International Journal of Agricultural Technology 2024 Vol. 20(5):1779-1786
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Successful production of Goat (*Capra hircus*) embryos: effects of cysteamine on maturation of goat oocytes and subsequent embryonic development following fertilization *in-vitro*

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Atabay, E. C., Atabay, E. P., Lazaro, P. G. M. and Rigos, L. M. (2024). Successful production of Goat (*Capra hircus*) embryos: effects of cysteamine on maturation of goat oocytes and subsequent embryonic development following fertilization *in-vitro*. International Journal of Agricultural Technology 20(5):1779-1786.

Abstract The results showed no significant differences in percent maturation of goat oocytes among control, 50 uM, and 100 µM cysteamine groups (81.97±7.11, 81.46± 5.28 and 84.44±3.03), respectively. This implies that the addition of IVM medium with cysteamine of goat oocytes matured *in-vitro* are not expressed at nuclear maturation level. However, following *in-vitro* fertilization, the cleavage rate of 100 µM cysteamine (50.00±2.40) was significantly higher than control and 50 µM (42.40 ± 3.27, 43.72± 2.77), respectively. Similarly, the blastocyst formation rate of 100 µM cysteamine (28.33±19.99) was significantly higher than those of control and 50 µM (19.84±6.91 and 19.64±6.18), respectively. The results indicated that the addition of 100 µM cysteamine into IVM medium is found to be beneficial in improving the cleavage rate of goat oocytes and subsequent development *in-vitro*, suggesting huge potential of the IVF technology in enhancing production and genetic improvement in goat species.

Keywords: Amino acid, Blastocyst, Cleavage, *In-vitro* maturation, Livestock

Introduction

Reproductive biotechnology in livestock production has been much desired as mainstream strategy towards greater animal productivity and income for the farming communities. Specifically in goats, the application of *in-vitro* embryo production method to complement artificial insemination technique was seriously considered to enhance genetic improvement of this species. The production of genetically superior goat embryos from oocytes derived from ovaries of slaughtered females which are no longer used for natural breeding process may be of interest to conserve the desirable genes and reproductive

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materials retrievable from these animals. The *in-vitro* technology in general is a potential alternative production scheme in livestock production towards addressing the industry needs for sustainable local supply of dairy and meat products.

However, *in-vitro* production of goat embryos is not a common practice in the Philippines since the procedure is not yet established in the country, although preliminary research works had been attempted on this area. There is a need therefore to establish goat embryo production system to complement current natural or artificial insemination programs. It should be emphasized however that the *in-vitro* embryo production is a multi-step procedure covering the following: *In-vitro* maturation, fertilization, and culture. Of the three major steps mentioned, the IVM part is considered critical as the IVM culture condition can influence the nuclear maturational capacity of the oocyte and further development of embryos *in-vitro*. *IVM* medium commonly used is TCM 199 supplemented with Foetal Calf Serum, gonadotropins, growth factor (Atabay *et al*., 2006) and antibiotics. Recently, the addition of antioxidants during IVM has become an important part of the IVEP system to overcome the adverse effects of the reactive oxygen substances generated along the process. Limited studies had been done locally; therefore, the study was conducted to develop the *in-vitro* embryo production system in goats. Specifically, the aim of the study was to determine the effects of different levels of cysteamine on the maturation rate based on first polar body extrusion and be able to produce embryos after fertilization *in-vitro*.

Materials and methods

Oocyte retrieval

Goat ovaries obtained from abattoir were separated from the reproductive tract before torching or scalding and transported in normal saline at 30-33^{0}C. Cumulus oocyte complexes (COCs) were retrieved using fine slicing the surface of the ovaries using scalpel and were washed with HEPES-buffered modified Tyrode's medium (TALP-HEPES, Bavister *et al.,* 1983). COCs were washed with TALP-HEPES supplemented with 3 mg/ml bovine serum albumin, 0.2 mM sodium pyruvate, 50 μg/ml gentamicin sulfate. COCs were evaluated and classified under stereomicroscope and selected as described by Atabay *et al.* (2006). Only Grades A and B oocytes were matured *in-vitro.*

In-vitro maturation

Three different maturation conditions for goat oocytes were used as follows: Treatment 1 (control), HEPES-buffered TCM 199 (Gibco Laboratories, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, Gibco), 0.2 mM sodium pyruvate (Sigma), 0.02 units/ml follicle stimulating hormone (FSH, from porcine pituitary, Sigma), 1µg/ml estradiol-17 β (Sigma),10 ng/ml epidermal growth factor (EGF, Sigma), and 50 µg/ml gentamicin sulfate (Sigma); Treatment 2, Control + 50 µM cysteamine; Treatment 3, Control + 100 µM cysteamine. Ten to twelve COCs were cultured for 26 to 28 h in 50-µl droplet of maturation medium overlaid with mineral oil under a humidified atmosphere of 5% CO_2 in air at 38.5 °C. Treatments were replicated four times. Some of the oocytes after *in-vitro* maturation were denuded (complete removal of cumulus cells) to determine the proportion of oocytes with extruded 1st polar body and assess the nuclear maturation rate.

In-vitro fertilization

Matured COCs were subjected to fertilization following the Percoll discontinuous density gradient of sperm separation. Briefly, the 45% Percoll-Bracket and Oliphant solution (BO) was prepared by 1:1 dilution of 1 ml of 90%Percoll (Sigma) to 1 ml Bracket and Oliphant Defined Medium (BODM) without Bovine Serum Albumin (BSA). Two ml of 45% Percoll was placed above the 90% Percoll and the tube was then transferred to the O_2 incubator at 38.5 °C until use. Two 0.5 ml straws of frozen goat semen were thawed at 37 °C for 1 min and then layered on top of the 45%Percoll gradient and centrifuged at 2000 rpm for 20 min. Thereafter, supernatant was discarded with sterile micropipette following centrifugation. The sample was centrifuged in 6 ml BODM at 1000 rpm for 5 min. Supernatant was discarded until 200 µl sample remained in the tube. Concentration of semen sample was adjusted to 10,000,000 sperm/ml. Fifty µl of the semen suspension were added to fertilization drops to have a final concentration of 1 x 10^6 sperm/ml. Ten to twelve matured oocytes were transferred to the fertilization drops and underwent fertilization *in-vitro* for 16 to 18 h at 38.5 °C with 5% CO_2.

In-vitro culture and assessment of development

After 16 to 18 h of sperm cells and oocytes co-incubation, presumptive zygotes were washed by centrifugation to remove the cumulus cells and spermatozoa. Thereafter, the zygotes were cultured in embryo culture medium: a

modified synthetic oviductal fluid (mSOF) containing 20 amino acids (Takahashi *et al.,* 1996; Gardner *et al.*, 2000), 10 µg/ml insulin and further supplemented with 5mM glycine, 5 mM taurine, 1 mM glucose, 1 mM L-glutamine, 3 mg/ml fatty acid-free BSA for seven days at 38.5ºC under 5% CO_2, 5% O_2 and 90% N_2 gas system. The above chemicals were Sigma products. The percentage of cleavage to 2-cell stage and blastocyst development were taken on 2nd and 7th days of *in-vitro* culture, respectively.

Statistical analysis

Data were expressed as the Means ± SD of four replications and were subjected to Duncan's multiple range tests using SAS version 9.9. A probability of P<0.05 was considered statistically significant.

Results

Percent maturation of goat oocytes at varying concentrations of cysteamine is shown in Table 1. Results revealed that the percent nuclear maturation of the control, 50 µM, and 100 µM cysteamine were 81.97±7.11, 81.46±5.28, and 84.44±3.03, respectively. Maturation rates among treatments were not significantly different (P>0.05) however, 100 µM cysteamine tended to be higher in the nuclear maturation rate.

Table 1. Percent maturation of goat oocytes in different concentrations of cysteamine

Cysteamine Concentrations	Number of Oocytes examined	Number of Oocytes with 1st Polar Body	Percent Nuclear Maturation
T1 (0 µM)	44	36	81.97± 7.11
T2 (50 µM)	46	38	81.46 ± 5.28
T3 (100 µM)	45	38	84.44 ± 3.03

Note: Values on percent nuclear maturation are presented as means±SD of four replicates.

Cleavage and blastocyst development rates during subsequent culture *in-vitro* are presented in Table 2. The rate of first cleavage division (two cell divisions) was significantly higher in Treatment 3 (50.00±2.40) as compared to Treatment 1 (42.40±3.27) and Treatment 2 (43.72±2.77). Moreover, goat oocytes matured with 100 µM of cysteamine had significantly higher ($P<0.05$) development to blastocyst (28.33±10.99) compared to Treatment 2 (19.84±6.91) and 50 µM cysteamine groups (19.64±6.18) groups.

Table 2. Development of goat oocytes in maturation medium with different concentrations of cysteamine

Cysteamine Concentrations	Number of Oocytes cultured	Percent Cleavage (at 2 cell stage)	Percent Blastocyst Development
T1 (0 µM)	71	42.40 ± 3.27[b]	19.84 ± 6.91[b]
T2 (50 µM)	69	43.72± 2.77[b]	19.64 ± 6.18[b]
T3 (100 µM)	70	50.00± 2.40[a]	28.33 ±10.99[a]

Note: [a, b] Values with different superscripts within a column differ significantly ($P< 0.05$)

Discussion

Several *in-vitro* studies demonstrated that the addition of cysteamine could have influenced nuclear maturation and subsequent development following IVF based on the species and culture medium used. Findings of the present study on *in-vitro* oocyte maturation conforms with that of De Matos *et al.* (2003) wherein 50, 100, and 200 µM of cysteamine did not affect in-vitro maturation of mouse oocytes but the rates of cleavage to 2 cell embryo and blastocyst formation increased after IVF. Another study had shown that 500 µM of cysteamine in TCM 199 based maturation medium decreased maturation rates of mouse oocytes which could be toxic affecting oocyte development (Roushandeh and Roudkenar, 2009). On the other hand, increased maturation rates and normal embryonic development were achieved after IVF when 500 µM cysteamine was used during IVM in porcine oocytes (Grupen *et al.*, 1995). Furthermore, the present findings corroborate with previous work, wherein 100 µM cysteamine in TCM 199 failed to improve maturation rates of equine oocytes (Luciano *et al.*, 2006); while 50

μM cysteamine for bubaline and 500 μM for porcine effectively increased IVM and IVC following IVF using TCM 199-based medium (Yoshida *et al*., 1993). The discrepancy of results of the previous studies could be attributed to species differences, different medium, and medium contents used.

It has been pointed out that complete cytoplasmic and nuclear maturation is necessary for the development of embryos *in-vitro* (Purohit *et al.,* 2005). Following fertilization, glutathione participates in sperm decondensation, oocyte activation, and transformation of the fertilizing sperm head into the male pronucleus (Yoshida *et al.,* 1993). In addition, unpacking of sperm DNA in the oocyte at the time of fertilization is necessary to participate in embryonic development (Zirkir *et al.,* 1985). On the other hand, disulfide bond reduction in sperm nuclei can be due to GSH (Mahi and Yanagimachi, 1975) which constitutes the first step in sperm nuclear decondensation process (Perreault *et al.,* 1984). Meanwhile, cysteamine generally serves as a precursor for the synthesis of glutathione and its high concentrations in oocytes improve their ability to overcome oxidative damage, enhance cytoplasmic maturation, and support successful fertilization with more blastocysts formed than embryos matured in medium without cysteamine (Grupen *et al.,* 1995; De Matos *et al*., 2003).

Moreover, supplementation of cysteamine during *in-vitro* maturation of oocytes as an antioxidant improved maturation rate and development of goat oocytes during subsequent embryo culture. Supplementation of cysteamine during culture can avoid if not minimize oxidative stress which is detrimental to oocytes quality and maturation (Guerin *et al*., 2001).

To summarize, the present study demonstrated successful production of goat embryos *in-vitro*. Current results concluded that goat oocytes matured in TCM medium containing 100 μM of cysteamine achieved the highest development rate to blastocyst stage among the treatments. Subsequent effort will be focused on the transfer of these embryos to recipient does to generate live kids. The embryo production technologies complementing with other reproductive techniques for goat production will potentially propel this so called "sunrise industry" into the mainstream of the livestock industry in the Philippines.

Acknowledgements

The authors express their heart-felt gratitude to Philippine Carabao Center of the Department of Agriculture for financial and logistical support that led to the completion of this study.

References

Atabay, E. P., Atabay, E. C., Duran, D. H., de Vera, R. V. and Cruz, L. C. (2006). Enhanced developmental competence of buffalo oocytes in the presence of hormones and epidermal growth factor during *in-vitro* maturation. Philippine Journal of Veterinary and Animal Sciences, 32:155-166.

Bavister, B. D. (1989). A consistent successful procedure for *in-vitro* fertilization of golden hamster eggs. Gamete Research, 23:139-158.

De Matos, D. G., Nogueira, D., Cortvindt, R., Herrera, C., Adriaensens, T., Pasqualini R. S. and Smitz, J. (2003). Capacity of adult and prepubertal mouse oocytes to undergo embryo development in the presence of cysteamine. Molecular Reproduction and Development, 64:214-218.

Gardner, D. K., Pool, T. B. and Lane, M. (2000). Embryo nutrition and energy metabolism and its relationship to embryo growth, differentiation, and viability. Seminars in Reproductive Medicine, 18:205-218.

Grupen, C. G., Nagashima, H. and Nottle, M. B. (1995). Cysteamine enhances *in-vitro* development of porcine oocytes matured and fertilized *in-vitro*. Biology of Reproduction, 53:173-178.

Guerin, P., Mouatassim, E. S. and Menezo, Y. (2001). Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Human Reproduction Update, 7:175-189.

Luciano, A. M., Goudet, G., Perazzoli, F., Lahuec, C. and Gerard, N. (2006). Glutathione content and glutathione peroxidase expression in *in-vivo* and *in-vitro m*atured equine oocytes. Molecular Reproduction and Development, 73:658-666.

Mahi, C. A. and Yanagimachi, R. (1975). Induction of nuclear decondensation of mammalian spermatozoa *in-vitro*. Journal of Reproduction and Fertility, 31:237-296.

Perreault, S. D., Bardee, R. R., Elsyein, K. H., Zucker, R. M. and Keefer, C. L. (1988). Interspecies differences in the stability of mammalian sperm nuclei were assessed *in-vivo* by sperm microinjection and *in-vitro* by flow cytometry. Biology of Reproduction, 39:157-167.

Purohit, G. N., Bradys, M. S. and Sharma, S. S. (2005). Influence of epidermal growth factor and insulin-like growth factor 1 on nuclear maturation and fertilization of buffalo cumulus oocyte complexes in serum free media and their subsequent development *In-vitro*. Animal Reproduction Science, 87:229-239.

Roushandeh, A. M. and Roudkenar, M. H. (2009). The influence of meiotic spindle configuration by cysteamine during *in-vitro* maturation of mouse oocytes. Iranian Biomedical Journal, 13:73-78.

Takahashi, M., Nagai, T., Okamura, N., Takahashi, H. and Okano, A. (2002). promoting the effect of beta-mercaptoethanol on *in-vitro* development under oxidative stress and cystine uptake of bovine embryos. Biology of Reproduction, 66:562-567.

Yoshida, M., Ishigaki, K., Nagai T. and Chikyu, M. (1993). Glutathione concentration during maturation and after fertilization in pig oocytes: relevance to the ability to form male pronucleus. Biology of Reproduction, 49:89-94.

Zirkir, B. R., Soucek, D. A., Chang, T. S. K. and Perreault, S. D. (1985). *In-vitro* and *in-vivo* studies of mammalian sperm nuclear decondensation. Gamete Res, 11:349-365.

(Received: 28 September 2023, Revised: 3 July 2024, Accepted: 6 July 2024)