INFLUENCE OF DIFFERENT CATEGORIES OF FOLLICLES AND PRESENCE OF CL ON RECOVERY RATE, QUALITY AND QUANTITY OF BUFFALO OOCYTES*

P.M. MAKWANA[1], R.G. SHAH[2], R.P. SINGH[3] AND A.J. DHAMI[4]

Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand-388001, Gujarat, India

Received : 10.08.2012

ABSTRACT

Accepted : 18.03.2013

The study was conducted on 408 freshly collected abattoir ovaries of Surti buffalo during peak breeding season. The total number of follicles recorded on these ovaries was 1369 and the overall mean number of follicles per ovary was found to be 3.36 ± 0.08. The mean number (percentage) of small, medium and large size follicles per ovary were 1.33 ± 0.05 (39.66), 1.32 ± 0.05 (39.44) and 0.70 ± 0.04 (20.89), respectively. Oocytes collected by slicing method were classified on the basis of cumulus investment and ooplasm homogeneity, viz., grade A (>3 layer of cumulus cells), grade B (1-3 layer of cumulus cells), grade C (less compact cumulus) and grade D (nude oocytes). The mean number of oocytes per ovary of grade A (0.82 ± 0.04) and B (0.79 ± 0.04) were significantly higher (P<0.05) than that of grade C (0.64 ± 0.04) oocytes. The small (<5 mm) follicles had highly significant (P<0.01) and positive correlations with grade A, B and C oocytes in decreasing order. Similarly, medium size follicles (5-8 mm) also had highly significant (P<0.01) and positive correlations with grade B and grade A oocytes. Large follicles (>8 mm) showed significant (P<0.01) and positive correlations with grade D and grade C oocytes. Significantly (P<0.05) greater number of oocytes per ovary were recovered (3.77 ± 0.14) when CL was absent compared with its presence (2.70 ± 0.12). Further, significantly higher percentage (P<0.01) of recovery rate of grade A (27 %) and grade B (26.5 %) oocytes was obtained from the ovaries in which CL was absent than the ovaries in which CL was present (grade A: 22.40 % and grade B: 20.53 %). The effect of presence Vs absence of CL on the ovaries revealed 24.53 Vs 20.60 per cent recovery rate for grade C and 32.53 Vs 25.83 per cent for grade D oocytes. The study showed that functional structure on the ovaries, i.e. corpus luteum and size of follicles affect the recovery rate and quality of buffalo oocytes.

Key words: Follicles, Corpus luteum, Recovery rate, Cumulus cells, Oocytes.

INTRODUCTION

The success of *in vitro* production of buffalo embryo has been hampered by many factors including low number of follicles on the ovaries (Jainuddin *et al.*, 1993), poor recovery rate of oocytes (Kumar *et al.*, 1997) and poor *in vitro* fertilization efficiency of buffalo oocytes (Holm *et al.*, 1999; Farin *et al.*, 2001). Whether these problems arise due to intrinsic quality of buffalo follicular oocytes themselves or due to inadequacies of culture system has yet to be understood. Research has shown that a decrease of storage temperature (Aman and Parks, 1994) or an increase in the storage time of ovary (Ravindranath *et al.*, 2003) decreases the embryonic development in oocytes aspirated from bovines. The recovery rate of immature oocytes in buffalo is poor than that of the cattle. The recovery of acceptable quality oocytes in buffalo is reported to vary from 0.4 (Totey *et al.*, 1992) to 2.4 (Gasparrini *et al.*, 2000), which is mainly attributed to very low primordial follicle reserve pool in the buffaloes (~ 12000; Danell,

* A part of MVSc thesis of first author approved by AAU, Anand

1 Embryologist, MBAHCS, Kamineni Fertility Center, Hyderabad, Andhra Pradesh, India-500001.
2 Associate Research Scientist, ARGO. 3 Field Veterinarian, Ghughli, Maharajgunj, UP.
4 Professor and Head , ARGO, AAU, Anand.

1987) compared to cows (~150000; Erickson, 1966) and high incidences of follicular atresia in the Swamp (Ocampo *et al.*, 1994) and Riverine (Palta and Chauhan, 1998) buffaloes. Despite this major factor, high rates of 70-90 % IVM (Chauhan *et al.*, 1998), 60-70 % IVF (Chohan and Hunter, 2003), and 40-50 % cleavage (Chauhan *et al.*, 1998) have been observed. However, blastocyst development is still very poor and ranges between 10 and 30 % (Chauhan *et al.*, 1997). Hence, the present study was planned to know the influence of different categories of follicles and presence of CL on the recovery rate, quality and quantity of buffalo oocytes.

MATERIALS AND METHODS

Four hundred and eight ovaries from the adult Surti buffaloes were collected during peak breeding season within half an hour of slaughter from the local abattoir and were transported within an hour to the laboratory in a vacuum flask containing normal saline (0.9 % NaCl) at pH 7.0 supplemented with gentamicin 50 µg/ml (Sigma, G 3632) and the temperature of the solution was maintained at 25-30°C (Totey *et al.,* 1992). Upon arrival at the laboratory, extraneous tissue and fat were removed and the ovaries were washed with 70 % ethanol to check contamination, followed by three washes of the normal saline (39°C). Ovaries were mopped with sterilized paper and were evaluated for presence of corpus luteum (CL) and their follicle size as <5 mm, 5-8 mm and >8 mm follicle (Selvaraju *et al.,* 1992). The follicle size was measured by digital Vernier Caliper. To investigate the influence of corpus luteum on the quantity and quality of oocytes recovered per ovary, the ovaries were divided into two groups i.e. the ovaries with and without corpus luteum. The total as well as usable COCs recovered from each ovary of the two groups were recorded.

After measurement of follicle size and evaluation of CL, ovaries were sliced with a fine BP blade and transferred in to 100 mm disposable searching petri dish (Tarson® INDIA) with warm normal saline (Totey *et al.*, 1992). The contents of all sliced ovarian follicles recovered were searched for cumulus oocyte complexes (COCs) in petri dish. A stereozoom microscope (Olympus SZX9, Tokyo, Japan) was used to identify oocytes. The oocytes were transferred to pre-warmed drops of 100 µl of gonadotrophin free Hepes-buffered TCM-199 medium, which was covered with 3 ml of silicon oil (Sigma) in 35 x 10 mm (Sonar® Axiva) plastic petri dish.

Classification of oocytes was done on the basis of cumulus investment and ooplasm homogeneity, as grade A (>3 layer of cumulus cells), grade B (1-3 layer of cumulus cells), grade C (less compact cumulus) and grade D (nude oocytes) (Gordon, 1995).

The data generated were used to work out, mean ± SE, percentages and correlation coefficients between follicles size and oocytes quality.

RESULTS AND DISCUSSION

In the present study, the total number of follicles recorded from 408 ovaries was 1369 and the overall mean number of follicles per ovary was found to be 3.36 ± 0.08. The mean number (percentage) of small, medium and large size follicles per ovary was 1.33 ± 0.05 (39.66), 1.32 ± 0.05 (39.44), and 0.70 ± 0.04 (20.90), respectively. The overall mean number of the follicles observed per ovary was found to be lesser than that reported by Amer *et al.* (2008) in buffalo. They reported 6.8 follicles in type III ovaries (without CL), followed by 5.2 in type II (regressed CL) and 4.4 in type I ovaries (functional CL). The follicular distribution in the present ovaries was found to be different than that reported by Sarvaiya (1997) for small, medium and large size follicles as 63.74, 30.22 and 6.04 %, respectively. The earlier investigations on Surti buffalo ovaries showed that there were great individual differences and the number of primordial follicles varied between 1200 and 40300 in an ovarian pair (Danell, 1987). Comparable figure in a Swedish cattle breed was about 50000 (Settergren, 1964) and in a few buffalo heifers of the Nili-Ravi breed 19100 (Samad and Nasseri, 1979). The present result also shows that buffaloes have less number of follicles available on the ovaries.

Oocytes collected were classified in to 4 grades (A to D) on the basis of cumulus investment and

ooplasm homogeneity. The mean number of oocytes per ovary of grade A (0.82 ± 0.04) and B (0.79 ± 0.04) were significantly higher (P<0.05), followed by grade D (0.76 ± 0.05), than that of grade C (0.64 ± 0.04) oocytes. The overall mean number of oocytes recovered per ovary was 3.01 ± 0.10. These findings are in agreement with the report of Sarvaiya (1997), who recovered 3.55 oocytes per ovary by aspiration and slicing method. The oocytes recovery by slicing method in the present study was higher than that (2.17 oocytes) reported by Dutta *et al.* (1996). The mean number of good quality oocytes was 0.82 ± 0.04, which was in agreement with the earlier report of Madan *et al.* (1994), Dutta *et al.* (1996) and Sarvaiya (1997), who found the value of 0.21, 0.75 and 0.78, respectively. The correlation findings between follicles size and oocytes qualities indicated that small (<5 mm) follicles had highly significant (P<0.01) and positive correlations with grade A, B and C oocytes in decreasing order. Similarly, medium size follicles (5-8 mm) also showed highly significant (P<0.01) and positive correlations with grade B and A oocytes. Large follicles (>8 mm) showed significant (P<0.05) and positive correlations with grade D and C oocytes. Thus, there was recovery of good quality oocytes (i.e. grade A) from small and medium size follicles, whereas large size follicles yielded B and C grade oocytes. These results demonstrate the correlation between follicular size and oocyte developmental competence. It is apparent that oocytes obtained from small bovine follicles do not have the same capacity to develop *in vitro* as the oocytes acquired from larger follicles.

Three hundred and twenty two ovaries were examined to know the effect of presence or absence of corpus luteum (CL) over the ovary on recovery rate and quality of oocytes. A careful examination of the ovaries revealed that CL was present on 139 and absent on 183 ovaries, which yielded 375 and 689 oocytes, respectively. Significantly (P<0.05) greater number of oocytes per ovary was recovered (3.77 ± 0.14) when the CL was absent compared with ovaries on which CL was present (2.70 ± 0.12). Thus, the effect of presence Vs absence of CL on the ovaries had significant effect on recovery rate and quality of buffalo oocytes. The grade A, B, C, D oocytes recovered from ovaries bearing CL were 0.60 ± 0.07, 0.55 ± 0.06, 0.66 ± 0.07 and 0.88 ± 0.09 compared to oocytes recovered from ovaries in absence of CL as 1.02 ± 0.07, 1.00 ± 0.07, 0.78 ± 0.06 and 0.97 ± 0.08, respectively. The recovery rate of all the four grade of oocytes was significantly higher in ovaries without CL than ovaries with CL. Further, significantly higher percentage (P<0.01) of recovery of grade A (27 %) and grade B (26.5 %) oocytes was obtained from the ovaries in which CL was absent than the ovaries in which CL was present (grade A: 22.40 % and grade B: 20.53 %). The effect of presence Vs absence of CL on the ovaries revealed 24.53 Vs 20.63 % recovery rate for grade C and 32.53 Vs 25.83 % for grade D oocytes. Nandi *et al.* (2000) reported decreased recovery rate of oocytes when ovary had a CL. This is because the follicular development is restricted, as the lutein cells occupy most of the portion of the ovary (Kumar *et al.*, 1997). The dominant follicle is usually observed in the CL bearing ovaries and the other follicles are very small and remain mostly inaccessible (Gasparrini *et al.*, 2000). Thus it was concluded that the functional structure on the ovaries, i.e. corpus luteum and size of follicles affect the recovery rate and quality of oocytes, since significantly higher recovery rate and good quality of oocytes were obtained from ovaries with absence of CL.

ACKNOWLEDGEMENTS

We thank Professor of Animal Biotechnology Dr. C.G. Joshi and Ex-Dean of the Faculty Dr. J.V. Solanki for the co-operation and facilities provided for this work.

REFERENCES

Aman, R.R. and Parks, J.E. (1994). Effects of cooling and rewarming on the meiotic spindle and chromosomes of *in vitro* matured bovine oocytes. *Biol. Reprod.*, **50**: 103-110.

Amer, H.A., Hegab, A.O. and Zaabal, S.M. (2008). Effects of ovarian morphology on oocyte quantity and quality, granulosa cells, *in vitro* maturation, and steroid hormone production in buffaloes. *Anim. Reprod.*, **5**: 55-56

Chauhan, M., Katiyar, P., Singla, S., Manik, R. and Madan, M. (1998). Production of buffalo calves through *in vitro* fertilization. *Indian J. Anim. Sci.*, **67**: 306-308.

Chauhan, M.S., Singla, S.K., Manik, R.S. and Madan, M.L. (1997). Production of buffalo calves through *in vitro* fertilization. *Indian J. Anim. Sci.*, **67**: 306-308.

Danell, B. (1987). Oestrus Behaviour, Ovarian Morphology and Cyclical Variation in Follicular System and Endocrine Pattern in Water Buffalo Heifers. Thesis, Uppsala University, Sweden, pp.54-94.

Dutta, D.J., Sarmah, B.K. and Sarmah, B.C. (1996). A Comparative study on the number and type of prepubertal goat oocytes recovered by three different methods. *Indian J. Anim. Reprod.*, **17**(2): 105-106.

Erickson, B.H. (1966). Development and radio response of pre-natal bovine ovary. *J. Reprod. Fert.*, **10**: 97-105.

Farin, P.W., Crosier, A.E. and Farin, C.E. (2001). Influence of *in vitro* system on embryo survival and fetal development in cattle. *Theriogenology,* **55**: 151-170.

Gasparrini, B., Neglia, G., Di Palo, R., Campanile, G. and Zicarelli, L. (2000). Effect of cysteamine during *in vitro* maturation on buffalo embryo development. *Theriogenology*, **54**: 1537-1542.

Gordon I. (1995). Laboratory Production of Cattle Embryos. CAB International, Wallingford, UK.

Holm, P., Booth, P.J., Sehmidt, M.H., Greve, T. and Callesen, H. (1999). High bovine blastocyst development in a static *in vitro* production system using SOF medium supplemented with sodium citrate and myoinositol with or without serum protein. *Theriogenology*, **52**: 683-700.

Jainuddin, M.R., Takahashi, Y., Nihayah, M. and Kanagawa, H. (1993). *In vitro* maturation and fertilization of swamp buffalo (*Bubalus bubalis*) oocytes. *Anim. Reprod. Sci.*, **31**: 205-212.

Kumar, A., Solanki, V.S., Jindal, S.K., Tripathi, V. N. and Jain, G.C. (1997). Oocytes retrieval and histological studies of follicular population in buffalo ovaries. *Anim. Reprod. Sci.,* **47**: 189-195.

Madan, M.L., Singla, S.K., Chauhan M.B., Manik, R.S. (1994). *In vitro* production and transfer of embryos in buffaloes. *Theriogenology*, **41**: 139-143.

Nandi, S., Chauhan, M.S. and Palta, P. (2000). Effect of a corpus luteum on the recovery and developmental potential of buffalo oocytes. *Vet. Rec.*, **147**: 580-581

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

Palta, P. and Chauhan, M.S. (1998). Laboratory production of buffalo (*Bubalus bubalis*) embryos. *Reprod. Fert. Dev.,* **10**: 379-391.

Ravindranath, B.M., Nandi, S., Raghu, H.M. and Reddy, S.M. (2003). *In vitro* maturation and fertilization of buffalo oocytes: Effects of storage of ovaries, IVM temperatures, storage of processed sperm and fertilization media. *Reprod. Dom. Anim.,* **38**: 21-26.

Samad, H.A. and Nasseri, A.A. (1979). A quantitative study of primordial follicles in buffalo heifer ovaries. *Compendium 13th FAO/SIDA International Course on Animal Reproduction,* Uppsala, Sweden.

Sarvaiya, N.P. (1997). A special problem on "Analysis of factors involved in number and quality of oocyte recovered from buffalo ovaries of abattoir origin" submitted to GAU, Anand (unpublished data).

Selvaraju, R., Majumdar, A.C. and Ansari, M.R. (1992). Quality of oocytes from different size of buffalo ovaries obtained from a slaughter house. *Indian J. Anim. Sci.*, **62**: 434-435.

Settergren, I. (1964). The ovarian morphology in clinical bovine gonadal hypoplasia with some aspects of its endocrine relations. Thesis, *Acta Vet. Scand.*, **5**: *Suppl.*

Totey, S.M., Singh, G., Taneja, M., Pawshe, C.H. and Talwar, G.P. (1992). *In vitro* maturation, fertilization and development of follicular oocytes from buffalo (*Bubalus bubalis*). *J. Reprod. Fert.*, **95**: 597-607.

Yadav, P.S., Saini, A. and Jain, G.C. (1996). Developmental ability of oocytes during *in vitro* maturation and fertilization in buffaloes. *Proc. XIII National Convention of ISSAR and National Symposium on Anim. Biotech.*, 4-6 Dec. Pantnagar, India, p.10.