FACTORS AFFECTING RECOVERY OF BUFFALO FOLLICULAR OOCYTES

H.A. Samad and A. Raza
Department of Animal Reproduction, University of Agriculture, Faisalabad-38040, Pakistan

ABSTRACT

These studies were conducted to determine the effect of various factors i.e., age, size of the ovary, presence or absence of corpus luteum (CL) and the season affecting the oocytes harvesting from ovaries of slaughtered buffaloes. Follicular oocytes were harvested from 2-6mm diameter follicles by scouring the ovarian surface in modified tyrode lactate medium TL-Hepes supplemented with 20% oestrus buffalo serum, sodium pyruvate and gentamycin sulphate. The effect of season, age and size of the ovary on the harvesting of oocytes was non-significant, while the absence of CL on the ovary had a significantly positive effect. The ovaries with CL yielded significantly lower good quality and total oocytes per ovary (2.85 ± 0.35 and 3.94 ± 0.28) than ovaries without CL (4.52 ± 0.52 and 5.90 ± 0.39).

INTRODUCTION

Embryo collection from the uteri of superovulated buffaloes by non-surgical transcervical technique has had discouraging results (Jainndeen, 1990). An alternative approach for buffalo embryo production is *in vitro* fertilization (IVF) of immature oocytes from slaughtered buffaloes. For *in vitro* maturation (IVM), fertilization and development of buffalo follicular ooctyes abundant recovery of good quality oocytes is one of the prerequisites. Unfortunately, poor recovery of immature oocytes in buffalo is a primary problem for the development of *in vitro* system.

Moreover, a great deal of study is required to establish various factors responsible for optimum maturation of buffalo follicular oocytes, ability to efficiently prepare and capacitate spermatozoa for fertilization and then subsequent culture of these *in vitro* fertilized oocytes.

Keeping these facts in view, the present investigation was undertaken to study different factors affecting oocytes harvesting from the ovaries of slaughtered buffaloes.

MATERIALS AND METHODS

Collection of Ovaries

Buffalo ovaries were collected from a local abattoir within 1-2 hours post-slaughtering and were transported immediately to the laboratory in a thermos containing sterile normal saline with added antibiotics (100 IU/ml penicillin G, 100 μg/ml streptomycin sulphate and 0.25 μg/ml amphotericin B) held at 30-35°C. Extraneous tissue was removed and the ovaries were cleaned in normal saline. Prior to oocyte collection the ovaries were rinsed in 70% ethanol to minimize the risk of contamination followed by three rinses with sterile normal saline to remove the traces of ethanol.

Recovery of Oocytes

Follicular oocytes were recovered from 2-6 mm diameter follicles by scoring (slicing) the ovarian surface with a sterile surgical blade, with instant rinsing and tapping the ovary to release oocytes in a sterile 60 x 15 mm petridish containing modified tyrode-lactate medium, Tl-Hepes (Bavister, 1989), supplemented with 20% oestrus buffalo serum, sodium pyruvate (0.20mM) and gentamycin sulphate (10μg/ml). The pH of the medium was adjusted to 7.4. For the classification of oocytes under steriomicroscope the criteria of De Loos *et al.* (1989) and Lonergan *et al.* (1991) based on their cumulus investment and ooplasm homogeneity were adopted (Table-1). Category A, B and C oocytes were considered morphologically good for IVF whereas the category D oocytes being the poor candidate for IVM/IVF were not included for IVM studies.

Factors Studied

Various factors i.e., age, size of ovary, presence or absence of corpus luteum and season were studied for their effect on the quantity and quality of oocytes. Age of the buffalo was noted by counting rings of the horns and physical appearance. In the laboratory the

dimensions of the ovaries were measured by vernier calliper and the presence or absence of corpus luteum was noted. One hundred thirty eight (138) ovaries were studied during normal breeding season (September and October) while 150 during low breeding season (May and June).

Statistical Analysis

The mean values of oocyte harvesting were computed using t-test.

RESULTS

The effect of age of the buffalo and the total number of good quality oocytes obtained per ovary by the scoring method of oocyte harvesting technique is given in Table-2. There was no significant difference in the number of oocytes collected per ovary from heifers (5.80±0.01) and adult buffaloes (5.24±0.24). Similarly good quality oocytes per ovary were also non-significantly higher in heifers (4.06±0.21) than adult buffaloes (3.40±0.30).

The effect of size of ovary on the total and good quality oocytes recovered per ovary is given in Table-2. The ovaries <2.25x1.75x1.25cm yielded 4.40±0.23 and 5.75±0.19 good quality and total ocytes per ovary while the ovaries >2.25x1.75x1.25cm yielded 4.22±0.41 and 5.46±0.29 good quality and total ooytes per ovary, respectively. However, the difference was non-significant.

Data regarding the effect of the presence or absence of CL on the oocyte recovery is given in Table-3. The ovaries with CL yielded significantly lower ($P<0.05$) good quality and total oocytes per ovary (2.85±0.35, 3.94±0.28) than ovaries without CL (4.52±0.52, 5.90±0.39).

The effect of normal breeding season (NBS) and low breeding season (LBS) on the oocyte recovery is given in Table-3. In NBS, the good quality and total oocytes recovered were 3.68±0.94 and 5.02±0.66, whereas these values were 3.41±1.12 and 4.86±0.88 per ovary in the LBS. Statistically the difference was non-significant.

DISCUSSION

The quantity and quality of oocytes recovered per ovary has been an important consideration in the production of IVM-IVF embryos. The buffalo ovary contains thousands of oocytes, but only a small number is utilized during the life span of the animal and most of these go waste after the slaughter of buffalo. Hence the effect of different factors like age of the buffalo, size of the ovary, presence or absence of CL and the season on the quantity and quality of oocytes recovered per ovary from slaughtered buffaloes was studied. The results showed that age of the

Table 1: Classification of oocytes based on their cumulus investment and ooplasm homogeneity

Category	Morphological features of follicular oocytes
A	Compact and dense multilayered (≤5) cumulus investment with homogeneous ooplasm
B	Compact and dense multilayered (3-4) cumulus investment with homogeneous ooplasm
C	Less compact (2-3) comulus cell layers with less homogeneous ooplasm
D	Denuded or naked oocytes with evenly granulated ooplasm

Table 2: Effect of age of the buffalo and size of the ovary on quantity and quality of follicular oocytes obtained for IVM.

	Age of the buffalo		Size of the ovary	
	Heifer	Adult	<2.25x1.75x1.25 cm	>2.25x1.75x1.25cm
No. of ovaries	31	37	41	54
Total oocytes recovered per ovary	5.80±0.01a	5.24±0.24a	5.75±0.19a	5.46±0.29a
Useable (A,B & C) oocytes recovered per ovary	4.06±0.21a	3.40±0.30a	4.40±0.23a	4.22±0.41
Oocytes recovered: Total	180	194	236	295
A	49	61	54	88
B	32	40	38	80
C	45	25	70	60
D	54	68	74	67

Values sharing same letters within a row are non-significant ($P<0.05$).

Table 3: Effect of corpus luteum and season on quantity and quality of buffalo follicular oocytes obtained for IVM.

	Corpus luteum on the ovary		Season	
	Present	Absent	Normal Breeding Season	Low Breeding Season
No. of ovaries	74	88	138	150
Total oocytes recovered per ovary	3.94±0.28a	5.90±0.39b	5.02±0.66a	4.86±0.88a
Useable (A,B & C) oocytes recovered per ovary	2.85±0.35a	4.52±0.52b	3.68±0.94a	3.41±1.12a
Oocytes recovered:				
Total	292	520	693	710
A	74	144	200	257
B	64	158	194	141
C	73	95	114	94
D	81	123	185	218

Values with different letters within a row differ significantly ($P<0.05$).

buffalo did not affect the quantity and quality of oocytes. The total and number of good quality oocytes recovered from heifers were 5.80±0.01 and 4.06±0.21 per ovary, while from adult ovary it was 5.24±0.24 and 3.40±0.30 respectively. The difference being non-significant. The results of the present study are comparable to those of Kataska and Smory (1984) in cattle, who observed no effect of age on the number and the quality of oocytes recovered. Similarly, Wani (1995) found no significant effect of age on the number and quality of sheep follicular oocytes. Armstrong *et al.* (1994) collected the oocytes from 6-8 week old ewes for in vitro fertilization and were able to produce pregnancies from such embryos. They further found that age was not a constraint and the oocytes could be obtained even from pre-pubertal animals at a rate equivalent or even higher than pubertal animals.

The ovaries <2.25x1.75x1.25cm in size yielded 4.00±0.23 and 5.75±0.19 good quality and total oocytes per ovary, while the ovaries >2.25x1.75x1.25cm yielded 4.22±0.41 and 5.46±0.29 good quality and total ooytes per ovary. Wani (1995) reported that in sheep larger ovaries yielded significantly more number of oocytes than smaller ovaries by aspiration technique, while the difference was non-significant with the scoring (slicing) method. Since no work has been reported about the effect of size of ovary on the number and the quality of oocytes in buffaloes, the results can not be compared.

In the present study the oocyte recovery rate of good quality and total oocytes from the ovaries having CL was poor than ovaries without CL (2.85±0.35 and 3.94±0.28 vs 4.52±0.52 and 5.90±0.39). The cause of lower oocytes in ovaries with CL may be attributed to the fact that CL reduces the growth of follicles and increases the atresia of follicles (Hafez, 1993).

The recovery rate of good quality and total oocytes in normal breeding season was 3.68±0.94 and 5.02±0.66 as compared to 3.41±1.12 and 4.86±0.88 in low breeding season. Parmar and Mehta (1992) studied the development of ovarian follicles in Surti buffalo ovaries in different seasons and found no significant effect on the number of developing follicles during different seasons. It was concluded that buffalo ovaries can be collected for harvesting follicular oocytes at any age and season after puberty. However, the ovaries without CL be preferred over those having CL.

ACKNOWLEDGEMENT

Authors of this manuscript are extremely thankful to Pakistan Science Foundation, Islamabad for financial help to carry out these studies under Research Project No. P-AU-(Agr) 175.

REFERENCES

Armstrong, DT, B. Irvine and CR Earl, 1994. In vitro fertilization of follicular oocytes from juvenile lambs and their developmental competence in vitro and in vivo. Biol. Reprod., 50: Suppl.1 189 abstract.

Bavister, B.D., 1989. A consistently successful procedure for *in vitro* fertilization of golden hamster eggs. Gamete Res., 23: 139-158.

De Loos, F., P. Maurck, T. Beneden and T.A.M. Kruip, 1989. Structural aspects of bovine oocyte maturation in vitro. Molecular Reprod. and Development, 31: 208-214.

Hafez, ESE., 1993. Folliculogenesis, egg maturation and ovulation. Reproduction in Farm Animals, Lea and Febiger, Philadelphia. pp: 114-143.

Jainndeen, MR, 1990. A review of embryo transfer technology in the buffalo nuclear techniques to improve domestic buffalo production in Asia. Panel Proceedings Series International Atomic Energy Agency, Vienna. pp: 103-112.

Kataska, L. and Z. Smory, 1984. Number and quality of oocytes in relation to age of cattle. Anim. Reprod. Sci., 90: 615-625.

Lonergan, P., E. Vergos, A. Kinis, H. Sharif, M. Gallagher and I. Gordon, 1991. The effect of recovery method on the type of bovine oocyte obtained for in vitro maturation. Theriogenology, 35: 231.

Parmar, A.P. and V.M. Mehta, 1992. Study of biometery of Surti buffalo ovaries and development of ovarian follicles in relation to different seasons. Indian J. Anim. Reprod., 13: 157-160.

Wani, N.A., 1995. *In vitro* maturation and in vitro fertilization of ovine oocytes. MVSc thesis, Faculty of post-graduate studies, Sher-e-Kashmir University of Agricultural Sciences and Technology, Srinagar