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Research Article

Harvesting and Evaluation of Riverine Buffalo Follicular Oocytes

Huma JAMIL*, Hafız Abdus SAMAD, Zafar Iqbal QURESHI, Najeeb Ur REHMAN, Laeed Akbar LODHI
Department of Animal Reproduction, University of Agriculture, Faisalabad, 38095, PAKISTAN

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Abstract: The low superovulatory response and poor recovery rate of oocytes are major impediments to the application of in vitro fertilization and embryo transfer technology in buffaloes. The present study aimed to evaluate the comparative efficacy of oocyte collection methods, season (low and peak breeding season), and ovarian status (presence or absence of corpus luteum) on the recovery rate and quality of recovered buffalo follicular oocytes. For this purpose riverine buffalo ovaries were collected from buffaloes slaughtered at Faisalabad Municipal Corporation Slaughter House. To study the efficiency of 3 different recovery methods the ovaries were dissected (n = 291), punctured (n = 301), or aspirated (n = 298). In all, 675 oocytes were recovered by dissection, 441 by puncture, and 363 by aspiration. The results of the present study revealed that a significantly higher ($P < 0.05$) oocyte recovery rate was obtained from the ovaries collected during the peak-breeding season and from those in which the corpus luteum was absent. Of the 3 oocyte recovery methods used, dissection yielded the highest percentage (36.74%) of type I oocytes, followed by puncture (32.87%), and aspiration (19.83%).

Key Words: Buffalo, oocytes harvesting method, ovary, breeding season, corpus luteum

Introduction

Buffaloes are reported to have low reproductive performance with inherent reproductive problems of weak/silent estrus signs, seasonal anestrous, delayed puberty, delayed first calving, late post partum conception, and a long calving interval (1). Improving the genetic potential of water buffaloes for milk and meat has been a major concern for decades in countries that produce and raise buffalo. Assisted reproductive technologies, such as artificial insemination (AI), multiple ovulation embryo transfer (MOET), and in vitro fertilization (IVF), have been introduced and applied as alternate approaches to overcome the reproductive inefficiencies of this animal (2). Due to the inefficiency of buffalo MOET programs in vitro production of embryos has received increasing interest (2). However, optimum exploitation of the benefits of IVF technologies depends on the constant supply of a large number of embryos, with the prerequisite of a large number of follicular oocytes. The success of in vitro production of buffalo embryos has been hampered by several factors, including a low quantity of follicles on the ovaries (3) and a poor oocyte recovery rate (4). Several methods of oocyte recovery, including aspiration of the follicles, dissection of the ovaries, and puncturing the follicles, have been used (4,5). However, the standardization of procedures for buffalo requires considerable developmental work. Hence, an optimum method has to be developed to recover the highest number of oocytes from a given ovary. The present study was aimed to determine a suitable method for recovering oocytes from buffalo ovaries during low and peak breeding seasons, while considering the stage of cyclic activity of the ovaries.

Materials and Methods

Collection of Ovaries

Ovaries from sexually mature buffaloes were collected within 30 min of slaughter from the abattoir. They were then transported within 2 h of slaughter to the laboratory in a vacuum flask containing sterilized phosphate buffered saline (PBS) (pH 7.35) supplemented with 100 IU penicillin G and 100 mg/ml streptomycin at 25-30 °C (5). Upon arrival at the laboratory extraneous tissue was removed from the ovaries. Then, the ovaries were washed with 70% ethanol to control contamination,

* E-mail: drhjamil@hotmail.com.

rinsed 3 times in PBS, and finally dried with sterilized paper towels. Ovary collection times were classified into 2 breeding seasons, i.e. peak breeding season (September, October, and November) and low breeding season (March, April, and May). To investigate the influence of the corpus luteum on the quantity and the quality of cumulus-oocyte complexes (COCs) recovered per ovary, the ovaries were divided into 2 groups, i.e. the ovaries with and without a corpus luteum. The total as well as usable COCs recovered from each ovary of the 2 ovary groups were recorded.

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To study the efficiency of 3 recovery methods the ovaries were dissected (6), punctured (4), or aspirated (7). Oocytes were studied with a stereomicroscope. The presence of cumulus cells surrounding immature oocytes is a pre-requisite for successful in vitro maturation of buffalo follicular oocytes. The total as well as usable COCs recovered per ovary were recorded according to the classification of Hasler et al. (8). The oocytes were classified as 4 types (I-IV). All type I and II oocytes were selected for in vitro maturation studies and were categorized as usable oocytes. The values obtained from oocyte harvesting methods were used in one-way analysis of variance to determine the effect of those factors on oocyte yield. Means were compared by least square design.

Results

When the quality of oocytes recovered with different methods was assessed, it was revealed that the highest percentage of type I oocytes was recovered by the dissection method (36.74%), followed by puncture (32.87%) and aspiration (19.83%); the difference between the 3 methods was significant ($P < 0.05$) (Table 1). For type II oocytes a significantly ($P < 0.05$) higher percentage was recovered by the aspiration method (36.36%) compared to puncture (29.25%) and dissection (27.40%); the difference between the later 2

Table 1. The effect of recovery method on the recovery rate and quality of recovered buffalo follicular oocytes.

Parameters	Method of Recovery of Oocyte		
	Dissection	Puncture	Aspiration
Number of ovaries	291	301	298
Total oocytes recovered	675	441	363
Oocytes recovered /ovary Mean ± SE	2.31 ± 0.9[a]	1.46 ± 0.57[b]	1.21 ± 0.45c
Total usable oocytes recovered	433	274	202
Usable oocytes recovered/ovary (type I + type II) Mean ± SE	1.48 ± 0.53[a]	0.91 ± 0.45[b]	0.67 ± 0.23[b]
Type I	36.74%[a]	32.87%[b]	9.83%c
(n)	(248)	(145)	(72)
Type II	27.40%[b]	29.25%[b]	36.36%[a]
(n)	(185)	(129)	(132)
Type III	10.81%[a]	10.65%[a]	36.63%[b]
(n)	(73)	(47)	(133)
Type IV	25.03%[a]	27.21%[a]	7.16%[b]
(n)	(159)	(120)	(26)

The values with different superscripts in the same row differ significantly ($P < 0.05$).
Type I: Oocytes surrounded by 4 or more layers of compact cumulus.
Type II: Oocytes surrounded by 1-3 layers of compact cumulus.
Type III: Nude oocyte without any cumulus.
Type IV: Oocyte surrounded by a cumulus that had undergone some degree of expansion and, in some cases, with pyknotic cumulus cells.

methods was not significant. There was no difference in the percentage of type III and type IV oocytes recovered by dissection and puncture; however, a significantly higher ($P < 0.05$) percentage of type III oocytes (36.63%) and a significantly lower ($P < 0.05$) percentage of type IV oocytes (7.16%) were recovered by the aspiration method.

The effect of season on the recovery rate of follicular oocytes is given in Table 2. In total, 344 ovaries were obtained during the peak breeding season and yielded 845 oocytes at the rate of 2.45 ± 0.91 oocytes per ovary, whereas the 308 ovaries obtained during the low breeding season yielded 602 oocytes at the rate of 1.93 ± 0.68 oocytes per ovary. The oocyte recovery rate per ovary was significantly higher ($P < 0.05$) during the peak breeding season compared to the low breeding season. Ovaries during the peak breeding season yielded significantly ($P < 0.05$) more type I oocytes (37.39%) than ovaries during the low breeding season (27.74%). However, significantly more type II oocytes were recovered during the low breeding season (33.00%) compared to the peak breeding season (28.75%). There was no significant effect of breeding season recorded for type III and type IV oocytes.

A significantly ($P < 0.05$) greater number of oocytes (2.29 ± 0.86) per ovary were recovered from ovaries without a corpus luteum than from ovaries with a corpus luteum (1.99 ± 0.82) (Table 3). In total, 272 usable oocytes were recovered from the ovaries without a corpus luteum (1.47 ± 0.54 oocytes per ovary), whereas 156 usable oocytes were recovered from ovaries with a corpus luteum (1.13 ± 0.45 oocytes per ovary). This rate of per ovary oocyte recovery was based on type I and type II oocytes, and the difference in the recovery rates between the 2 groups of ovaries was significant ($P < 0.05$). Detailed study of oocyte quality indicated that a significantly higher percentage of type I oocytes (35.15%) were recovered from the ovaries without a corpus luteum compared to the ovaries with a corpus luteum (23.02%).

Table 2. The effect of breeding season on the recovery rate and quality of buffalo follicular oocytes.

Parameters	Peak breeding season (Oct., Nov., Dec.)	Low breeding season (Mar., Apr., May)
Number of ovaries	344	308
Total oocytes recovered	845	602
Oocyte recovered/ovary		
Mean ± SE	2.45 ± 0.91[a]	1.93 ± 0.68[b]
Total usable oocytes recovered	559	366
Usable oocytes recovered / ovary (type I + type II)		
Mean ± SE	1.62 ± 0.56[a]	1.18 ± 0.45[b]
Type I	37.39%[a]	27.74%[b]
(n)	(316)	(167)
Type II	28.75%[b]	33.00%[a]
(n)	(243)	(199)
Type III	8.63%[a]	11.62%[a]
(n)	(73)	(70)
Type IV	25.20%[a]	28.07%[a]
(n)	(213)	(169)

The values with different superscripts in the same row differ significantly ($P < 0.05$)
Type I: Oocytes surrounded by 4 or more layers of compact cumulus.
Type II: Oocytes surrounded by 1-3 layers of compact cumulus.
Type III: Nude oocyte without any cumulus.
Type IV: Oocyte surrounded by a cumulus that had undergone some degree of expansion and, in some cases, with pyknotic cumulus cells.

Table 3. The effect of the presence or absence of a corpus luteum on the recovery rate and quality of buffalo follicular oocytes.

Parameters	Corpus luteum	
	Present	Absent
Number of ovaries	138	183
Total oocytes recovered	275	421
Oocyte recovered/ovary		
Mean ± SE	1.99 ± 0.82[b]	2.29 ± 0.86[a]
Total usable oocytes recovered	156	272
Usable oocytes recovered/ovary (type I + type II)		
Mean ± SE	1.13 ± 0.45[b]	1.47 ± 0.54[a]
Type I	23.02%[b]	35.15%[a]
(n)	(67)	(148)
Type II	32.36%[a]	26.36%[b]
(n)	(89)	(111)
Type III	12.71%[a]	11.04%[a]
(n)	(37)	(48)
Type IV	29.81%[a]	23.99%[b]
(n)	(82)	(101)

The values with different superscripts in the same row differ significantly ($P < 0.05$).
Type I: Oocytes surrounded by 4 or more layers of compact cumulus.
Type II: Oocytes surrounded by 1-3 layers of compact cumulus.
Type III: Nude oocyte without any cumulus.
Type IV: Oocyte surrounded by a cumulus that had undergone some degree of expansion and, in some cases, with pyknotic cumulus cells.

Discussion

The quantity and the quality of oocytes recovered per ovary are important considerations in the production of IVM-IVF embryos. Proper oocyte recovery and their selection in the laboratory are vital for successful embryo production. The presence of an intact complement of cumulus cells surrounding the oocyte and homogeneous appearing ooplasm have been considered the best criteria for the selection of oocytes most likely to undergo maturation and embryonic development. The competence of buffalo oocytes is influenced by a wide array of biological (9) and environmental (10) factors. Some influencing factors, i.e. recovery method, season, size, and stage of ovarian cycle on quantity and quality of buffalo follicular oocytes were investigated in the present study.

The present study demonstrated that dissection of the ovarian surface follicle is a convenient and effective method for collecting a high yield of buffalo follicular oocytes for in vitro culture. Similar findings have been reported for cattle (11), goats (12), and sheep (13). The number of oocytes per ovary recovered by dissection was lower in this study than that reported in cattle (11). This discrepancy may be attributed to the considerably smaller number of primordial follicle reserves in buffalo ovaries than in those of cattle. Buffalo ovaries have a fewer follicles (14) and as a result, fewer follicles are recruited in each cycle (15), contributing to the lower recovery of good quality oocytes (16). In comparison to a stock of 50,000 primordial follicles in cattle ovaries at the time of puberty (17), the number of these follicles in buffaloes has been reported to be only 12,000-19,000 (18). Moreover, anestrous associated with ovarian acyclicity is quite common in the buffalo and there are fewer

primordial follicles in the ovaries of non-cyclic buffaloes than in cyclic buffaloes. Poor recruitment of primordial follicles into growing and Graafian follicles, and more atresia in acyclic buffalo ovaries might be another cause for the low number of oocytes obtained in the present study. The number of oocytes recovered per ovary with aspiration and puncture methods was lower in the present study than that previously reported in cattle (19). In fact, the aspiration and puncture techniques mainly approach the large follicles, leaving small-embedded follicles inaccessible, which could be the cause for poor oocyte yield with these methods. The superiority of the dissection method over that of aspiration and puncture for recovering oocytes in the present study is in agreement with the results obtained by Sharma (20), and Datta and Goswami (21). The average number of oocytes recovered per ovary in the present study (2.31) was higher than the 0.73 reported by Totey et al. (5) and 0.96 reported by Datta and Goswami (21). The 1.48 usable oocytes recovered per ovary in the present study exceeded the 0.16, 0.85, and 0.42 reported by Datta and Goswami (21), Singh and Majumdar (15), and Madan et al. (2), respectively. This difference could be due to the selection criteria used for categorizing the oocytes in different studies. In conclusion, the dissection method was found to be a rather convenient technique for the reasonable retrieval of total and usable buffalo follicular oocytes.

Ovary collection times were classified into 2 breeding seasons, i.e. peak breeding season (September, October, and November) and low breeding season (March, April, and May). The recovery of oocytes was significantly higher ($P < 0.05$) during the peak breeding season than during the low breeding season. A similar trend was observed with the recovery of usable oocytes. Bovine reproduction has a complex dependency on soil, plant, and climatic factors (22), particularly in tropical and subtropical parts of the world. Although buffaloes are polyestrous, they exhibit a distinct seasonal variation in breeding activity. Results of other studies performed in Pakistan are consistent with those performed in India; about 64%-75% of buffalo exhibited estrus between September and December (23). It has been observed that the maximum number of services occurred during the fall season (September, October, and November), followed by the winter season (December, January, and February) (24). The seasonal effect on the incidence of estrus was very significant ($P < 0.05$). The poor recovery of oocytes during the low breeding season was probably due to the relatively inactive status of ovaries (25) that often renders buffalo as seasonal breeders (26). The results of the present study revealed that a significantly higher ($P < 0.05$) oocyte recovery rate per ovary was obtained from the ovaries collected during the peak breeding season.

In the present study a significantly greater number of oocytes per ovary were recovered from ovaries without a corpus luteum than from ovaries with a corpus luteum. According to Nandi et al. (9) the oocyte recovery rate decreased when ovaries had a corpus luteum. This is because follicular development is restricted, as lutein cells occupy most of the ovary (27). The dominant follicle is usually observed in the corpus luteum-bearing ovary, and the other follicles are very small and remain mostly inaccessible (28). Cow (29) and goat (30) ovaries containing a corpus luteum yielded a lower number of oocytes than ovaries without a corpus luteum. Several researchers have reported that the presence of a corpus luteum yields a lower number of oocytes per ovary and a lower proportion of usable oocytes (29). In contrast, Boediono et al. (19) and Das et al. (4) found no difference in the mean number of oocytes per ovary between corpus luteum-bearing and non-bearing ovaries. In conclusion it was determined that the method of oocyte collection, season, and ovarian status at the time of oocyte collection significantly affects the recovery of usable oocytes in buffalo for use in IVF programs.

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