the experiments. However, SOF-based medium also tested and found promising for goat oocyte maturation. Generally, selected oocytes are cultured in presence of CO_2 (5%) in air and humidity (95%) at 38 to 39°C. Many research studies have been carried out on goat oocyte maturation; however, IVM of goat oocytes show great deal of variation in maturity from laboratory to laboratory even though cultured in the same condition using the same protocol. Besides, IVM oocytes are still less competent than oocytes matured *in vivo*. Therefore, studies at molecular level should have prime importance which may lead to the discovery of factors affecting maturational competence of goat oocytes *in vitro* which consequently determine the fate of embryos.

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