EFFECT OF HARVESTING TECHNIQUE AND OTHER FACTORS ON OOCYTE RETRIEVAL IN BUFFALO *(BUBALUS BUBALIS)*

M.MUTHA RAO* AND Y. UMA MAHESH[1]

Embryo Transfer Laboratory, SV Veterinary University, Lam farm, Guntur - 522 034, AP

Received : 11.10.2010 Accepted : 4.1.2011

ABSTRACT

A study was undertaken to assess the ovarian biometry, follicular population and oocyte recovery in buffalo ovaries obtained from a local abattoir. Three harvesting techniques viz., aspiration, puncture and slicing methods were employed to recover oocytes. Effect of ovarian status (luteal phase vs. non-luteal phase), ovarian volume (<1.5 cm^3 vs. >1.5 cm^3), side of ovary (Right vs. left) and number of visible follicles (No follicles vs.1-5 follicles vs. 6-10 follicles) on oocyte yield and quality were analyzed. The ovarian biometry (length, width, thickness, volume and weight) and the number of visible follicles did not differ significantly between left and right ovaries. The number of visible follicles were significantly higher ($P<0.01$) in non-luteal phase ovaries (2.78 ±0.16) compared to luteal phase ovaries (1.97 ±0.19). Among the three collection methods, slicing technique yielded highest no. of total and good quality oocytes respectively (7.98 ± 0.70 and 3.23 ± 0.30) followed by puncture (3.46 ± 0.31 and 1.25 ± 0.17) and aspiration methods (2.38±0.19 and 0.84 ± 0.10). Irrespective of the method employed, a little over 1/3 of recovered oocytes were of poor quality (32.46 – 34.34%). Luteal phase ovaries (having CL) yielded lower no. of oocytes compared to non-luteal phase (no CL) ovaries. Both left and right ovaries contributed equally to total as well as different quality grades of oocytes in all the methods. Ovarian volum non-significantly affected the oocyte yield which was slightly higher in ovaries with a mean volume >1.5 cm^3. The results indicate that slicing method is superior to the other two methods employed in this study to harvest more number of good quality as well as culture grade oocytes from buffalo ovaries.

Key words: Oocyte Retrieval, Oocyte recovery, Visible follicles, Buffalo

INTRODUCTION

Availability of large number of culture grade oocytes is an essential prerequisite to realise more number of pre implantation embryos in *in-vitro* embryo production programme. With slaughter of more than 2 million buffaloes annually in India (Agnihotri, 1992), the ovaries obtained from them can provide the cheapest and the most abundant source of primary oocytes. However, the oocyte yield varies with the harvesting technique employed. Though follicle aspiration is widely used method due to its speed of operation, the oocyte yield is compromised in this method. Hence, apart from this conventional method of follicle aspiration other methods of oocyte retrieval like follicle dissection, follicle puncture and ovarian slicing have been attempted in many domestic species with equivocal results (Wani *et al.*, 1999). The aim of the present study was to assess the comparative efficacy of follicular aspiration, follicle puncture and ovarian slicing on oocyte yield and to evaluate retrospectively the influence of various factors like ovarian status, ovarian volume, side of ovary and the number of visible follicles on the recovery of different quality grades of oocytes in slaughter derived buffalo (*Bubalus bubalis*) ovaries.

*Corresponding author. Associate professor, Div. of Animal Reproduction, FVSc &AH, SKUAST-J, RS Pura, Jammu, 181102, India. 1.Scientist, Centre for Cellular and Molecular Biology, Uppal, Hyderabad, India.

MATERIALS AND METHODS

Buffalo ovaries (n=144) collected from a local slaughter house were transported within two hours to

the laboratory in a flask containing 0.9% normal saline at room temperature. In the laboratory they were washed twice in sterile PBS with antibiotics to remove superfluous tissue, bursa and blood clots. The weight (g) of each ovary was measured on a precision electronic balance (monopan) and biometry (cm) was recorded using vernier calipers separately for length, width and thickness. The volume of the ovary was deduced by the formula length x width x thickness x 0.523 as per Amir Lass and Peter Brindson (1999). The number of follicles visible to the naked eyes were counted and recorded. The ovaries were then placed in oocyte collection medium (TCM 199, Sigma, USA plus gentamycin sulphate 50µg /ml and heparin 10 IU/ml). Each ovary was processed individually and oocytes were collected by one of the following methods.

i) Aspiration of visible follicles (2-6 mm diameter) on the ovaries using a 20 gauze needle attached to a 5 ml sterile disposable plastic syringe containing 2 ml oocyte collection medium (OCM). Aspirated follicular fluid was then transferred to a sterile 35 mm petridish.

ii) Puncture of whole ovarian surface by a sterile 18 gauze hypodermic needle while the ovary is held completely submerged in OCM in a 90 mm petridish.

iii) Slicing of ovaries – in this method a hemostat was attached to the base of the ovary to hold it firmly in place and 2-3 mm deep incisions were made across the whole ovarian surface using a sterile scalpel blade. The ovary was swirled vigorously in a beaker containing OCM. The medium was then placed in a 90 mm petridish.

The petridish was kept undisturbed for 5 minutes to allow the cumulus–oocyte-complexes (COC) to settle. The petridish was then examined twice by at least two persons under stereo zoom microscope and the oocytes were scanned. The scanned oocytes were separated into a 35 mm petridish for grading at 63x magnification and then assessed as good, fair and poor based on cumulus corona investment and the homogeneity of ooplasm (Chauhan *et al.*, 1998). The number and quality of oocytes were recorded for each ovary. Good and fair quality oocytes were considered as culturable grade. Statistical analysis to analyze the ovarian biometry, to compare the efficacy of different collection methods and to assess the effect of various factors on oocyte recovery was performed by student's t-test and one way anova as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The mean length, width, thickness, volume and weight of the 144 abattoir derived buffalo ovaries were noted to be 1.75 ± 0.03 cm, 1.27 ± 0.02 cm, 1.07 ± 0.02 cm, 1.38 ± 0.07 cm^3 and 1.94 ± 0.08 g, respectively. No significant differences (P>0.05) were observed in respect of these parameters either between left and right ovaries or between luteal phase and non-luteal phase ovaries. The present findings on ovarian biometry are comparable to earlier reports (Razzaque *et al.*, 2008) in buffaloes. Similarly the mean number of visible follicles does not vary between left and right ovaries. However, non-luteal phase ovaries contained significantly more number of visible follicles (2.78 ± 0.16) than luteal phase ovaries (1.97 ± 0.19). In line with our observation, Amer *et al.* (2008) reported more number of vesicular follicles in ovaries without CL (6.8) than in ovaries with regressing CL (5.2) or those with functional CL (4.4), which may reflect the optimum level of gonadotrophins for follicular recruitment and absence of negative feed back effect of progesterone on anterior pituitary.

The number of visible follicles in buffalo ovaries was observed to be 2.56 ± 0.16 (range 0-9). Frequency distribution of these follicles indicate that 16 (11.11%), 111 (77.08%) and 17 (11.81%) ovaries bear no visible follicles, 1-5 follicles (2.31±0.11) and 6-10 follicles (6.59±0.23), respectively. These observations are similar to those of Das *et al.* (1996).

The mean oocyte recovery in the present study was 4.60±0.33 of which 1.77 ± 0.14 (38.46%), 1.28 ± 0.10 (27.75%), 1.55 ± 0.12 (33.79%) and 3.05 ± 0.23 (66.21%) were of good, fair, poor and culture grade oocytes, respectively (Table). The total as well as culture garde oocytes recorded in this study were much higher than the earlier findings (Jamil *et al.*, 2008) in buffaloes. Among the three harvesting techniques, slicing method appeared to be superior in terms of both total recovery and number of culture grade oocytes. This method

yielded significantly (P<0.01) higher number of oocytes (7.98 ± 0.70) per ovary compared to puncture (3.46 ± 0.31) and aspiration methods (2.38±0.19). This higher oocyte yield in slicing method was also reflected in more number of mean good, fair, poor quality and culture grade oocytes. Similar findings were reported in buffaloes (Das *et al.*, 1996 and Jamil *et al.*, 2008) and cattle (Carolan *et al.*, 1994). Higher oocyte recovery in ovarian slicing may be due to their release from both surface follicles as well as from deeper cortex (Das *et al.*, 1996). Our study also revealed higher oocyte yield in puncture method compared to aspiration. While it may be difficult to aspirate oocytes from small and medium sized follicles before cumulus expansion (Ball *et al.*, 1983) the extra pressure applied during puncture may release oocytes from these follicles.

Distribution of different quality grades indicate that approximately 1/3 oocytes each were of good, fair and poor quality in all the methods. On the other hand, Das *et al.* (1996) recorded only 11% good quality oocytes and 50% poor quality oocytes in bubaline ovaries. Age, season, nutritional status (body condition) and cyclicity of animals at the time of slaughter, size and functional status of follicles, method of oocyte retrieval etc. are some of the factors that might contribute to recorded variation in oocyte quality (Amer *et al.*, 2008).

Comparison of oocyte recovery rate in buffaloes with that of cows revealed that in the latter species as high as 11 -15 oocytes per ovary were recovered by follicle aspiration (Carolan *et al.*, 1994) and about 30-45 by various surface cutting techniques (Carolan *et al.*, 1994). The low oocyte recovery in buffalo in comparison to its bovine counterpart may be explained by various factors like smaller number of primordial follicle reserve (Dannel, 1987), few antral follicles at any stage of estrous cycle (Kumar *et al.*, 1997), higher rate of follicular atresia (Ocampo *et al.*, 1994) and culling of buffaloes only at terminally unproductive stage and age. In agreement with our results earlier workers also reported considerably low oocyte recovery rate from buffalo ovaries (Amer *et al.*, 2008).

Retrospective analysis of various factors that might influence oocyte recovery revealed that non-luteal phase ovaries yield significantly higher number of oocytes compared to luteal phase ovaries (5.16±0.41 vs.3.00±0.34). Also more number of usable oocytes could be obtained from ovaries not bearing CL. Similar findings were reported in buffaloes (Amer *et al.*, 2008 and Jamil *et al.*, 2008) and cows (Moreno *et al.*, 1993). Side of ovary and ovarian volume did not affect the oocyte yield in this study. Further it was observed that, ovaries having more than five surface follicles produced more number of total as well as usable oocytes than

Table : Effect of harvesting technique on the quantity and quality of oocytes recovered from buffalo ovaries

Attributes	Harvesting technique			Total
	Aspiration	Puncture	Slicing	
No. of ovaries used	48	48	48	144
No. of oocytes recovered	114	166	383	663
Mean No. of oocytes	2.38±0.19^a	3.46±0.31^b	7.98±0.70^c	4.60±0.33
Mean good oocytes	0.84±0.10^a (35.08)	1.25±0.17^b (36.14)	3.23±0.30^c (40.47)	1.77±0.14 (38.46)
Mean fair oocytes	0.77±0.09^a (32.46)	1.02±0.11^a (29.52)	2.04±0.23^b (25.59)	1.28±0.10 (27.75)
Mean poor oocytes	0.77±0.09^a (32.46)	1.19±0.14^b (34.34)	2.71±0.27^c (33.94)	1.55±0.12 (33.79)
Mean culture grade oocytes	1.61±0.14^a (67.54)	2.27±0.22^b (65.66)	5.27 ± 0.50^c (66.06)	3.05±0.23 (66.21)

Means with different superscripts within a row vary significantly (P<0.01)

Figures in parentheses indicate percentage.

those having either no follicles or 1-5 follicles. However, only 11.81% ovaries employed in this study contained more than five follicles and thus it is unlikely that greater oocyte production would be from this group of ovaries in buffaloes.

The results demonstrate that collection of oocytes from non-luteal phase ovaries by slicing technique produce more number of total as well as culture grade oocytes. Presence of more number of surface follicles would be a fringe benefit to ensure additional oocyte recovery from buffalo ovaries.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Principal Scientist, cattle project, Lam and the authorities of SV Veterinary University, Tirupati for providing facilities to carry out the research work.

REFERENCES

Agnihotri, M. K. (1992). Current status and future prospects of buffalo meat production in *India. Buffalo J.*, **8**:95-102.

Amer, H.A., Hegab, A.O. and Zabbal, S.M. (2008). Effects of ovarian morphology on oocyte quantity and quality, granulose cells, *in vitro* maturation, and steroiod hormone production in buffaloes. *Anim. Reprod. Sci.*, **5**: 55-62.

Amir Lass and Peter Brinsden. (1999). The role of ovarian volume in reproductive medicine. Human Reproduction Update, **5**:256-266.

Ball, G.D., Leibfred, M.L., Lenz, R.W., Ax, R.L., Bavister, B.D. and First, N.L. (1983). Factors affecting successful *invitro* fertilization of bovine follicular oocytes. *Biol. Repro.*, **28**: 717-725.

Carolan, C., Monaghan, P., Gallagher, M. and Gordon, I. (1994). Effect of recovery method on yield of bovine oocytes per ovary and their developmental competence after maturation, fertilization and culture invitro. *Theriogenology,* **41**: 1061-1068.

Chauhan, M.S., Singla, S.K., Palta, P., Manik, R.S. and Madan, M.L. (1998). *In vitro* maturation and fertilization, and subsequent development of buffalo (*Bubalus bubalis*) embryos; effect of oocyte quality and type of serum. *Reprod. Fertil. Dev.,* **10**:173-177.

Dannel, B. (1987). Estrous behaviour, ovarian morphology and cyclic variation in follicular system and endocrine pattern in water buffalo heifers. PhD dissertation. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Das, G.K., Jain, G.C., Solanki, V.S. and Tripathi, V.N. (1996). Efficacy of various collection methods for oocyte retrieval in buffalo. *Theriogenology,* **46**:1403-1411.

Jamil, H., Samad, A., Qureshi, Z.I., Rehman, N.U. and Lodhi, L.A. (2008). Harvesting and evaluation of riverine buffalo follicular oocytes. Turkish *J. of Vet. Anim. Sci.*, **32**: 25-30.

Kumar, A., Solanki, V.S., Jindal, S.K., Tripathi, V.N. and Jain, G.C. (1997). Oocyte retrieval and histological studies of follicular population in buffalo ovaries. *Anim. Reprod. Sci.*, **47**: 225-236.

Moreno, J.F., Flores-Foxworth, G., Westhusin, M and Kraemer, D.C. (1993). Influence of pregnancy and presence of a CL on quantity and quality of bovine oocytes obtained from ovarian follicles aspirated post-mortem. *Theriogenology,* **39**:271.

Ocampo, M.B., De Asis, A.T., Ocampo, L.C. and Kanagawa, H. (1994). Histological observation of follicular atresia in swamp buffalo. *Buffalo Bulletin,* **13**:51-55.

Razzaque, W.A.A., Sahatpure, S.K., Pawshe, C.H. and Kuralkar, S.V. (2008). Biometry of ovaries and follicular count in cycling and non-cycling Nagpuri buffaloes (*Bubalus bubalis*). *Buffalo Bulletin,* **27**:150-153.

Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods. 8th edition, Iowa University Press Ames, Iowa 50010.

Wani, N.A., Wani, G., Khan, M.Z. and Sidique, M.A. (1999). Effect of different factors on the recovery rate of oocytes for *in vitro* maturation and in vitro fertilization procedures in sheep. *Small Ruminant Res.*, 34:71-76.