In vitro production of bovine and caprine embryos and determining the cause of low cleavage rate

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Introduction

The number of high quality oocytes per ovary is an important consideration in the *in vitro* production of embryos. Similarly, various method of oocytes collection such as dissection, slicing and aspiration have been used to harvest high number of quality oocytes from abattoir ovaries. It is well established that the culture condition employed for *in vitro* maturation of mammalian oocytes can significantly influence the outcome of *in vitro* embryonic development. Supplementation of serum and hormones (combination of luteinizing hormone, follicle stimulating hormone (FSH) and oestradiol) in the culture medium in order to obtain high *in vitro* maturation rate remain controversial. Therefore, the objectives of this study were (i) to compare the oocytes collection methods in terms of the types of oocytes and the number of good-quality oocytes obtained per ovary, (ii) to determine the effect of oocytes quality on cumulus expansion and maturation rate, and (iii) to determine the effect of serum and hormone (FSH and oestradiol) supplementation on the *in vitro* maturation of cattle oocytes.

Materials and Methods

Cattle ovaries were collected from local abattoirs and transported in a thermos flask containing phosphate buffered saline with 100,000 IU/l penicillin and 100 mg/l streptomycin (PBS) at 35-37°C to the laboratory within 2-3 hours after slaughter. At the laboratory, the ovaries were washed 3 times in warm PBS and subsequently two methods of oocytes recovery were employed, aspiration and slicing (Experiment 1). Briefly, oocytes were aspirated from 2-8mm follicles by using an 18 G needle attached to a 10ml disposable syringe, which was previously primed with 1ml of TALP Hepes medium supplemented with 0.3% bovine serum albumin and 50ug/ml gentamycin. For slicing, ovaries were placed in a 90mm plastic disposable petri dish containing 8-10ml of TALP Hepes. Each ovary was cut into two halves and subsequently sliced with a scalpel blade to recover oocytes from follicle of <8mm in diameter. For both methods, oocytes were searched under a stereomicroscope at 10-40x and picked up using a sterile class micropipette and treansferred into a 35mm plastic disposable petri dish containing 2-3ml of fresh TALP Hepes. All oocytes recovered were classified into 4 categories on the basis of their cumulus cells attachment, as: A-Cumulus-oocyte-complexes (COCs) surrounded by $\geq$ 4 layers of cumulus cells, B-COCs surrounded by $\leq$ 3 layers of cumulus cells, C-deduned oocytes with partially or no cumulus cells, and D-oocytes surrounded by expanded cumulus cells (fibrinated oocytes). For each category, the number of oocytes per category and the total number of oocytes recovered per ovary were compared between the two collection methods. Experiment 2 – all oocytes collected for each category were matured *in vitro* for 22 hours at 39°C in humidified air and 5% CO_2 in 4-well dishes containing 500ul of *in vitro* maturation (IVM) medium under mineral oil. For *in vitro* maturation, Hepes buffered medium 199 containing 0.2mM sodium pyruvate and 50ug/ml gentamycin supplemented with 10% fetal bovine serum (FBS) and hormones 0.02 unit/ml FSH and 1 ug/ml oestradiol) was used as a basic medium. Experiment 3 - Only cumulus-oocyte-complexes (COCs) were selected for IVM with different combination of maturation media containing either 10% or 20% FBS and with or without hormones supplementation. For Experiment 2 & 3 , after IVM, the percentages of cumulus expansion and maturation rate of COCs of all treatments were compared.

Results and Discussion

In Experiment 1, from the total of 180 and 33 ovaries used for aspiration and slicing, respectively, the results indicated that significantly more oocytes were recovered per ovary by slicing than by aspiration (29.38 vs. 12.02 oocytes/ovary). The proportion of oocytes in each category was not significantly different except for category D (7.58% vs. 14.7% of total oocytes/ovary for aspiration and slicing, respectively. Significantly more good quality oocytes per ovary were obtained for slicing than aspiration, respectively (Category A; 9.82 $\pm$ 1.71 vs. 3.34 $\pm$ 0.47 and category B; 9.83 $\pm$ 1.35 vs. 4.87 $\pm$ 0.53). High recovery rate of good quality oocytes by slicing in this study was in agreement with earlier studies (Carolan, et al., 1994). Slicing is expected to obtained high recovery rate of oocytes due to the nature of this technique to harvest oocytes from small diameter follicles located in the ovarian cortex. High percentage of category D oocytes recovered by slicing in this study is because of no excessive pressure exerted by a syringe as in aspiration (Bols et al., 1997).

In Experiment 2, out of 536 oocytes recovered for various categories, categories A and B had significantly higher cumulus expansion rates (97.1% and 88.3%, respectively) compared with C and D (6.0% and 20.6%, respectively). Similarly, categories A and B had significantly higher maturation rates compared with C and D (91.4% and 82.3% vs. 35.0% and 0%, respectively). Similar maturation rates were obtained by previous study (Konishi et al., 1996). This result also clearly indicates that identification and selection of good quality oocytes for *in vitro* maturation procedure can be conducted based on their morphological appearance.

In Experiment 3, out of 2269 COCs randomly assigned to different maturation treatments, oocytes matured with hormones supplementation in either 10% or 20% FBS had significantly higher cumulus expansion rates (89.91% and 85.80%, respectively) than those without hormones (61.73% and 69.68%, respectively). A total of 408 oocytes were examined for evidence of nuclear maturation. The maturation rate of COCs was significantly higher in the presence of hormones than without hormones for 10% FBS (84.50% vs. 60.60%, respectively) and for 20% FBS (87.67% vs. 57.24%, respectively). Although some studies indicated that serum supplementation in the maturation medium had no effect on the oocytes maturation rates (Fukushima et al., 1991; Goto and Iritani, 1992), results in this study however, support that the oocytes cumulus expansion and maturation rate increased significantly in the presence of hormones (Izadyar et al., 1998).

Conclusions

Based on the findings of this study, it can be concluded hat slicing as a method of oocytes recovery capable to recover 2.4 times more acceptable oocytes per ovary than aspiration, and intact cumulus attachment should be considered as one of the most important criteria in selection of good quality oocytes for *in vitro* production of cattle embryos. This study also indicated that hormone supplementation (FSH and oestradiol) to *in vitro* maturation medium enhance cumulus expansion and nuclear maturation rates of cattle oocytes.

Benefits from the study

This study provides more opportunities to explore the possibility of producing high number of cattle embryo using *in vitro* procedures.

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Project Publications in Refereed Journals:

Nil

Expertise Development

Name of Graduate	Degree Awarded	Field of Expertise	Graduation Year
Riasari Gail Sianturi	MSc	Theriogenology	2001
Noraisah Marsan	DVM	Veterinary Medicine	2001

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