ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 11 (1): 1-9, 2012
ISSN 1682-296X / DOI: 10.3923/biotech.2012.1.9

Factors Affecting *in vitro* Culture of Goat Embryos with Special Reference to ICSI-derived Oocytes: A Review

Abu Nasar Md. Aminoor Rahman
Department of Gynecology, Obstetrics and Reproductive Health,
Faculty of Veterinary Medicine and Animal Science,
Bangabandhu Sheikh Mujibur Rahman Agricultural University,
Gazipur, 1706, Bangladesh

Abstract: Significant developments have been achieved in assisted reproductive technology during the last two decades to boost livestock production. *In vitro* production of embryos is currently the central focus of these modern ARTs in livestock industry including goat industry. For any successful IVP program, *in vitro* culture (IVC) of fertilized oocytes or embryos is very critical stage. An optimum environment in IVC medium which mimics the uterine environment is necessary for the proper growth and development of intreacyto plasmic spream injechlan on ICSI-derived goat embryos *in vitro*. The success of IVC depends on the various factors such as osmolarity and ionic composition, temperature, pH and CO_2, oxygen, carbohydrates, amino acids, lipids and fatty acids, proteins, growth factors and cytokines, etc. Any deviation from the appropriate environment could lead to embryonic arrest at any stage of development. This review describes the factors affecting growth and development of ICSI-derived goat embryos in IVC medium.

Key words: Goat, *in vitro* culture (IVC), factors, embryos, ICSI

INTRODUCTION

Oocyte or embryo *in vitro* culture (IVC) is an Assisted Reproductive Technology (ART) that enables embryo development *ex vivo*. This involves artificial culture of fertilized oocytes or embryos in essentially standard cell culture conditions until they are ready to transfer to the recipient animals. In mammals, embryos produced *in vitro* in sequential steps of *in vitro* maturation (IVM), conventional *in vitro* fertilization (IVF) or Intra cytoplasmic Sperm Injection (ICSI) and 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). Embryos obtained from ruminant species have been cultured in a number of defined and semidefined or undefined media. A defined medium is a medium which is prepared using identifiable components prior to embryo culture and that the components must address the biochemical needs of the embryo as it develops and the physical environment created must simulate the *in vivo* environment (Thompson *et al*., 1996). On the other hand, in semidefined or undefined medium some or most of the components are unidentified. Defined medium is the choice when the objective is to study aspects of embryonic development. However, if the objective is merely to support *in vitro* embryo survival, then the appropriate medium is that which is effective (Wright and O'Fallon, 1987). It is assumed that the IVC conditions required for goat ICSI-derived embryos are similar with that of IVF-derived embryos. Therefore, for goat embryo culture similar systems are mostly used as in bovine and ovine IVC systems. There are several factors which might influence the proper growth and development of goat embryos *in vitro*. The current study will discuss factors affecting growth and development of goat embryos with special reference to ICSI-derive embryos in IVC culture system.

Factors affecting embryo development *in vitro*: There are several factors which may influence in growth and development of fertilized oocytes or embryos in IVC system. These are discussed below:

Osmolarity and ionic composition: Intracellular osmolarity has been suggested to play an important role during *in vitro* development (Lawitts and Biggers, 1992;

Corresponding Author: Abu Nasar Md. Aminoor Rahman, Khorsheda Monjheel (Second Floor), 41/17, Hazi Afsar Uddin Road Zigatola, Dhaka-1209, Bangladesh Tel: +88 02 913 0323

Biggers *et al*., 1993). Addition of intracellular osmolyte such as glycine and alanine has been reported to enhance *in vitro* bovine embryo development (Moore and Bondioli, 1993). As reviewed by Wright and Bondioli (1981) bovine embryo development improved when the osmolarity of SOF medium was 270 mOsm kg^{-1} instead of 300 mOsm kg^{-1}. However, in ovine embryo culture, osmolarity within the range of 260 to 300 mOsm kg^{-1} had little effect on development (Walker *et al*., 1988). The effect of varying concentrations of K^+ (2.5-27.8 mM) and Ca^{2+} (1.3-5 mM) on ovine embryo culture were investigated by Walker *et al*. (1988) and found that K^+ at 13 mM and above was inhibitory but no effect for Ca^{2+}. On the other hand, the ionic composition of ovine and bovine oviductal fluid was also investigated (Grippo *et al*., 1992; Restall and Wales, 1996). It was found that bovine and ovine oviductal fluid contained lower concentration of Na^+ and higher concentration of K^+ compared with blood plasma (Leese, 1988). Grippo *et al*. (1992) reported that bovine oviductal fluid contained approximately 4.5 mM K^+ and a K^+ Na^+ ratio of 0.032. Ionic composition also varies along the estrous cycle and the length of the oviduct, especially in relation to Ca^{2+} (Grippo *et al*., 1992). However, not much had been done in IVC formulation to mimic changes in *in vivo* ionic composition (Thompson *et al*., 1996). The osmolarity in goat IVC medium is kept at 270 to 280 mOsm kg^{-1} (Tervit *et al*., 1972; Younis *et al*., 1991) or 280 to 300 mOsm kg^{-1} (Brackett and Oliphant, 1975; Takahashi and First, 1992).

Temperature: Although, it has been well established that optimal temperature of IVF is around 39°C (Ball *et al*., 1983; Lenz *et al*., 1983) there is very little research done on the optimal temperature for caprine or bovine embryo development. Wang *et al*. (1991) investigated *in vitro* development of bovine embryos at 36 to 40°C and found that more embryos developed to the blastocyst and hatching blastocyst stages at 39°C. In addition, work was done on temperature sensitivity and thermo-tolerance of embryos. Ealy and Hansen (1994) and Ealy *et al*. (1995) demonstrated that embryos become more resistant to heat shock as they progress through development. Generally, ICSI-derived goat embryos are cultured at 38.5°C (Jimenez-Macedo *et al*., 2005; Jimenez-Macedo *et al*., 2006; Jimenez-Macedo *et al*., 2007; Abdullah *et al*., 2008; Rahman, 2008; Rahman *et al*., 2009) or 38.5 to 39°C (Keskintepe *et al*., 1997; Wang *et al*., 2003) in the CO_2 incubator.

pH and CO_2: Information on the alteration of intracellular pH on ruminant embryo development is scanty (Thompson *et al*., 1996). However, it is known that the pH of oviductal and uterine fluid is regulated by bicarbonate and equilibration with CO_2 which varies according to the estrous cycle stage (Olds and VanDemark, 1957). Therefore, bicarbonate is likely to be involved in many biochemical pathways and should always be included in the IVC medium. It was also reported that ovine (Walker *et al*., 1989) and bovine (Thompson *et al*., 1996) embryos would progress to the blastocyst stage when buffered with zwitterionic buffers (Good *et al*., 1966) in CO_2-free atmosphere. Thus, dependence on CO_2 is not entirely necessary for embryonic development provided a suitable buffering system is available. However, goat embryos generally cultured in presence of 5% CO_2 inside the incubator (Keskintepe *et al*., 1997; Wang *et al*., 2003; Jimenez-Macedo *et al*., 2005). As mentioned in Urdaneta (2005) a pH of 7.2 to 7.4 is maintained in the IVC medium for culturing goat embryos (Tervit *et al*., 1972; Brackett and Oliphant, 1975; Younis *et al*., 1991; Takahashi and First, 1992).

Oxygen: To date, several reports (Tervit *et al*., 1972; Wright and Bondioli, 1981; Thompson *et al*., 1990; Nagao *et al*., 1994; Watson *et al*., 1994b) on the effect of atmospheric oxygen concentration on ruminant embryo development have pointed to reduced oxygen concentration (below 10%) which reflects the level found *in vivo* and is associated with increased developmental rates (Fischer and Bavister, 1993). Based on oxygen consumption data (Thompson *et al*., 1996) and deduction from degree of pyruvate and glutamine utilization (Rieger, 1992; Gardner *et al*., 1993; Thompson *et al*., 1993; Thompson *et al*., 1996) bovine embryos prior to compaction are dependent on oxidative phosphorylation for generating ATP. When embryos are cultured in air, all blastomeres are likely to be exposed to adequate levels of oxygen (Byatt-Smith *et al*., 1991). However, the inner blastomeres may be in anoxic environment when larger embryos (such as that of human and livestock species) are incubated in reduced oxygen concentration of 5% reviewed by Thompson *et al*. (1996).

Oxygen requirement of embryos changes throughout development. According to a review by Thompson *et al*. (1996) embryos at post-compaction may require less oxygen than those at pre-compaction based on the evidence that the proportion of ATP generated from oxidative phosphorylation decreases in bovine embryos once compaction begins (Thompson *et al*., 1996). Furthermore, blood flow through the capillary bed on the eighth day of the sheep estrous cycle is significantly lower than at the time of estrus (Brown *et al*., 1985) which suggests the anoxicity of the uterine environment at implantation (Leese, 1995). For culturing ICSI-derived goat embryos oxygen level in the CO_2 incubator is maintained

at 5% (Keskintepe *et al*., 1997; Wang *et al*., 2003; Jimenez-Macedo *et al*., 2005; Jimenez-Macedo *et al*., 2006; Jimenez-Macedo *et al*., 2007; Rahman *et al*., 2007, 2009).

Carbohydrates: Carbohydrate utilization is generally increased as development progresses, especially with the onset of compaction due to surge of demand for energy placed by Na^+, K^+-ATPase required for blastocoel formation (Thompson *et al*., 1996). Kim *et al*. (1993) demonstrated that D-glucose supplementation in bovine IVC medium is probably not necessary until approximately 3 or 4 days of development, at which stage supplementation improves development. However, using glucose at 5 to 6 mM in IVC medium (i.e., glucose concentration in blood plasma) is generally inhibitory to development (Thompson *et al*., 1992; Kim *et al*., 1993; Matsuyama *et al*., 1993). For ovine embryos, carbohydrates are not compulsory for blastocyst development if cultured in the presence of oxidisable amino acids (Thompson *et al*., 1993). Limited amount of glucose is oxidized via the tricarboxylic acid cycle which results in lactate production (Rieger, 1992; Thompson *et al*., 1992; Gardner *et al*., 1993; Thompson *et al*., 1996).

Supplementation of pyruvate and/or L-lactate in IVC medium also enhances development, although not a compulsory requirement (Kim *et al*., 1993; Matsuyama *et al*., 1993; Thompson *et al*., 1993). Thompson *et al*. (1993) suggested that the optimal pyruvate (0.3 mM) to L-lactate (3.3 mM) ratio in ovine embryo culture is 1:10 (Tervit *et al*., 1972; Takahashi and First, 1992). Same level of sodium pyruvate, glucose and let L-lactate used in cattle and sheep IVC medium are also used in goat (Urdaneta, 2005).

Amino acids: Supplementation of groups of amino acids, that is, the essential and non-essential amino acids have been reported to have beneficial effects on embryo development. Much progress has been attributed to work done in Barry D. Bavister's laboratory that discovered the supplementation of essential and non-essential amino acids to stimulate *in vitro* development of hamster embryos and that certain amino acids inhibit development (Carney and Bavister, 1987; Bavister and Arlotto, 1990). Chatot *et al*. (1989) also made a significant finding that glutamine is the key component in medium that supported development of embryos from mouse strains that normally arrest at 2-cell stage. For bovine and ovine embryos, the addition of pooled amino acids is reported to improve embryo development (Gardner, 1994; Gardner *et al*., 1994; Rosenkrans and First, 1994). Moore and Bondioli (1993) also reported increased developmental rate by using culture medium that simulates alanine and glycine concentrations like those found in oviductal and uterine fluid. However, so far it is less understood how amino acid requirement changes as development progresses (Thompson *et al*., 1996). Like in cattle and sheep, same concentration of BME essential (20 μL mL^{-1}) and MEM non-essential (10 μL mL^{-1}) amino acids are added in the IVC medium for culturing goat embryos (Urdaneta, 2005).

Lipids and fatty acids: Supplementation of lipids and fatty acids may not be an absolute requirement because ruminant embryos had been successfully cultured in lipid-free medium (Seidel *et al*., 1991). There seems little requirement for exogenous lipids and fatty acids because bovine embryos mostly utilize carbohydrates as source of ATP (Thompson *et al*., 1996). However, the presence of many ooplasmic lipid droplets in ovine embryos has been suggested to be directly associated to the mitochondria and may serve to be an important energy source *in vivo* (Dorland *et al*., 1995). Thompson *et al*. (1996) suggested that these ooplasmic lipids may act as fuel reservoir in situations when *in vivo* substrate availability is low. Addition of lipid and fatty acids in goat IVC medium has not been reported.

Proteins: The function of a non-specific protein source in embryo culture is not well understood. Typically, serum albumin is used as protein supplement in IVC medium because it is the major protein found in reproductive tract fluid. Moreover, serum albumin supplementation is beneficial in that it chelates metal cations and prevents embryos from adhering to the Petri dish surface. In goat, Estiws Goat Serum (EHS) is traditionally supplemented in oocyte or embryo culture media as a protein source to provide additional unidentified beneficial growth factors, hormones and peptides that may be present in the serum (Phua, 2006). However, protein is not an absolute requirement for culturing bovine embryos (Seidel *et al*., 1991; Bavister *et al*., 1992) because most of the demand for ATP is likely to be fulfilled by carbohydrate metabolism (Thompson *et al*., 1996). However, for culturing ICSI-derived goat embryos BSA (0.3-1.0%, w/v) (Wang *et al*., 2003), GS (20%, v/v) (Wang *et al*., 2003), FBS (0.1 μL embryo) (Jimenez-Macedo *et al*., 2005; Jimenez-Macedo *et al*., 2006; Jimenez-Macedo *et al*., 2007) or heat-inactivated goat serum (10%, v/v) (Rahman *et al*., 2007; Abdullah *et al*., 2008; Rahman *et al*., 2009) have been reported to use as protein source in the IVC medium.

Growth factors and cytokines: Only certain growth factors supplemented in bovine IVC medium appear to enhance embryo development. Such growth factors are

EGF, transforming growth factor-β (TGF-β) and Platelet-Derived Growth Factor (PDGF) (Larson *et al*., 1992a, b; Flood *et al*., 1993; Thibodeaux *et al*., 1995). The involvement of these growth factors in enhancing development is further confirmed by the reports of Watson *et al*. (1992) and Watson *et al*. (1994a) that mRNA of several growth factors and their receptors are transcribed at different developmental stages of ruminant embryos. The embryos also demonstrate autocrine and paracrine activities which enhance development when cultured in groups (Gardner *et al*., 1994; Keefer *et al*., 1994). A further report by Thibodeaux *et al*. (1995) indicated the PDGF to act as a paracrine growth factor. Cytokines are also reported to interact with growth factors in facilitating embryo development (Fry *et al*., 1992). No such report has been revealed for culturing ICSI-derived goat embryos.

Embryonic arrest of *in vitro* produced goat embryos: Early goat embryos cultured *in vitro* fail to develop past the 8-16 cell stages in traditional culture media. This block occurred around the time of activation of the embryonic genome (Pivko *et al*., 1995). This is also true for ICSI-derived goat and sheep embryos. It was found that 100% of goat (Jimenez-Macedo *et al*., 2005) and 80% of sheep (Gomez *et al*., 1997) embryos produced by ICSI underwent development arrest on day 4 (16-cell to morula stage). This could be due to a number of factors such as oocyte maturation, culture system, oocyte activation and abnormal fertilization (Gomez *et al*., 1997; Gomez *et al*., 1998; Jimenez-Macedo *et al*., 2005). In one study, more than 50% of goat embryos produced *in vitro* showed chromosomal abnormalities (Villamediana *et al*., 2001).

The reasons for embryonic arrest generally can be classified into two categories internal and external factors. Internal factors for embryonic arrest involve innate defects occurring in the oocyte or embryo itself. Examples of innate defects are poor oocyte quality, enzymatic deficiencies or dysfunctional regulation in an embryo which is of genetic origin, non-disjunction in the oocyte and inadequate chromatin packaging in the sperm. External factors for embryonic arrest are factors pertaining to the environment surrounding the embryo. The environment deemed 'hazardous' to the embryo is usually artifacts produced either as end products or by-products of the embryo's metabolic processes that accumulate to toxic levels over time cited in Phua (2006).

The generation of oxygen-derived free radicals has been proposed to be the cause of some cases of abnormal or 'blocked' development in ruminant embryos (Rieger, 1992; Johnson and Nasresfahani, 1994). This proposition is made based on investigations on the effect of various free radical scavengers (Nasr-Esfahini *et al*., 1990; Noda *et al*., 1991; Goto *et al*., 1993; Johnson and Nasresfahani, 1994) or reduced oxygen concentration (Thompson *et al*., 1990; Johnson and Nasresfahani, 1994; Nagao *et al*., 1994; Watson *et al*., 1994b) on intracellular H_2O_2 levels. According to Thompson *et al*. (1996) the implication of these investigations is that either a mechanism exists *in vivo* to scavenge free radicals, or free radicals are not generated *in vivo* at all in the first place due to limited availability of oxygen (Byatt-Smith *et al*., 1991).

The inclusion of amino acids in IVC medium leads to the inevitable accumulation of ammonium ions (Gardner, 1994; Gardner *et al*., 1994). Although, the ammonium toxicity level has not yet been determined for bovine embryos, but what is known is that ammonium toxicity causes fetal abnormalities in mouse following embryo transfer (Gardner, 1994). Currently, the problem of ammonium accumulation is handled in either of two ways: changing to a fresh medium before it accumulates to toxic level or removing ammonium from the culture medium by enzymatic means (Gardner, 1994). Similarly, accumulation of L-lactic acid may be harmful to the embryo because using high levels of Na D, L-lactate (33 mM) has been shown to inhibit ovine *in vitro* embryo development (Thompson *et al*., 1993).

***In vitro* culture of ICSI-derived goat embryos:** The information on IVC of ICSI-derived goat embryos is scanty. The IVC media those are used for IVF are also used to culture ICSI-derived goat embryos. To date, the culture media reported to be used successfully for IVC of ICSI-derived goat embryos to the morula/blastocyst stage are SOF medium (Keskintepe *et al*., 1997; Jimenez-Macedo *et al*., 2005; Jimenez-Macedo *et al*., 2006; Jimenez-Macedo *et al*., 2007) modified Tyrode's albumin lactate pyruvate medium plus modified potassium simplex optimization medium (mTALP-mKSOM) (Wang *et al*., 2003) and Gardner's sequential media G1.3-G2.3 (Wang *et al*., 2003; Jimenez-Macedo *et al*., 2005). In IVF, culture of goat embryos in co-culture system has been reported in TCM199 (or M199) together with different cell layer system, for example, GC monolayers (Mogas *et al*., 1997a, b), CC monolayers (Izquierdo *et al*., 1998) or Goat Oviduct Epithelial Cell (GOEC) monolayers (Rajikin, 1995; Rho *et al*., 2001; Katska-Ksiazkiewicz *et al*., 2004). In contrast, one study reported that ICSI-derived goat embryos can be cultured in TCM199 together with GC monolayers (Jimenez-Macedo *et al*., 2005). In IVF studies, the inclusion of somatic cell monolayers is believed to provide growth-promoting factors

Table 1: Timeline of significant finding in IVC of ICSI-derived goat oocytes

Year	Author	Significant findings
1997	Keskintepe *et al.* (1997)	Culturing in modified SOF medium, 57.7, 35.5 and 24.4% cleavage, morula and blastocyst rates, respectively were obtained from oocytes injected with broken tail sperm without any activation treatment
2003	Wang *et al.* (2003)	Using Piezo-ICSI with tail-cut sperm and culturing in G1.3-G 2.3 medium, 74 cleavage, 15 morula and 9% blastocyst were obtained. While culturing in mTALP-mKOSM medium cleavage, morula and blastocysts development rates increased to 89, 41 and 35%, respectively using same ICSI technique.
2004	Zhou *et al.* (2004)	Treating sperm with 0.0005% Triton X-100 before ICSI, significantly higher rates of fertilization, cleavage and morula/blastocyst were obtained than with other concentrations and manual immobilization. Again, post-injection activation of oocytes with ionomycin alone or ionomycin plus 6-DMAP significantly increased the rates of fertilization, cleavage and morula/blastocyst after ICSI. However, name of the cultured medium used in this study could not be known as only abstract was available.
2005	Jimenez-Macedo *et al.* (2005)	Culturing in three different media, 73.4 cleavage and 3.8% morula were obtained from mSOF; 66.6% cleavage and 5.2% morula from G1.3-G2.3 and 82% cleavage and 2.6% morula were obtained from TCM199+GC monolayers. These results were obtained using conventional ICSI and activation of injected oocytes with Ca^{2+} ionophore and DMAP.
2006	Jimenez-Macedo *et al.* (2006)	Treating sperm before ICSI with heparin plus Ca^{2+} ionophore and culturing them in mSOF medium, 23.7% cleavage, 8.2% morula and 5.1% blastocyst were obtained. Using same protocol when ICSI was performed according to diameter cleavage, morula and blastocyst development rates obtained were 60.3, 6.2 and 0%, respectively, for oocyte of 110-125 μm; 66.9, 30.4 and 15.9%, respectively for oocyte of 125-135 μm and 75.0, 22.2 and 11.1%, respectively for oocyte >135 μm in diameter.
2007	Jimenez-Macedo *et al.* (2007)	Treating sperm before ICSI with heparin plus Ca-ionophore, performing ICSI according to diameter and culturing them in mSOF medium, cleavage and blastocyst development rates obtained were 51.0 and 14.7%, respectively for oocyte of 110-125 μm and 66.2 and 34.5%, respectively for oocyte >135 μm in diameter.
2008	Abdullah *et al.* (2008)	Significantly higher cleavage (71-79%) and morula development (9-32%) rates were obtained when LOPU was done between 60-72 h after FSH/HCG treatment than commonly used 36 h.
2009	Rahman *et al.* (2009)	Higher fertilization (61%), cleavage (83%) and morula development (20%) rates were obtained in LOPU-derived goat embryos compared to abattoir one.

(Gandolfi, 1994) act as nutritive support (Mogas *et al.*, 1997b) or to eliminate inhibitory components such as glucose and oxygen (Watson *et al.*, 1994b). According to Cognie *et al.* (2003) there is still much room for improvement of IVC conditions considering that the survival rate of transferred fresh IVP goat embryos was significantly lower (47%) than their *in vivo* counterparts (71%). The timeline of significant finding in IVC of ICSI-derived goat oocytes has been depicted in Table 1.

CONCLUSIONS

The present state of IVC of ICSI-derived goat embryos shows some promise. A number of studies have been done to investigate the growth and development of goat embryos or fertilized oocytes in different IVC medium with both IVF and ICSI-derived embryos, from both adult and prepubertal goat. Although IVF and ICSI technique has a great deal of difference, however, for embryo culture more or less similar medium are used for embryos derived from both the sources. From different studies it is obvious that using similar media IVF embryos shows better growth and development than ICSI-derived goat embryos. Although both the methods are *in vitro*, however, in IVF sperm have freedom in fertilizing oocytes. On the other hand in ICSI, sperm are either defective or immotile and there is no freedom at all. Therefore, compared to IVF, ICSI-derived embryos are more vulnerable as every oocyte experienced an injury with the injection needle. Therefore, ICSI-derived goat embryos may need more care than the IVF embryos. Till now, no single IVC medium showed much promise in growth and development of ICSI-derived goat embryos. Therefore, more studies are needed to identify the unique ingredients necessary for the better growth and development of ICSI-derived goat embryos *in vitro*.

ACKNOWLEDGMENTS

The author wish to thank Islamic Development Bank (IDB) for providing an IDB Merit Scholarship to the author. He also wishes to thank Professor Ramli Bin Abdullah and Professor Wan Khadijah Wan Embong of University of Malaya for supervising his PhD work. The help and advice of Professor Ng Soon Chye, National University of Singapore and Dr. S.L. Liow, Embryonics International Pte Ltd., Singapore, are greatly acknowledged.

REFERENCES

Abdullah, R.B., S.L. Liow, A.N.M.A. Rahman, W.K. Chan, W.E. Wan-Khadijah and S.C. Ng, 2008. Prolonging the interval from ovarian hyperstimulation to laparoscopic ovum pick-up improves oocyte yield, quality and developmental competence in goats. Theriogenology, 70: 765-771.

Ball, G.D., M.L. Leibfried, R.W. Lenz, R.L. Ax, B.D. Bavister and N.L. First, 1983. Factors affecting successful *in vitro* fertilization of bovine follicular oocytes. Biol. Reprod., 28: 717-725.

Bavister, B.D. and T. Arlotto, 1990. Influence of single amino acids on the development of hamster one-cell embryos *in vitro*. Mol. Reprod. Dev., 25: 45-51.

Bavister, B.D., T.A. Rose-Hellekant and T. Pinyopumminter, 1992. Development of *in vitro* matured *in vitro* fertilized bovine embryos into morulae and blastocysts in defined culture media. Theriogenology, 37: 127-146.

Biggers, J.D., J.A. Lawitts and C.P. Lechene, 1993. The protective action of betaine on the deleterious effects of NaCl on preimplantation mouse embryos *in vitro*. Mol. Reprod. Dev., 34: 380-390.

Brackett, B.G. and G. Oliphant, 1975. Capacitation of rabbit spermatozoa *in vitro*. Biol. Reprod., 12: 260-274.

Brown, B.W., I.S. Fraser and P.E. Mattner, 1985. Capillary blood flow in the endometrium and myometrium of conscious sheep: Effect of catheterization of uterine arteries. Aust. J. Biol. Sci., 38: 209-214.

Byatt-Smith, J.G., H.J. Leese and R.G. Gosden, 1991. An investigation by mathematical modelling of whether mouse and human preimplantation embryos in static culture can satisfy their demands for oxygen by diffusion. Hum. Reprod., 6: 52-57.

Carney, E.W. and B.D. Bavister, 1987. Stimulatory and inhibitory effects of amino acids on the development of hamster eight-cell embryos *in vitro*. J. *In vitro* Fert. Embryo Transf., 4: 162-167.

Chatot, C.L., C.A. Ziomek, B.D. Bavister, J.L. Leais and I. Torres, 1989. An improved culture medium supports development of random-bred 1-cell mouse embryos *in vitro*. J. Reprod. Fert., 86: 679-688.

Cognie, Y., G. Baril, N. Poulin and P. Mermillod, 2003. Current status of embryo technologies in sheep and goat. Theriogenology, 59: 171-188.

Dorland, M., D.K. Gardner and A.O. Trounson, 1995. Serum in synthetic oviduct fluid causes mitochondrial degeneration in ovine embryos. J. Reprod. Fertil., 13: 70-70.

Ealy, A.D. and P.J. Hansen, 1994. Induced thermotolerance during early development of murine and bovine embryos. J. Cell. Physiol., 160: 463-468.

Ealy, A.D., J.L. Howell, V.H. Monterroso, C.F. Arechiga and P.J. Hansen, 1995. Developmental changes in sensitivity of bovine embryos to heat shock and use of antioxidants as thermoprotectants. J. Anim. Sci., 73: 1401-1407.

Fischer, B. and B.D. Bavister, 1993. Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits. J. Reprod. Fertil., 99: 673-679.

Flood, M.R., T.L. Gage and T.D. Bunch, 1993. Effect of various growth-promoting factors on preimplantation bovine embryo development *in vitro*. Theriogenology, 39: 823-833.

Fry, R.C., P.A. Batt, R.J. Fairclough and R.A. Parr, 1992. Human leukemia inhibitory factor improves the viability of cultured ovine embryos. Biol. Reprod., 46: 470-474.

Gandolfi, F., 1994. Autocrine, paracrine and environmental factors influencing embryonic development from zygote to blastocyst. Theriogenology, 41: 95-100.

Gardner, D.K., M. Lane and P. Batt, 1993. Uptake and metabolism of pyruvate and glucose by individual sheep preattachment embryos developed *in vivo*. Mol. Reprod. Dev., 36: 313-319.

Gardner, D.K., 1994. Mammalian embryo culture in the absence of serum or somatic cell support. Cell Biol. Int., 18: 1163-1179.

Gardner, D.K., M. Lane, A. Spitzer and A. Batt, 1994. Enhanced rates of cleavage and development for sheep zygotes cultured to the blastocyst stage *in vitro* in the absence of serum and somatic cells: Amino acids, vitamins and culturing embryos in groups stimulate development. Biol. Reprod., 50: 390-400.

Gomez, M.C., J.W. Catt, L. Gillan, G. Evans and W.M.C. Maxwell, 1997. Effect of culture, incubation and acrosome reaction of fresh and frozen-thawed ram spermatozoa for *in vitro* fertilization and intracytoplasmic sperm injection. Reprod. Fertil. Dev., 9: 665-673.

Gomez, M.C., J.W. Catt, G. Evans and W.M. Maxwell, 1998. Sheep oocyte activation after Intra Cytoplasmic Sperm Injection (ICSI). Reprod. Fertil. Dev., 10: 197-205.

Good, N.E., G.D. Winget, W. Winter, T.N. Connolly, S. Izawa and R.M. Singh, 1966. Hydrogen ion buffers for biological research. Biochemistry, 5: 467-477.

Goto, Y., Y. Noda, T. Mori and M. Nakano, 1993. Increased generation of reactive oxygen species in embryos cultured *in vitro*. Free Radic. Biol. Med., 15: 69-75.

Grippo, A.A., M.A. Henault, S.H. Anderson and G.J. Killian, 1992. Cation concentrations in fluid from the oviduct ampulla and isthmus of cows during the estrous cycle. J. Dairy Sci., 75: 58-65.

Hendriksen, P.J.M., P.L.A.M. Vos, W.N.M. Steenweg, M.M. Bevers and S.J. Dieleman, 2000. Bovine follicular development and its effect on the *in vitro* competence of oocytes. Theriogenology, 53: 11-20.

Izquierdo, D., P. Villamediana, M. Palomo, T. Mogas and M.T. Paramio, 1998. Effect of sperm capacitation and fertilization media on IVF and early embryo development of prepubertal goat oocytes. Theriogenology, 49: 1501-1513.

Jimenez-Macedo, A.R., D. Izquierdo, B. Anguita and M.T. Paramio, 2005. Comparison between intracytoplasmic sperm injection and *in vitro* fertilization employing oocytes derived from prepubertal goats. Theriogenology, 64: 1249-1262.

Jimenez-Macedo, A.R., B. Anguita, D. Izquierdo, T. Mogas and M.T. Paramio, 2006. Embryo development of prepubertal goat oocytes fertilized by intracytoplasmic sperm injection (ICSI) according to oocyte diameter. Theriogenology, 66: 1065-1072.

Jimenez-Macedo, A.R., M.T. Paramio, B. Anguita, R. Morato, R. Romaguera, T. Mogas and D. Izquerdo, 2007. Effect of ICSI and embryo biopsy on embryo development and apoptosis according to oocyte diameter in prepubertal goats. Theriogenology, 67: 1399-1408.

Johnson, M.H. and M.H. Nasresfahani, 1994. Radical solutions and cultural problems: Could free oxygen radicals be responsible for the impaired development of preimplantation mammalian embryos *in vitro*? BioEssays, 16: 31-38.

Katska-Ksiazkiewicz, L., B. Rynska, B. Gajda and Z. Smorag, 2004. Effect of donor stimulation, frozen semen and heparin treatment on the efficiency of *in vitro* embryo production in goats. Theriogenology, 62: 576-586.

Keefer, C.L., S.L. Stice, A.M. Paprocki and P. Golueke, 1994. *In vitro* culture of bovine IVM-IVF embryos: Cooperative interaction among embryos and the role of growth factors. Theriogenology, 41: 1323-1331.

Keskintepe, L., P. Morton, S.E. Smith, M.J. Tucker, A.A. Simplicio and B.G. Brackett, 1997. Caprine blastocyst formation following intracytoplasmic sperm injection and defined culture. Zygote, 5: 261-265.

Kim, J.H., H. Funahashi, K. Niwa and K. Okuda, 1993. Glucose requirement at different developmental stages of in vitro-fertilized bovine embryos cultured in semi-defined medium. Theriogenology, 39: 875-886.

Larson, R.C., G.G. Ignotz and W.B. Currie, 1992a. Platelet-Derived Growth Factor (PDGF) stimulates development of bovine embryos during the fourth cell cycle. Development, 115: 821-826.

Larson, R.C., G.G. Ignotz and W.B. Currie, 1992b. Transforming growth factor-β and basic fibroblast growth factor synergistically promote early bovine embryo development during the fourth cell cycle. Mol. Reprod. Dev., 33: 432-435.

Lawitts, J.A. and J.D. Biggers, 1992. Joint effects of sodium chloride, glutamine and glucose in mouse preimplantation embryo culture media. Mol. Reprod. Dev., 31: 189-194.

Lazzari, G., C. Wrenzycki, D. Herrmann, R. Duchi, T. Kruip, H. Niemann and C. Galli, 2002. Cellular and molecular deviations in bovine *in vitro* produced embryos are related to the large offspring syndrome. Biol. Reprod., 67: 767-775.

Leese, H.J., 1988. The formation and function of oviduct fluid. J. Reprod. Fertil., 82: 843-856.

Leese, H.J., 1995. Metabolic control during preimplantation mammalian development. Human Reprod. Update, 1: 63-72.

Lenz, R.W., G.D. Ball, M.L. Leibfried, R.L. Ax and N.L. First, 1983. *In vitro* maturation and fertilization of bovine oocytes are temperature-dependent processes. Biol. Reprod., 29: 173-179.

Matsuyama, K., H. Miyakoshi and Y. Fukui, 1993. Effect of glucose levels during the *in vitro* culture in synthetic oviduct fluid medium on *in vitro* development of bovine oocytes matured and fertilized *in vitro*. Theriogenology, 40: 595-605.

Mogas, T., M.J. Palomo, M.D. Izquierdo and M.T. Paramio, 1997a. Developmental capacity of *in vitro* matured and fertilized oocytes from prepubertal and adult goats. Theriogenology, 47: 1189-1203.

Mogas, T., M.J. Palomo, M.D. Izquierdo and M.T. Paramio, 1997b. Morphological events during *in vitro* fertilization of prepubertal goat oocytes matured *in vitro*. Theriogenology, 48: 815-829.

Moore, K. and K.R. Bondioli, 1993. Glycine and alanine supplementation of culture medium enhances development of *in vitro* matured and fertilized cattle embryos. Biol. Reprod., 48: 833-840.

Nagao, Y., K. Saeki, M. Hoshi and H. Kainuma, 1994. Effects of oxygen concentration and oviductal epithelial tissue on the development of *in vitro* matured and fertilized bovine oocytes cultured in protein-free medium. Theriogenology, 41: 681-687.

Nasr-Esfahini, M.H., J.R. Aitken and M.H. Johnson, 1990. Hydrogen peroxide levels in mouse oocytes and early cleavage stage embryos developed *in vitro* or *in vivo*. Development, 109: 501-507.

Noda, Y., H. Matsumoto, Y. Umaoka, K.J. Tatsumi, J. Kishi and T. Mori, 1991. Involvement of superoxide radicals in the mouse two-cell block. Mol. Reprod. Dev., 28: 356-360.

Olds, D. and N.L. VanDemark, 1957. Luminal fluids of bovine female genitalia. J. Am. Vet. Med. Assoc., 31: 555-556.

Phua, A.C.Y., 2006. Develpoment of a PCR-based method for sex determination of caprine embryos produced from *in vitro* maturation, fertilization and culture techniques. M.Sc. Thesis, University of Malaya, Kuala Lumpur.

Pivko, J., P. Grafenau and V. Kopecny, 1995. Nuclear fine structure and transcription in early goat embryos. Theriogenology, 21: 126-137.

Rahman, A.N.M.A., R.B. Abdullah and W.E. Wan Khadijah, 2007. Goat embryo development from *in vitro* matured oocytes of heterogeneous quality through intracytoplasmic sperm injection technique. Biotechnology, 6: 373-382.

Rahman, A.N.M.A., 2008. Goat Embryo Production from *In Vitro* Matured Heterogeneous Oocytes Fertilised by Intracytoplasmic Sperm Injection (ICSI) Technique. Ph.D. Thesis, University of Malaya, Kuala Lumpur.

Rahman, A.N.M.A., R.B. Abdullah and W.E. Wan Khadijah, 2009. Effect of oocyte source on the developmental competence of *in vitro* matured goat oocytes fertilized by intracytoplasmic sperm injection technique. Turk. J. Vet. Anim. Sci., 33: 323-331.

Rajikin, M.H., 1995. *In vitro* studies of maturation and fertilisation of oocytes and subsequent culture of embryos in goats. Ph.D Thesis, University of Malaya, Kuala Lumpur.

Restall, B.J. and R.G. Wales, 1996. The fallopian tube of the sheep III. The chemical composition of the fluid from the fallopian tube. Aust. J. Biol. Sci., 19: 687-698.

Rho, G.J., A.C. Hahnel and K.J. Betteridge, 2001. Comparisons of oocyte maturation times and three methods of sperm preparation for their effects on the production of goat embryos *in vitro*. Theriogenology, 56: 503-516.

Rieger, D., 1992. Relationship between energy metabolism and development of the early embryo. Theriogenology, 37: 75-93.

Rosenkrans, Jr. C.F. and N.L. First, 1994. Effect of free amino acids and vitamins on cleavage and developmental rate of bovine zygotes *in vitro*. J. Anim. Sci., 72: 434-437.

Seidel, G.E., Jr., T. Glass and S.E. Olson, 1991. Culture of 1-cell bovine embryos to blastocysts in chemically defined media. Biol. Reprod., 44: 155-155.

Takahashi, Y. and N.L. First, 1992. *In vitro* development of bovine one-cell embryos: Influence of glucose, lactate, pyruvate, amino acids and vitamins. Theriogenology, 37: 963-978.

Tervit, H.R., D.G. Whittingham and L.E.A. Rowson, 1972. Successful culture *in vitro* of sheep and cattle ova. J. Reprod. Fertility, 30: 493-497.

Thibodeaux, J.K., M.W. Myers and W. Hansel, 1995. The beneficial effects of incubating bovine embryos in groups are due to platelet-derived growth factor. Theriogenology, 43: 336-336.

Thompson, J.G.E., A.C. Simpson, P.A. Pugh, P.E. Donnelly and H.R. Tervit, 1990. Effect of oxygen concentration on *in vitro* development of preimplantation sheep and cattle embryos. J. Reprod. Fertil., 89: 573-578.

Thompson, J.G., A.C. Simpson, P.A. Pugh and H.R. Tervit, 1992. Requirement for glucose during *in vitro* culture of sheep preimplantation embryos. Mol. Reprod. Dev., 31: 253-257.

Thompson, J.G., A.C.S. Bell, P.A. Pugh and H.R. Tervit, 1993. Metabolism of pyruvate by pre-elongation sheep embryos and effect of pyruvate and lactate concentrations during *in vitro* culture. Reprod. Fertility Dev., 5: 417-423.

Thompson, J.G., R.J. Partridge, F.D. Houghton, C.I. Cox and H.J. Leese, 1996. Oxygen uptake and carbohydrate metabolism by *in vitro* derived bovine embryos. J. Reprod. Fertility, 106: 299-306.

Trounson, A., C. Anderiesz and G. Jones, 2001. Maturation of human oocytes *in vitro* and their developmental competence. Reproduction, 121: 51-75.

Urdaneta, A., 2005. Use of thiol compounds in the *in vitro* embryos production from prepubertal goat oocytes. Universitat Autonoma de Barcelona, Barcelona, Spain.

Villamediana, P., F. Vidal and M.T. Paramio, 2001. Cytogenetic analysis of caprine 2- to 4-cell embryos produced *in vitro*. Zygote, 9: 193-199.

Walker, S.K., R.F. Seamark, P. Quinn, G.M. Warnes, R.J. Ashman and D.H. Smith, 1988. Culture of pronuclear embryos of sheep in a simple medium. Proceedings of the 11th International Congress of Animal Reproduction and Artificial Insemination, June 26-30, 1988, Dublin, Ireland, Pp: 483.

Walker, S.K., R.J. Lampe and R.F. Seamark, 1989. Culture of sheep zygotes in synthetic oviduct fluid medium with different concentration of sodium bicarbonate and HEPES. Theriogenology, 32: 797-804.

Wang, W.L., H.S. Jiang, D.S. Shi, K.H. Lu, I. Gordon and C. Polge, 1991. The effect of temperature on the *in vitro* maturation, fertilization and development of bovine oocytes. Proceedings of the 7th Scientific Meeting of European Embryo Transfer Association, December 15-17, 2008, Cambridge, UK, Pp: 216.

Wang, B., H. Baldassarre, J. Pierson, F. Cote, K.M. Rao and C.N. Karatzas, 2003. The *in vitro* and *in vivo* development of goat embryos produced by intracytoplasmic sperm injection using tail-cut spermatozoa. Zygote, 11: 219-227.

Watson, A.J., A. Hogan, A. Hahnel, K.E. Wiener and R.M. Schultz, 1992. Expression of growth factor ligand and receptor genes in the preimplantation bovine embryo. Mol. Reprod. Dev., 31: 87-95.

Watson, A.J., P.H. Watson, M. Arcellana-Panlilio, D. Warnes, S.K. Walker and R.M. Schultz, 1994a. A growth factor map for ovine preimplantation development. Biol. Reprod., 50: 725-733.

Watson, A.J., P.H. Watson, D. Warnes, S.K. Walker, D.T. Armstrong and R.F. Seamark, 1994b. Preimplantation development of *in vitro* matured and *in vitro* fertilized ovine zygotes: Comparison between coculture on oviduct epithelial cell monolayers and culture under low oxygen atmospheres. Biol. Reprod., 50: 715-724.

Wright, Jr. R.W. and K.R. Bondioli, 1981. Aspects of *in vitro* fertilization and embryo culture in domestic animals. J. Animal Sci., 53: 702-729.

Wright, Jr. R.W. and J.V. O'Fallon, 1987. Growth of Domesticated Animal Embryos *in vitro*. In: The Mammalian Preimplantation Embryo: Regulation of Growth and Differentiation *in vitro*, Bavister, B.D., (Ed.). Plenum Press, New York, USA, pp: 251-271.

Younis, A.I., K.A. Zuelke, K.M. Harper, M.A.L. Oliveira and B.G. Brackett, 1991. *In vitro* fertilization of goat oocytes. Biol. Reprod., 44: 1177-1182.

Zhou, J.B., Y.G. Wu, D. Han, L.Q. Liu and X.W. Tan *et al.*, 2004. Effects of sperm and oocyte quality control on intracytoplasmic sperm injection (ICSI) in goats. Shi Yan Sheng Wu Xue Bao, 37: 367-374.