TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Short Communication

Turk J Vet Anim Sci
(2013) 37: 468-471

doi:10.3906/vet-1204-11

Effect of corpus luteum: quality and recovery rate of buffalo (*Bubalus bubalis*) oocytes

Lakshman SAHOO[1,*], Suresh K. SINGLA[2]

[1]Fish Genetics and Biotechnology Division, Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Odisha, India
[2]Animal Biotechnology Centre, National Dairy Research Institute (Deemed University), Karnal, Haryana, India

Received: 07.04.2012 • **Accepted:** 12.10.2012 • **Published Online:** 29.07.2013 • **Printed:** 26.08.2013

Abstract: Recovery rate and quality of oocytes were investigated from abattoir collected ovaries with and without corpora lutea (CL). The oocytes were categorized into four grades: grade-A, grade-B, grade-C, and grade-D based on the presence of the cumulus cells complex around the oocytes. The number of oocytes per ovary were 1.7 ±0.1 and 1.5 ±0.1, obtained from ovaries without CL and with CL, respectively. Recovery rate of different grades of oocytes from ovaries with CL and without CL were almost identical.

Key words: Buffalo, ovary, oocyte, CL

India has the highest buffalo population in the world. In terms of meat, milk, and draft work, the indigenous river buffalo is the major contributor and is considered the leading dairy animal in the country. Due to its ability to survive in harsh climatic conditions, in comparison with cattle the buffalo is considered as the animal of the future in India. While buffalo have several advantages over cattle, poor availability of superior germplasm is a major problem in buffaloes. Inherent problems like delayed maturity, seasonality of breeding, poor conception rate, silent estrus, low germ cell reserve, higher rates of follicular atresia, high rate of incidence of early embryonic death, longer inter-calving period (1), poor freezability of semen, and susceptibility to thermal stress (2) are all limitations to the reproductive performance and productivity in the species. Therefore, attempts have been made to produce elite animals through advanced reproductive technologies, requiring large number of oocytes from ovaries of abattoir origin/live animals. Retrieval of good quality oocytes and average number of oocytes per ovary in buffaloes are low (3–6). Recovery rate and quality of oocytes is influenced by season, and significantly higher numbers of good quality oocytes were obtained during the winter season as opposed to the summer season. Moreover, during the winter season, significantly higher numbers of good quality oocytes were obtained from the ovaries during days where the temperature was lower than 25 °C (7). Little information on recovery rates of different grades of oocytes from buffalo ovaries is available. The present study highlights number of oocytes recovered per ovary having CL and without CL in buffaloes, and their relevant percentage of different grades of oocytes recovered from abattoir-collected ovaries.

Buffalo ovaries from slaughtered animals were immediately collected from the Delhi slaughterhouse between February and April of 2001.The ovaries were thoroughly washed to remove tissue debris and blood clots, and transported to Karnal in a thermos flask at 32–35 °C with fresh saline solution within 3–4 h of collection.

The ovaries were again thoroughly washed in normal saline and were classified into 2 groups, with CL and without CL, and transferred separately into 2 sterile separate glass beakers. Later they were put on sterile filter paper to remove excess water. All the surface follicles of 3–10 mm diameter were aspirated in phosphate buffered saline albumin (PBSA) medium using a 5-mL plastic syringe (tissue culture grade) and a 19-gauge needle. The oocytes thus collected were kept in a petri dish with PBSA and examined under a microscope with 45× magnification.

The oocytes were searched, separated, washed thoroughly, and classified into 4 grades. The criteria used for grading was as described by Singla (8) with some modification:

Grade-A: Oocytes with a compact cumulus complex and 3 or more layers of cumulus cells.

Grade-B: Oocytes with compact cumulus complex and 1–3 layers of cumulus cells.

Grade-C: Oocytes with expanded and partially denuded cumulus cells.

Grade-D: Oocytes without cumulus cells.

* Correspondence: lakshmansahoo@gmail.com

Among 1345 ovaries, 960 were without CL (71.4%) and 385 were with CL (28.6%) (Table 1). The average frequency of CL was 28.6% during the period of study, suggesting that about 57% of buffaloes were either cycling or had functional ovaries. Total number of ovaries taken, total number of oocytes, different grades of oocytes, and their percentage recovered from ovaries without and with CL are presented in Tables 2–4. Statistical analysis suggests no significant difference in recovery rate of different grades of oocytes from ovaries without and with CL. Higher number of oocytes were recovered from ovaries without CL (1.7 ± 0.11, 1.01 to 2.2) than ovaries having CL (1.5 ± 0.1, 1.2 to 2). Frequency of distribution of different grades of recovered oocytes from ovaries with and without CL was almost identical.

Reproductive efficiency and cyclicity in buffaloes were greatly affected by the month of the year (9). Number of oocytes retrieved and quality of oocytes were affected by the season (9). Good quality and greater number of oocytes were obtained from buffalo ovaries during the day where the temperature was lower than 25 °C. Our result showed that 28.6% of ovaries had CL in contrast to 23.7% of ovaries reported by Singla (8). Further, the authors also have reported that the percentage of ovaries having CL was greater in the winter season than in the summer season. As ovaries were collected from the slaughterhouse between February and April, that may be one of the causes for having a higher number of ovaries with CL during the study. The ratio of ovaries with CL to that of ovaries without CL was 1:2.5 as compared to 1:3 (8).

Retrieval of 9.5%, 11.1%, and 22.7% of Grade-1; 17.0%, 37.8%, and 5.8% of Grade-2; and 55.2%, 51.1%, and 25.5% of Grade-3 oocytes was reported (3,6,9). However, there were no data available for the recovery of different

Table 1. Distribution of ovaries with and without CL.

Trial no.	Total no. of ovaries	Ovaries with CL (%)	Ovaries without CL (%)
1	92	30 (32.6)	62 (67.4)
2	123	39 (31.7)	84 (68.3)
3	128	45 (35.2)	83 (64.8)
4	103	34 (33.0)	69 (67.0)
5	101	25 (24.8)	76 (75.2)
6	83	17 (20.5)	66 (79.5)
7	44	-	44 (100.0)
8	218	65 (29.8)	153 (70.2)
9	176	63 (35.8)	113 (64.2)
10	109	28 (25.7)	81 (74.3)
11	168	39 (23.2)	129 (76.8)
Total	1345	385 (28.6)	960 (71.4)

Table 2. Total number ovaries with and without CL and total number of oocytes recovered.

Ovaries	Numbers	No. of oocytes recovered
With CL	385	572
Without CL	960	1578
Total	1345	2150

Table 3. Number of oocytes per ovary and the percent distribution of their quality obtained from ovaries without CL.

Trial no.	Oocytes per ovary	Percent oocytes			
		Grade-A (No.)	Grade-B (No.)	Grade-C (No.)	Grade-D (No.)
1	1.66	6.8 (7)	37.9 (39)	31.6 (28)	24.3 (25)
2	1.82	25.5 (39)	50.3 (77)	18.3 (18)	5.9 (9)
3	1.01	26.2 (22)	38.1 (32)	21.4 (30)	14.3 (12)
4	1.5	30.0 (21)	45.5 (55)	14.5 (19)	10.0 (8)
5	1.5	18.4 (29)	48.2 (47)	26.3 (23)	7.1 (4)
6	1.16	29.3 (49)	47.5 (94)	19.2 (21)	4.0 (13)
7	1.59	23.2 (63)	56.7 (90)	17.5 (45)	2.1 (6)
8	2.11	27.4 (52)	52.5 (62)	12.8 (24)	7.3 (12)
9	2.23	35.0 (71)	50.0 (144)	11.7 (17)	3.3 (6)
10	2.20	30.4 (23)	36.3 (55)	26.3 (17)	7.0 (2)
11	1.6	24.7 (33)	50.0 (50)	23.3 (16)	2.1 (11)
Mean	1.7 ± 0.11	24.7 ± 1.6	46.6 ± 1.2	19.9 ± 1.3	9.5 ± 2.9

Table 4. Number of oocytes per ovary and the percent distribution of their quality obtained from ovaries with CL.

Trial no.	Oocytes per ovary	Percent oocytes			
		Grade-A (No.)	Grade-B (No.)	Grade-C (No.)	Grade-D (No.)
1	1.3	12.8 (5)	43.6 (17)	35.9 (14)	7.7 (3)
2	1.53	31.7 (19)	53.3 (32)	11.7 (7)	3.3 (2)
3	1.17	18.9 (10)	50.9 (27)	18.9 (10)	11.3 (6)
4	2.0	19.6 (10)	44.6 (25)	23.2 (12)	12.5 (3)
5	1.58	20.0 (8)	50.0 (9)	24.0 (9)	6.0 (1)
6	1.2	29.6 (18)	33.3 (31)	33.3 (17)	3.7 (12)
7	1.58	23.1 (35)	39.7 (50)	21.8 (8)	15.4 (7)
8	1.96	35.0 (14)	50.0 (22)	8.0 (15)	7.0 (4)
9	1.38	24.5 (13)	40.0 (28)	27.3 (9)	7.3 (4)
10	1.64	24.1 (11)	51.8 (25)	16.7 (13)	7.4 (7)
Mean	1.53 ± 0.1	23.6 ± 1.4	45.7 ± 1.2	21.4 ± 2.0	7.8 ± 1.3

grades of oocytes classified into 4 grades with respect to buffaloes. Our study revealed that 23.6% of Grade-A, 45.7% of Grade-B, 21.4% of Grade-C, and 7.8% of Grade-D oocytes were recovered from ovaries with CL and the corresponding figures were 24.7%, 46.6%, 19.9%, and 9.5% in ovaries without CL. However, on average, 24.1% of Grade-A, 46.14% of Grade-B, 20.88% of Grade-C, and 8.6% of Grade-D oocytes were recovered. The percentage of recovery of different grades of oocytes did not differ significantly from ovaries with and without CL in contrast to the result reported by Singla (8), where data were presented at 1% level of significance. This may be attributed due to the fact that in this study we graded oocytes into 4 grades as opposed to 3 grades (8), which might have contributed in establishing the difference. We observed that there was no significant difference in the percentage of recovery rate of different grades of oocytes irrespective of presence of CL. In contrast to 0.41 and 0.67 oocytes per ovary, a higher rate of recovery was observed in our study (10). Nevertheless, further investigation is needed to shed light on how this affects further developmental ability of the oocytes.

Acknowledgments

The authors are thankful to the Director at the National Dairy Research Institute, Karnal, for providing the necessary facilities to carry out the present study. Our sincere thanks to Dr SKH Ray for his valuable input while preparing the manuscript.

References

1. Madan, M.L.: Status of reproduction in female buffalo. In: Buffalo Production and Health: a compendium of latest research information based on Indian studies. ICAR Publication, New Delhi, India, 1988; 89–100.
2. Kurup, M.P.G.: Present status of embryo transfer in buffalo and future expectations. Proc. of the Second World Buffalo Congress, New Delhi, 1998; II: 587–590.
3. Suzuki, T., Singla, S., Sujata, T., Madan, M.: In vitro fertilization of water buffalo follicular oocytes and their ability to cleave in vitro. Theriogenology, 1992; 38: 1187–94.
4. Madan, M.L., Singla, S.K., Chauhan, M.S., Manik, R.S.: In vitro production and transfer of embryos in buffaloes. Theriogenology, 1994; 41: 139–143.
5. Totey S.M., Singh G., Taneja M., Pawshe C.H., Talwar G.P.: In vitro maturation, fertilization and development of follicular oocytes from buffalo (*Bubalus bubalis*). J. Reprod. Fert, 1992; 95: 597–607.
6. Jain, G.C., Das G.K., Solanki V.S., Tripathi V.N.: Comparative efficiency of three different collection techniques for oocyte retrieval in buffaloes. Theriogenology, 1995; 43: 240.
7. Datta, T.K., Goswami, S.L.: Feasibility of harvesting oocytes from buffalo (*Bubalus bubalis*) ovaries by different methods. Buffalo J., 1998; 14: 277–284.
8. Singla, S.K.: PhD thesis submitted to National Dairy Research Institute, 1995.
9. Madan, M.L.: Embryo transfer technology in buffaloes. Indian J. Anim. Reprod., 1992; 13: 255.
10. Singh, S., Dhanda, O.P., Malik, R.K.: Effect of the presence of the corpus luteum on oocyte recovery and subsequent in vitro maturation and fertilization. Asean-Aust. J. Anim. Sci. 2001; 14: 1675–1677