RECOVERY OF CANINE OOCYTES BY SLICING METHOD *

J. UMAMAGESWARI[1], CECILIA JOSEPH[2], K. KULASEKAR[3] . S.BALASUBRAMANIAN[4] AND P.SRIDEVI[5]

Department of Animal Reproduction, Gynaecology and Obstetrics
Madras Veterinary College, Chennai - 600 007.

Received : 19.03.2013 Accepted : 31.10.2013

ABSTRACT

Ovaries were collected from bitches undergoing routine ovario-hysterectomy and transported in physiological saline at 35°C to 38°C within 2 to 3 h of collection. A total of 1616 oocytes were retrieved by slicing of 52 ovaries with a recovery rate of 31.07 ± 3.47 oocytes per ovary. Among the collected oocytes 25.93, 43.07 and 31.00 percent of oocytes were graded as I, II and III respectively. The overall good quality oocytes (grade I and II COCs) recovered from bitch ovaries by slicing method irrespective of reproductive cycle in the present study was 69.00 percent.

Key words: Ovaries, Bitch, Oocyte, Recovery.

The reproductive mechanism of bitch is unique among the mammals. Canine oocytes are ovulated at the germinal vesicle stage and require 2 to 5 days for the completion of meiosis. Basic research on oocyte and follicular development and the application of *invitro* technology for *invitro* maturation and fertilization in canine are enhanced by techniques for oocyte recovery that are efficient and repeatable. The recovery procedures adopted for canine are slicing of ovarian cortex (Nickson *et al.,* 1993), aspiration of follicle and follicular dissection (Gardon, 1994). The present study was undertaken to study the quantity and quality of oocytes retrieved by slicing method.

Ovaries were collected from bitches undergoing routine ovario-hysterectomy for the purpose of neutering in the local veterinary practice and transported to the laboratory in physiological saline at 35°C to 38°C within 2 to 3 h of collection. Following removal of the ovarian bursa the exposed ovary was rinsed with saline to remove blood and other contaminants.

Cumulus oocyte complexes (COCs) were retrieved from canine ovaries by slicing method (Nickson *et al.,* 1993), where in ovaries were held firmly with an artery forceps in a 60 mm petridish containing 10 ml of Phosphate Buffer Saline (PBS) supplemented with 1 per cent Fetal Calf Serum (FCS) and several crisscross incisions (~ 1mm^3) were made on the surface of each ovary with a No.11 Bard Parker blade to release oocytes. The collected immature oocytes were screened under stereo zoom microscope at 10 X magnification, rinsed in modified HEPES-buffered Tyrodes medium (TCMh) in 35 mm petridish and graded (Figure 1) as described by Hewitt *et al.* (1998).

Grade I - COCs were darkly pigmented and completely surrounded by one or more layers of cumulus cells (Fig. 1a)

*Part of M.V.Sc. Thesis of first author.

1. Corresponding Author, Assistant Professor, Department of Clinics, M.V.C,Chennai-07.
2. Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, M.V.C.
3. Professor and Head, Department of Animal Reproduction, Gynaecology and Obstetrics
4. Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, M.V.C.
5. Professor, Department of Clinics, M.V.C, Chennai-07.

Grade II - COCs were lightly pigmented with incomplete layers of cumulus cells (Fig. 1b).

Grade III - COCs were pale coloured, often misshapen and without any cumulus cells attached (Fig.1c).

The quantity and quality of oocytes retrieved by slicing method were analysed and statistically interpreted as per Snedecor and Cochran (1994).

Hewitt *et al.* (1998) reported that oocytes released directly from punctured follicles would provide a smaller and more uniform population and would be impossible to puncture individual follicles, as they remained below the surface of the ovary and become apparent only a few days before ovulation. Since oocytes retrieval by follicular puncture was difficult, it was decided to go for slicing method of oocytes retrieval in the present study.

In this study, a total of 1616 oocytes were recovered by slicing of 52 ovaries with an average yield of 31.07 ± 3.47 oocytes per ovary. The recovery rate of 25.93, 43.07 and 31.00 percent with a mean oocyte yield of 8.05 ± 1.00, 13.38 ± 1.79 and 9.63 ± 1.39 per ovary was observed for Grade I, II and III oocytes respectively. The percentage of grade I oocytes retrieved by slicing method in the present study was 25.93 which was lower than the values of 33.00 and 53.50 percent reported by Mahi and Yanagimachi (1976) and Hishinuma *et al.*, (2004) respectively. The relatively low incidence of grade I oocytes recorded in the present study could be due to the slicing method followed, wherein oocytes released from follicles at different stages of development and atretic follicles were highly heterogenous, containing oocytes of different characteristics as suggested by Mogas *et al.*, (1992).

The percentage of good quality oocytes (grade I and grade II) retrieved by slicing method in the present study was 69.00 which was higher than the valus of 49.90 percent reported by Gabriela *et al.* (2002). Slicing method resulted in retrieval of mixed populations of oocytes that were obtained from the ovaries of bitches at various stages of the estrous cycle, where in follicles with different characteristics at different stage of development were present which could account for the variation observed in the present study.

Thus, the results of the present study shows that good quality oocytes (grade I and II COCs) could be recovered from bitch ovaries by slicing method irrespective of reproductive cycle and these oocytes can be used for further processing in *invitro* technology.

REFERENCES

1. Hewitt, D.A., Watson, P.F. and England,G.C.W. (1998). Nuclear staining and culture requirements for *invitro* maturation of domestic bitch oocytes. *Theriogenology,* **49** : 1083-1101.

2. Hishinuma, M., Minami, S., Okamoto, Y., Miyatake,K. and Sekine, J. (2004). Recovery, morphological quality, and *in vitro* maturation of follicular oocytes from bitches with pyometra. *Theriogenology,* **62** : 1652-1662.

3. Gabriela, F., Hay, M.A. and Goodrowe, K.L. (2002). The effects of oocyte storage and cumulus cell presence on canine zona penetration by domestic dog spermatozoa. *Theriogenology,* **57** : 1123-1134.

4. Gordon, I. (1994). Laboratory production of cattle embryos. Wallingford: Cab International.

5. Mahi, C.A. and Yanagimachi, R. (1976). Maturation, fertilization and development of dog oocytes *in vitro. Biol. Reprod.,* **46** : 853-858.

6. Mogas, T., Martino, A., Palomo, M.J. and Paramio, M.T. (1992). Effect of method of recovery on the numbers and type of oocytes obtained for *in vitro* maturation. *J. Reprod. Fert.,* **9** : 52 (Abst.).

7. Nickson, D.A., Boyd, J.S., Eckersall, P.D., Ferguson, J.M., Harvey M.J.A. and Renton, J.P. (1993). Molecular biological methods for monitoring oocyte maturation and *in vitro* fertilization in bitches. *J. Reprod. Fert. Suppl.*, **47** : 231-240.

8. Snedecor, G.W. and Cochran, W.G. (1994). Statistical methods (8th Ed.), Iowa State University Press, USA.

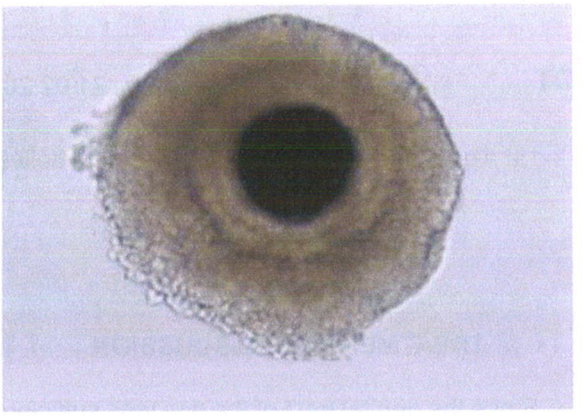

Fig.1a : Grade I - COCs were darkly pigmented and completely surrounded by one or more layers of cumulus

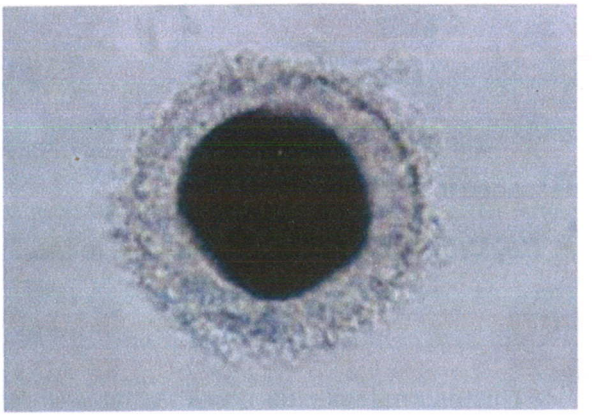

Fig.1b : Grade II - COCs were lightly pigmented with incomplete layers of cumulus cells

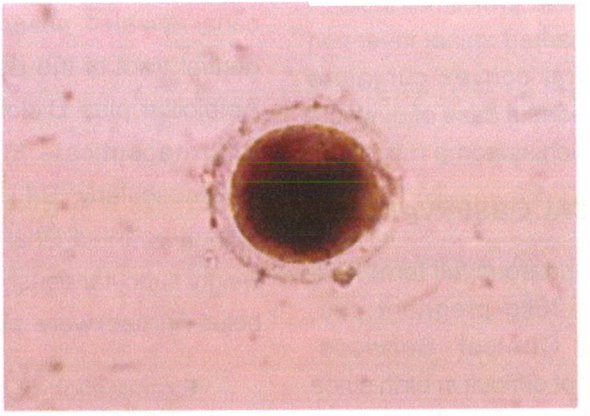

Fig.1c : Grade III - COCs were pale coloured, often misshapen and without any cumulus cells attached