International Journal of Scientific Research in Knowledge, 3(1), pp. 021-029, 2015
Available online at http://www.ijsrpub.com/ijsrk
ISSN: 2322-4541; ©2015; Author(s) retain the copyright of this article
http://dx.doi.org/10.12983/ijsrk-2015-p0021-0029

Full Length Research Paper

Improved Bovine Blastocyst Developmental Potential by L-carnitine Supplementation

Paul Christian Ver J. Manzano[1], Marlon B. Ocampo[2]*, Lerma C. Ocampo[2], Excel Rio S. Maylem[1], Jonathan V. Lazaro[1]

[1]College of Veterinary Science and Medicine, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines 3120
[2]Philippine Carabao Center, Reproductive Biotechnology Unit, Science City of Munoz, Nueva Ecija, Philippines 3120
*Corresponding author: ocampomarlon29@yahoo.com; Tel No.: 09994984211

Received 03 December 2014; Accepted 08 February 2015

Abstract. In this study, the potential role of L-carnitine supplementation in the maturation of oocytes and pre-implantation development of embryos was investigated using bovine as a model. In Experiment 1, bovine oocytes recovered from the abattoir were matured in the absence (control) or presence of L-carnitine, subjected to *in vitro* fertilization and assessed on their developmental potential up to the blastocyst stage. The nuclear maturation and cleavage rate observed between the control and L-carnitine supplemented group ranged from 83.1% – 87.1% and 57.1% – 67.9%, respectively. Significantly higher blastocyst formation rate and improved total cell count in 0.1- 0.5 mg/ml L-carnitine supplemented groups were observed versus the control (51.0 – 54.2% vs 29.5% and 97.4 – 110.1 ± 2.2 vs 82.5 ± 1.6, respectively (P<0.05). In Experiment 2, zygotes resulting from in vitro fertilization of bovine oocytes were cultured in modified synthetic oviductal fluid medium with or without L-carnitine supplementation. Results showed no significant difference on the blastocyst formation rate among treatment groups, but the total cell count of blastocyst derived from 0.1 – 0.5 mg/ml L-carnitine supplemented groups were higher than the control (98.4 – 115.4 ± 3.1 vs 84.6 ± 3.2, respectively (P<0.05). Overall, the results demonstrated the usefulness of the procedures utilized in the maturation, fertilization and culture of bovine oocytes and early- stage embryos. That, L-carnitine supplementation at the level of 0.1 – 0.5 mg/ml concentration in the maturation and culture media tend to enhance the developmental potential of oocytes and early- stage embryos to the blastocyst stage as indicated by a higher total cell count (improved cell activity).

Keywords: blastocyst, bovine, cell count, L-carnitine, oocytes

Abbreviations:
LC – L-carnitine
IVM – in vitro maturation
IVF- in vitro fertilization
IVC – in vitro culture
mSOF – modified synthetic oviductal fluid
COCs – cumulus-oocyte complexes
BSA – bovine serum albumin
NaCl – sodium chloride
GLM – general linear model
CPT1B – carnitine palmitoyl transferase 1B
TCM – tissue culture medium
ATP – adenosine triphosphate
BO – Brackett-Oliphant medium

1. INTRODUCTION

In many mammalian species, the critical factors of utmost importance in the development of *in vitro* produced embryos are the culture conditions for the maturation of immature cumulus-oocyte complexes (COCs) and early- stage embryos. Most improvements in the success rate of *in vitro* embryo production were attributed to the development of media based on metabolic requirements of the oocytes and embryos and the composition of oviductal and uterine fluids. For instance, the energy requirement needed by the oocytes and embryos for the acquisition of developmental competence differ (Biggers et al., 1967; Thompson, 2000). COCs preferentially utilize glucose (Sutton et al., 2003; Harris et al., 2007), whereas pre-compaction embryos use pyruvate and lactate (Thompson et al., 1996) and return to glucose after compaction (Krisher et al., 1999; Purcell and Moley, 2009). Additionally, COCs derived in the ovaries collected from the slaughterhouse matured "spontaneously" *in vitro*. Such oocyte maturation *in vitro* occurs in the absence of certain crucial oocyte cytoplasmic events and components that are required for complete developmental competence of the oocyte (Gilchrist and Thompson, 2007). Hence, the purpose

of many researchers dealing on reproduction is to improve oocyte and pre-implantation embryos developmental competence by delaying or temporarily preventing spontaneous maturation while at the same time promoting ooplasm development.

The use of L-carnitine (LC) has been claimed to have a beneficial role in the cellular metabolism and embryonic development of some mammalian species (Kruip et al., 1983; Fergusson and Leese, 2006; Abdelrazik et al., 2009) due to its involvement in lipid metabolism, an alternative source for energy production. Also, LC plays a role in reducing oxidative stress by enhancing the activity of numerous antioxidant enzymes, eg., superoxide dismutase, catalase and glutathione peroxidase (Rizzo et al., 2010). As demonstrated in porcine, supplementation of maturation medium with LC increased the gluthathione and decreased the reactive oxygen species levels in the oocytes which has deleterious effect on embryo development (Wu et al., 2011). In this study, two approaches have been tried to determine the possible role of LC on the acquisition of developmental competence of immature bovine COCs and early- stage embryos resulting from *in vitro* fertilization (IVF). The first approach is on the incorporation of LC in the maturation medium, since it is during maturation that oocytes produce adenosine triphosphate (ATP), mostly by mitochondrial metabolism to fuel the energy needed for meiotic processes and fertilization supporting further development of resulting embryos to the blastocyst stage (Krisher et al., 2007). And secondly, on the inclusion of LC in the culture medium for early- stage embryos, since it is during this period that the "cell-block" phenomenon inhibit further development to the blastocyst stage of most mammalian embryos when cultured *in vitro*. The objective of the study was to determine if LC supplementation could enhance the developmental potential of bovine COCs matured *in vitro* and improved the blastocyst formation of cleaved embryos resulting from IVF.

2. MATERIALS AND METHODS

2.1. Media

The basic media for maturation of oocytes was tissue culture medium (TCM-199; Gibco Co., Grand Island, N.Y., USA) with Earle's salts and L-glutamine (Krisher et al., 1999), for fertilization was a Brackett-Oliphant medium (BO; Brackett and Oliphant, 1975) and for culture was the modified synthetic oviductal fluid medium (mSOF, Tervit et al., 1972).

2.2. Oocyte collection and maturation

Bovine ovaries were collected immediately postmortem at local abattoirs and transported to the laboratory in 0.9 % saline solution at 30 - 35º C within 4 - 6 hrs. The ovaries were pooled irrespective of the donors estrus cycle. COCs were aspirated from antral follicles (3 - 5 mm in diameter) by using 18- gauge needle attached to a 10- ml sterile plastic syringe, washed three times in the maturation medium before selecting using a stereomicroscope based on the criteria described by Ocampo et al., (1993). A group of 10 - 15 COCs were transferred into 50 µl droplets of maturation medium under mineral oil (Sigma Chem Co., St. Louis, USA) in a 35 x 10 mm Falcon polysterene culture dish (Becton and Dickinson Labware, N.J., USA) which had been previously pre-incubated to equilibrate for at least 2 hrs in a CO_2 incubator. COCs were cultured at 39º C under an atmosphere of 5 % CO_2 and 95 % air with high humidity. After culture for 22 hrs, the cumulus and corona cells were removed by pipetting.

2.3. Sperm preparation

Straws of locally processed frozen semen from the Sperm Processing Unit of the Philippine Carabao Center at Central Luzon State University Ranch in Digdig, Carranglan, Nueva Ecija were thawed at 39º C water bath for 15 sec and washed twice with fertilization medium (BO medium) containing 1 mg/ml bovine serum albumin (BSA, Initial fractionation by heat shock, Sigma, st. Louis, MO, USA) by centrifugation at 800 rpm for 5 min. After the final wash, the sperm was re-suspended at 2 x 10^6 sperm/ml in fertilization medium with 5 mM theophylline and 6 mg/ml BSA, and pre-incubated for 1 hr at 39º C; 95 % air atmosphere in a tightly capped test tube.

2.4. *In vitro* fertilization and culture

Selected oocytes were transferred to the fertilization droplets (50 µl) with 15 oocytes per drop under mineral oil. A portion of sperm suspension was added to the droplets giving a final sperm concentration of 1 x 10^6 cells/ml, 2.5 mM theophylline and 3 mg/ml BSA. The motility rate of sperm during insemination was more than 50%. After sperm-oocyte co-culture for 24 hr, the extra sperm cells attached to the oocytes were removed by pipetting using a finely drawn glass pipette. Subsequently, the oocytes were washed twice with culture medium before transferring to 50 µl drops of mSOF medium and cultured for 7 days in a humidified incubator with a gas phase of 5% O_2, 5% CO_2 and 90% N_2 level at 39°C.

Table 1: Effect of LC supplementation in the maturation medium on the rate of nuclear maturation

LC	No. of	No. of oocytes (%)	

(mg/ml)	replicates	cultured	matured
-	5	50	42 (84.0)
0.1	5	77	64 (83.1)
0.5	5	85	74 (87.1)
1.0	5	62	53 (85.5)

Table 2: Effect of LC supplementation in the maturation medium on the development rate to the blastocyst stage

LC	No. of oocytes (%)			Total cell count

(mg/ml)	inseminated	cleaved	blastocyst	(mean±SEM)
-	182	112 (61.5)	33 (29.5)^{a}	82.5 ± 1.6^{a}
0.1	78	48 (61.5)	26 (54.2)^{b}	110.1 ± 2.2^{b}
0.5	105	60 (57.1)	31 (51.0)^{b}	97.4 ± 4.9^{b}
1.0	81	55 (67.9)	22 (40.0)^{a,b}	86.4 ± 3.1^{a}

a,b Values with different superscripts in the same column are significantly different ($P < 0.05$)

2.5. Cell counting

Blastocyst appearing on the 7th day were collected and subjected to a differential staining protocol for embryos (Thouas et al., 2001) with modification. Briefly, blastocyst were washed in PBS-PVP, then placed in 1 ml of Hoechts working solution (0.75 ml of 2.3% Na citrate dehydrate solution; 0.25 ml of ethanol; 10 µl of Hoechts 33342 stock solution of 1 mg/ml concentration dissolved in ethanol) in an Effendorf tube, wrapped in aluminum foil and stored in the refrigerator (4°C) for at least 24 hrs. Subsequently, the blastocyst were recovered and washed in glycerol, mounted on a glass slide, flattened in glycerol by a cover slip to a level where all nuclei appeared at the same focal plane and examined by using a fluorescent microscope (Eclipse E-600; Nikon) under ultraviolet light. A digital image of each embryo was taken and the total cells (both inner cell mass and trophectoderm) counted.

2.6. Experimental design

Effect of LC supplementation in the IVM medium on nuclear maturation, fertilization and subsequent development to the blastocytst stage.

COCs were matured in IVM medium supplemented with LC (0.1, 0.5 and 1.0 mg/ml) or without (control) for 22 hrs. Nuclear maturation of the oocytes was based on the presence of 1st polar body. Selected matured oocytes were subjected for fertilization and the cleavage and blastocyst formation rate analyzed followed by staining for cell counting. The experiment was replicated five times.

Effect of LC supplementation in the IVC medium on the blastocyst formation of cleaved embryos following fertilization.

Early- stage embryos (2- to 4- cell stage) found 32 hrs post insemination were randomly assigned to droplets of IVC medium with LC (0.1 mg, 0.5 mg and 1.0 mg/ml) or without (control) LC supplementation for 7 days. Resulting blastocyst were recorded and their cell count analyzed. The experiment was replicated five times.

2.7. Statistical analysis

Randomized experiments were conducted and each experiment replicated five times. Each treatment was analyzed by a general linear model (GLM) procedure in the SAS for Windows (Version 6.2, Cary, NC: SAS institute Inc.) that could standardize biases affecting the experimental results. Significant effect observed among treatment groups were compared by the least square method in the SAS. Statistical significance was determined, where the *P* value was less than 0.05.

Table 3: Effect of LC supplementation in mSOF on the development of early- stage embryos to the blastocyst stage

LC	No. of embryos (%)		Total cell count
(mg/ml)	cultured	blastocyst	(mean±SEM)
-	76	34 (44.7)	84.6 ± 3.2^{a}
0.1	81	39 (48.2)	115.4 ± 3.1^{b}
0.5	91	48 (52.7)	94.3 ± 4.2^{a}
1.0	76	36 (47.4)	$98.4 \pm 4.7^{a,b}$

a,b Values with different superscripts in the same column are different significantly (P>0.05)

3. RESULTS

In Experiment 1, a total of 720 COCs were cultured in maturation medium with or without LC supplementation for 22 hrs. Of these, 274 COCs were fixed for meiotic status evaluation. No significant differences were noticed between the control (84.0%) and LC supplemented groups (83.1% – 87.1%) in terms of nuclear maturation (Table 1), as evidenced by the presence of 2nd metaphase plate and 1st polar body. Subsequently, 446 COCs that appeared morphologically normal with 1st polar body (Fig. 1) were selected and assigned randomly to the control (n=182) and LC supplemented groups (eg., 0.1 mg/ml (78); 0.5 mg/ml (105); 1.0 mg/ml (81) and subjected for insemination *in vitro*. The cleavage rate observed between the control (61.5%) and LC supplemented groups (57.1% - 67.9%), similarly had no difference. However, higher blastocyst formation rate was observed in LC supplemented groups versus the control. The total cell count of blastocyst embryos derived in 0.1 mg/ml (110.1±2.2) LC supplemented group was significantly higher than the control (82.5±1.6) and 1.0 mg/ml (86.4±3.1) LC supplemented group (Table 2).

In Experiment 2, a total of 324 cleaved embryos resulting from fertilization of COCs matured in maturation medium alone were assigned randomly in droplets of IVC medium with or without LC supplementation. Higher blastocyst formation rate was observed in LC supplemented groups but did not differ significantly with the control. The total cell count of blastocyst embryos (Fig. 2) derived in 0.1 mg/ml (115.4 ± 3.1) LC supplemented group was significantly higher than the control (84.6 ± 3.2) (Table 3).

4. DISCUSSION

In this study, we looked for a correlation between the developmental potential of immature bovine COCs and early- stage embryos resulting from IVF after culture *in vitro* in the presence or absence of LC. LC plays an important role in the lipids/fatty acid metabolism as an endogenous energy source for the oocytes and embryos. The cytoplasm of ruminant oocyte and pre-implantation embryos are rich in lipid droplets, which, in COCs are associated with the endoplasmic reticulum and mitochondria and are degraded to some extent during maturation (Kruip et al., 1983; McEvoy et al., 2000).The ß-oxidation pathway that metabolizes lipids/fatty acids within the mitochondria to generate cellular ATP has an essential role in determining oocyte quality and its ability to support embryo development (Van Blerkom et al., 1995; Stojkovic et al., 2001; Fergusson and Leese, 2006). The initial, and rate limiting step in ß-oxidation is the entry of activated fatty acids into the mitochondrion, which is catalyzed by carnitine palmitoyl transferase (CPT1B). CPT1B attaches carnitine, enabling the entry of the fatty acid into the mitochondrial matrix where carnitine is removed by CPT2 and the fatty acid enters the ß-oxidation spiral producing multiple Acetyl-CoA molecules from which ATP is generated via the TCA cycle and the electron transport chain. Therefore, in order to fully understand on how the oocytes and embryos utilize lipid substrates for energy production and whether this form of metabolism is developmentally beneficial, more experimentation is required. In this study, LC was utilized as a supplement in the maturation of COCs and culture of bovine early- stage embryos, partly to determine its effective concentration in improving its developmental potential *in vitro*. The choice of limiting the LC concentrations used to 0.1 mg/ml – 1.0 mg/ml was based on previous observations that LC concentration at 2 mg/ml during IVM would be inhibitory to the meiotic progression of the oocytes, as demonstrated in porcine (Wu et al., 2011). Furthermore, excessive LC concentration may result to depletion of lipid density which may be detrimental to development per se (Sutton et al., 2012) in *in vitro* condition.

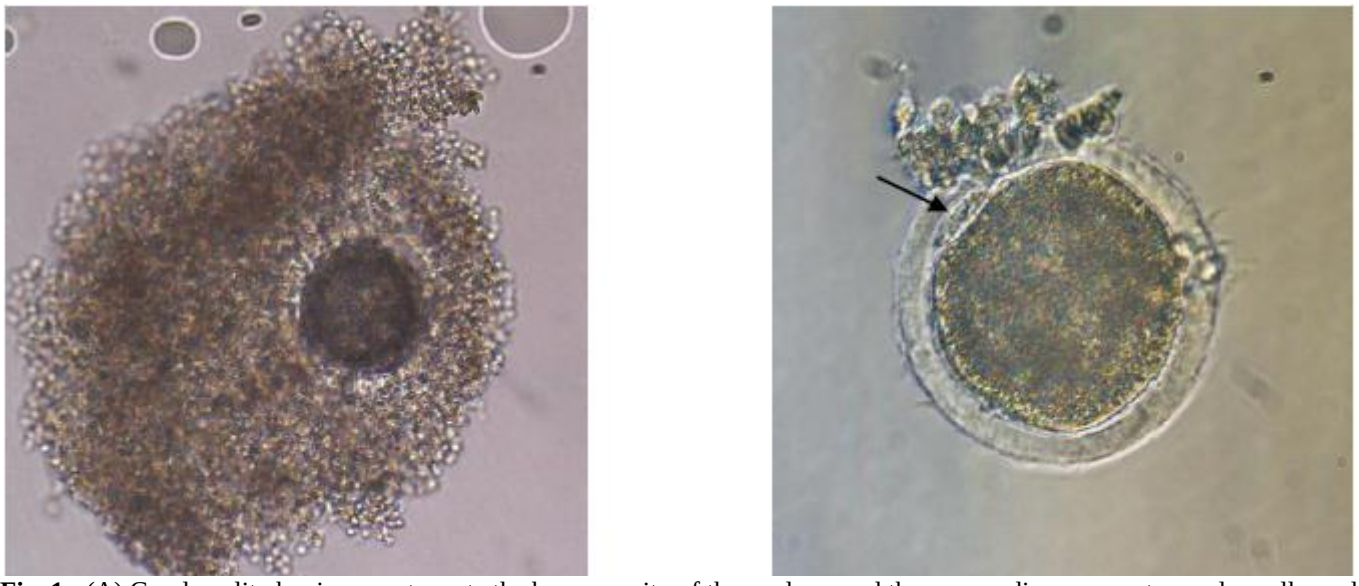

Fig. 1: (A) Good quality bovine oocyte, note the homogeneity of the ooplasm and the surrounding compact cumulus cells, and (B) matured oocyte 22 hrs after culture with the emission of the 1st polar body (arrow).

Our results showed that COCs nuclear maturation and cleavage rate were not influenced by the presence of LC during the initial maturation period. This indicates that the current maturation system being used is efficient enough in terms of immature COCs completion of 1st meiosis up to the level of 1st cleavage following fertilization. The high maturation rate (80.0% or more) obtained could be attributed to the strict selection of COCs for maturation using only the good quality oocytes (Ocampo et al., 1993). It has been suggested that LC treatment exert its positive effect on oocytes with compromised developmental competence, eg., fair quality COCs, vitrified oocytes, but not on oocytes with otherwise higher developmental competence, eg., good quality COCs, non-vitrified oocytes, (Chankitisakul et al., 2013). Nonetheless, immature mammalian COCs matured with LC was found to have improved glutathione level during maturation (You et al., 2012), indicating that its influence is more prominent on the cytoplasmic maturation rather than nuclear maturation. That LC improved the meiotic competence of mammalian oocytes by holding back the apoptosis of granulosa cells and enhancing its mitochondrial activity (Hashimoto, 2009; Somfai et al., 2011). In this study, we have shown that LC supplementation was effective at supporting blastocyst formation and improving its quality as evidenced by having a higher total cell count. These findings suggest that the beneficial effect of LC was prominent on the improvement of embryonic development rather than completion of 1st meiosis. Based on the data gathered, it appears that 0.1 – 0.5 mg/ml concentration of LC is sufficient in improving the developmental potential of bovine oocytes and early- stage embryos. In porcine, treatment of immature oocytes with 10 mM (1.98 mg/ml) LC during IVM resulted to improved pre-implantation development of parthenogenetically activated and somatic cell nuclear transferred embryos. Whereas using LC as a supplement at 0.6 mg/ml concentration on oocytes and 8- cell embryos significantly improved the integrity of microtubule and chromosome structure aside from decreasing the level of apoptosis (Mansour et al., 2009). In mice, supplementation of culture medium with 0.3 mg/ml LC improved blastocyst formation by reducing tumor necrosis factor-α, blocking effects of actinomycin-D and hydrogen peroxide plus decreasing levels of DNA damage (Abdelrazik et al., 2009).

The results in Experiment 2 showed that LC addition in the IVC of early- stage bovine embryos in trying to overcome the “cell-block” stage at 8- to 16-cell stage, is less than necessary since the blastocyst formation rate in the control and LC groups had no significant difference regardless of the concentrations used.. Interestingly, the total cell numbers of blastocyst embryos derived in LC supplemented groups were observed higher than the control indicating an improved cell activity.

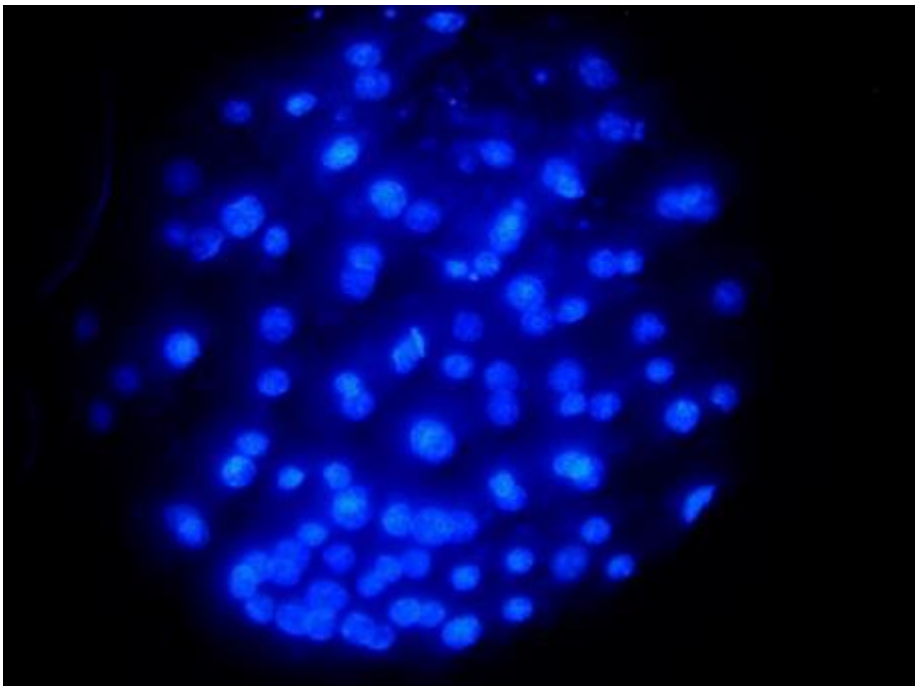

Fig. 2: Day 7 expanded blastocyst embryo fixed for cell counting

This observation was more distinct when using 0.1- 0.5 mg/ml LC concentration than 1.0 mg/ml concentration. In porcine, supplementation of medium with 10 mM LC showed higher rates of blastocyst formation and improves its developmental competence. Such effect has been assumed to be due to increased intracellular gluthathione synthesis which reduces the ROS levels (You et al., 2012). ROS can be generated by handling or culturing embryos *in vitro*, and it is well understood that high level of ROS causes cell membrane lipid peroxidation (Nasr-Esfahani et al., 1990; Noda et al., 1991), DNA fragmentation and influences RNA transcription and protein synthesis (Takahashi et al., 2000), which lead to in vitro developmental blocks and early embryonic death (Noda et al., 1991; Goto et al., 1993). Also, supplementation of the culture medium with LC significantly increases β-oxidation which is associated with oocyte quality improvement and blastocyst development as indicated by having a higher number of cells in the inner cell mass (Dunning et al., 2010, 2011). In addition, some factors that promote embryo development could be related in one way or another to lipid metabolism, such as increased production of ATP and conversion of lipids to steroids and hormones that serve as major contributing factors for embryo development during *in vitro* culture. Endogenous lipids are a potential source of energy within the oocytes and embryos via β-oxidation but require transportation of fatty acids into the mitochondria by CPT1, thus the addition of LC, a cofactor of CPT1 has become necessary (Sutton et al., 2012).

4. CONCLUSION

LC supplementation in the IVM and IVC medium had no significant effect on the completion of 1st meiosis (nuclear maturation) and cleavage following IVF but support the development of early- stage embryos resulting from IVF to the blastocyst stage and sustain its potential as evidenced by having a higher cell count, an indication of improved cell activity. In addition, supplementation of the media with 0.1 – 0.5 mg/ml LC is sufficient to enhance the developmental potential of bovine oocytes and embryos *in vitro*.

REFERENCES

Abdelrazik H, Sharma R, Mahfouz R, Agarwal A (2009). L-carnitine decreases DNA damage and improves the *in vitro* blastocyst development rate in mouse embryos. Fertil Steril., 91: 589-596.

Biggers JD, Whittingham DG, Donahue RP (1967). The pattern of energy metabolism in the mouse oocyte and zygote. Zoology, 58: 560-567.

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

Chankitisakul V, Somfai T, Inaba Y, Techakumphu M, Nagai T (2013). Supplementation of maturation medium with L-carnitine improves cryo-tolerance of bovine *in vitro* matured oocytes. Theriogenology, 79: 590-598.

Dunning KR, Cashman K, Russell DL, Thompson JG, Norman RJ, Robker RL (2010). β-oxidation is essential for mouse oocyte developmental

competence and early- embryo development. Biol Reprod., 83: 909-918.

Dunning KR, Akison LK, Russell DL, Norman RJ, Robker RL (2011). Increased β-oxidation and improved oocyte developmental competence in response to L-carnitine during ovarian *in vitro* follicle development in mice. Biol Reprod., 85: 548-555.

Ferguson EM, Leese HJ (2006). A potential role for triglyceride as an energy source during bovine oocyte maturation and early- embryo development. Mol Reprod Dev., 73: 1195-1201.

Gilchrist RB, Thompson JG (2007). Oocyte maturation: Emerging concepts and technologies to improve developmental potential *in vitro*. Theriogenology, 67: 6-15.

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

Harris SE, Adriaens I, Leese HJ, Gosden RG, Picton HM (2007). Carbohydrate metabolism by murine ovarian follicles and oocyte grown *in vitro*. Reproduction, 134: 415-424.

Hashimoto S (2009). Application of *in vitro* maturation to assisted reproductive technology. J Reprod Dev., 55: 1-10.

Krisher RL, Lane M, Bavister BD (1999). Developmental competence and metabolism of bovine embryos cultured in semi-defined and defined culture media. Biol Reprod., 60: 1345-1352.

Krisher RL, Brad AM, Herrick JR, Sparman ML, Swain JE (2007). A comparative analysis of metabolism and viability in porcine oocytes during *in vitro* maturation Anim Reprod Sci., 98: 72-96.

Kruip TAM, Cran DG, Van Beneden TH, Dieleman SJ (1983). Structural changes in bovine oocytes during final maturation *in vivo*. Gamete Res., 8: 29-47.

Mansour G, Abdelrazik H, Sharma RK, Radwan E, Falcone T, Agarwal A (2009). L-carnitine supplementation reduces oocyte cytoskeleton damage and embryo apoptosis induced by incubation in peritoneal fluid from patients with endometriosis. Fertil Steril., 91: 2079-2086.

McEvoy TG, Coull GD, Broadbent PJ, Hutchinson JS, Speake BK (2000). Fatty acid composition of lipids in immature cattle, pig and sheep oocytes with intact zona pellucida. J Reprod Fertil., 118: 163-170.

Nasr-Esfahani MH, Aitken JR, Johnson MH (1990). Hydrogen peroxide levels in mouse oocytes and early- cleavage stage embryos developed *in vitro* or *in vivo*. Development, 109: 501-507.

Noda Y, Matsumoto H, Umaoka Y, Tatsumi K, Kishi J, Mori T (1991). Involvement of superoxide radicals in the mouse 2- cell block. Mol Reprod Dev., 28: 356-360.

Ocampo MB, Ocampo LC, Ryu IS, Mori T, Ueda J, Kanagawa H (1993). Effects of culture time, ovarian activity, cumulus cells and sera on the nuclear and cytoplasmic maturation of pig oocytes *in vitro*. Anim Reprod Sci., 34: 135-146.

Purcell SH, Moley KH (2009). Glucose transporters in gametes and pre-implantation embryos. Trends in Endoc Metabol., 20: 483-489.

Rizzo AM, Berselli P, Zava S, Montorfano G, Negroni M, Corsetto P (2010). Endogenous antioxidants and radical scavenger. Adv Exp Med Bio., 698:52-67.

Somfai T, Kaneda M, Akagi S, Watanabe S, Haraguchi S, Mizutani E (2011). Enhancement of lipid metabolism with L-carnitine during IVM improves nuclear maturation and cleavage ability of porcine oocytes. Reprod Fertil Dev., 23: 912-920.

Stojkovic M, Machado SA, Stojkovic P, Zakhartchenko V, Hutzler P, Goncalves PB (2001). Mitochondrial distribution and ADP content of bovine oocytes before and after maturation: correlation with morphological criteria and developmental capacity after fertilization and culture. Biol Reprod., 64: 904-909.

Sutton ML, Cetica P, Gilchrist RB, Thompson JG (2003). The metabolic profiles of bovine COCs: effects of oocyte-secreted factors and stimulation of cumulus expansion. Theriogenology, 59: 500.

Sutton ML, Feil D, Robker RL, Thompson JG, Dunning KR (2012). Utilization of endogenous fatty acid stores for energy production in bovine pre-implantation embryos. Theriogenology, 77: 1632-1641.

Tervit HR, Whittingham DG, Rowson LE (1972). Successful culture *in vitro* of sheep and cattle ova. J Reprod Fertil., 30: 493-497.

Thompson JG, Partridge RJ, Houghton FD, Cox CI, Leese HJ (1996). Oxygen uptake and carbohydrate metabolism by *in vitro* derived embryos. J Fertil Steril., 106: 299 -306.

Thompson JG (2000). *In vitro* culture and embryo metabolism of cattle and sheep embryos – a decade of achievement. Anim Reprod Sci., 60: 263-275

Thouas GA, Korfiatis NA, French AJ, Jones GM, Trounson AO (2001). Simplified technique for differential staining of inner cell mass and

trophectodem cells of mouse and bovine blastocysts. Reprod Biomed., Online 3: 25-29.

Van Blerkom J, Davis PW, Lee J (1995). Fertilization and early embryology: ATP content of human oocytes and developmental potential and outcome after IVF and ET. Hum Reprod., 10: 415-424.

Wu GQ, Jia BY, Li JJ, Fu XW, Zhou GB, Hou YP (2011). L-carnitine enhances oocyte maturation and development of parthenogenetic embryos in pigs. Theriogenology, 76: 785-793.

You J, Lee J, Hyun S, Lee E (2012). L-carnitine treatment during oocyte maturation improves in vitro development of cloned pig embryos by influencing intracellular glutathione synthesis and embryonic gene expression. Theriogenology, 78: 235-243.

Dr. Paul Christian V. Manzano obtained his Doctor of Veterinary Science and Medicine degree at Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. Also, he received his Bachelor of Science in Animal Husbandry degree in the same university in 2012. His research focuses on the application of reproductive biotechnologies in mammalian species (eg., superovulation-embryo transfer, in vitro fertilization, cryopreservation of gametes).

Dr. Jonathan V. Lazaro obtained his Doctor of Veterinary Science and Medicine at Central Luzon State University in 1994 and Master of Veterinary Studies (Pathology) at the University of Queensland, Australia in 2002. He has been the research coordinator of his college for five years and had served as adviser of undergraduate students. He has collaborated with several research projects with the Philippine Carabao Center and College of Fisheries in the field of pathology, parasitology and reproductive physiology that resulted in some international publications.

Excel Rio S. Maylem is currently taking up her Master's Degree in Biology at Central Luzon State University, where she finished her Bachelor's degree in the same field with academic honors. Currently, she is a Science Research Assistant at the Reproductive Biotechnology Laboratory of the Philippine Carabao Center. Her main activities focus on the use of Computer Assisted Sperm Analyzer, Gametes and Embryo Quality Assessment using Fluorescence Microscopy, IVM-IVF, and Embryo Cryopreservation. She is also participating in the Fixed Time Artificial Insemination and Multiple Ovulation and Embryo Activities of the Unit.

Dr. Lerma C. Ocampo is a Senior Science Research Specialist in the Philippine Carabao Center-Department of Agriculture, Reproductive Biotechnology Unit. Her Post-Doctoral Training was in Colorado State University, College of Veterinary Medicine and BioMedical Science-Animal Reproduction and Biotechnology Laboratory. Her PhD was in Agriculture (Animal Science) – BioResource and Product Science, Graduate School of Agriculture – Laboratory of Animal Breeding and Reproduction, Hokkaido University, Japan. Her researchesfocuses on,cryobanking of genetic materials and related fields resulting to numerous publications (both international and national) in refereed journals. She obtained her DVSM and BSAH degrees at Central Luzon State University, Philippines.

Dr. Marlon B. Ocampo is a Supervising Science Research Specialist in the Philippine Carabao Center-Department of Agriculture, Reproductive Biotechnology Unit. His Post-Doctoral Training under the PAEF-Fulbright Program is in the University of Illinois – ACES,USA. He obtained his PhD in Veterinary Medicine at Hokkaido University in 1993 under the MONBUSHO Program. His DVSM and BSAH degrees were from the CVSM, CLSU, Philippines. His research focuses on Theriogenology, Surgery and Medicine resulting to numerous publications (both international and national) in refereed journals.