Qualitative and quantitative analysis of buffalo ovaries, follicles and oocytes in view of *in vitro* production of embryos

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Abstract

This study was conducted to evaluate the buffalo ovaries, follicles and cumulus-oocyte-complexes (COCs) with the view of *in vitro* production (IVP) of embryos. Buffalo (*Bubalis bubalis*) ovaries were collected from local slaughterhouse and categorized according to the position of ovaries in the genital tract (left vs. right) and presence or absence of corpus luteum (CL). Moreover, collected ovaries were also evaluated for length, width and weight, number of follicles, aspirated follicles and state of COCs. The length (cm) of right ovary (2.32±0.06) was significantly (P<0.05) higher than left ones (2.14±0.05). Number of follicles were higher (P<0.05) in the left (7.25±0.31) compare to the right (6.22±0.32) ovaries. Other parameters, including width, weight and number of COC aspirated did not differ significantly (P<0.05) between right and left ovaries. The length, width and weight of ovaries with CL were higher (P<0.05) whereas, number of observed follicles, aspirated follicles, number of COCs and number of normal COCs were significantly (P<0.05) higher in ovaries without CL than their counter parts.

Key words: Buffalo, Corpus luteum, Follicles, Cumulus-oocyte-complexes

 Bang. J. Anim. Sci. 2011. 409 (1-2): 23-27

Introduction

Buffalo (*Bubalis bubalis*) is an important part of agricultural economy of Bangladesh and it plays a significant role in livestock production in Bangladesh (Huque et al. 2010). Reproductive efficiency is the primary factor affecting productivity and is hampered in female buffalo by (1) inherent late maturity, (2) poor estrous expression in summer, (3) distinct seasonal reproductive patterns and (4) prolonged calving intervals. Reproductive efficiency can be improved by introducing embryos produced *in vitro* (Raza et al., 2001). Oocytes are the main raw materials for in vitro embryo production (IVP) experiments. So, slaughter house ovaries can be an economic source for the collection of oocytes. Oocyte quality has long been considered as a main limiting factor for IVP. Therefore, the success of any IVP program in buffalo production, either in vitro fertilization (IVF) or in vitro culture (IVC) of embryos largely depends on the continuous supply of quality oocytes in optimum quantity. The oocytes from ovaries without CL had greater developmental competence than ovaries with CL (Tasripoo et al. 2005). Jamil et al. (2008) reported that a significantly higher oocyte recovery rate was obtained from the ovaries of buffalo collected during the peak-breeding season and from those without CL. In addition, Warriach and Chohan (2004) observed that the oocytes of buffalo with ≥3 layers of cumulus cells showed higher maturation rates than oocytes with partial or no cumulus cells and oocytes co-cultured with cumulus cells but did not differ from oocytes having 1-2 layers of cumulus cells. The degeneration rates were higher for oocytes with partial or no cumulus cells than those surrounded by healthy cumulus cell layers. This result reveals that buffalo oocytes with intact layers of cumulus cells show better IVM rates than oocytes without cumulus cells and the co-culture of poor quality oocytes with cumulus cells improves their meiotic competence. In Bangladesh, some research works on *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture (IVC) of embryos of mouse, cattle and goat have already been performed. For successful *in vitro* production (IVP) of buffalo embryos, the evaluation of ovaries, the efficient collection and grading of oocytes is important. From this point

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of view, this study was undertaken for the collection and evaluation of slaughter house ovaries, follicles and COCs with the view of IVP of embryos.

Materials and Methods

Collection and Processing of ovaries

Buffalo ovaries of unknown reproductive history were collected from local slaughterhouse. The ovaries were kept in collection vial containing 0.9% physiological saline (5 lac iu of penicillin and 100 mg of streptomycin were added per liter of saline on the day of collection) in a thermo flask at 25–30^{o}C and transported to the laboratory within 4-5 hours. The ovaries were then transferred in the sterilized petridishes containing same saline and rinsed thoroughly by physiological solution at room temperature and marked as right and left ovary (right and left ovary was tagged during slaughter with the help of butcher). The presence or absence of corpus luteum (CL) was also recorded.

Evaluation of ovary

Measurement of length, width and weight

The length and width of ovaries (right and left ovaries; ovaries with CL and ovaries without CL) were measured with the help of a slide calipers and expressed in cm (Figure 1). Weight of individual ovary was measured by placing them on a digital balance and was recorded in a tabular form.

Follicle counting on the surface of the ovary

There are many follicles on the surface of both ovaries. The number of visible follicles on the surface of different category of ovaries were counted and recorded accordingly.

COCs aspiration and grading

The ovaries were washed 2-3 times in saline solution at room temperature. They were then placed in a beaker and kept in a water bath at 30^{0}C. One ovary was picked up in hand. The 10 ml syringe was loaded with 1.0-1.5ml of PBS (Sigma, USA) and the needle (18G) was put in the ovary parenchyma near the vesicular follicles of 2-6 mm diameter and all follicles were aspirated near the point. After aspirating the follicles from one ovary, the aspirated follicular materials were transferred slowly into a 90-mm petridish, avoiding damage to the cumulus cells and the COCs were searched and graded under microscope at low magnification. The COCs were then classified into 4 grades (Figure 2) according to the slight modification of the method of Khandoker et al. (2001), where grade A: oocytes completely surrounded by cumulus cells; grade B: oocytes partially surrounded by cumulus cells, grade C: oocytes not surrounded by cumulus cells and grade D: degeneration observed both in oocytes and cumulus cells. Grade A and B were considered as normal COCs and grade C and D as abnormal. In the meantime another petridish of D-PBS was prepared for pooling COCs and the COCs were picked up with an appropriate glass micropipette. The tip diameter of the pipette was checked under the microscope to ensure COCs, which could be easily aspirated without damaging the cumulus cells. Then the COCs were washed 2-3 times into D-PBS.

Statistical analysis

Results are expressed as mean ± SD and $p<0.05$ was considered to be statistically significant unless otherwise stated. Data were analyzed using one way analyses of variance (ANOVA) with the SPSS program (Version 11.5; SPSS Inc., Chicago, IL, USA).

Results and Discussion

Among 136 ovaries (consisting 68 on each side) a number of 93 belonged without CL and others with CL. Among 136 ovaries, CL was present on 24 of right ovaries and 19 of left ovaries. The results of the different parameters are summarized in Table 1 and Table 2.

The length (cm) of right ovary (2.32±0.06) was significantly ($p<0.05$) higher than the left (2.14±0.05) ones but no significant ($p<0.05$) differences were found in the width (cm) and weight (g) of left (1.63±0.06 and 2.87±0.32) and right ovaries (1.60±0.05 and 3.59±0.31), respectively (Table 1). Similar results were found in goat (Islam et al. 2007). Normal physiological explanation of ovarian activity is that right ovaries are more active than left ones (Rahman et al. 1977 and Sarker 1993). On the other hand, the length, width and weight were significantly ($p<0.05$) higher in ovaries without CL than those of with ovaries with CL(Table 2). The CL is an extra cellular material within the ovary which made the differences of its length, width and weight (Jablonka-Sharif et al. 1993).

Table 1. Dynamics (mean±SE) in buffalo right and left ovaries

Classification (No. ovaries)	Length (cm)	Width (cm)	Weight (g)	No. follicles	No. follicles aspirated	Collected COCs per ovary		
						Normal	Abnormal	Total
Left (68)	2.14^{b}±0.05	1.63±0.06	2.87±0.32	7.25^{a}±0.31	6.19±0.24	1.54±0.12	0.88±0.10	2.42±0.14
Right (68)	2.32^{a}±0.06	1.60±0.05	3.59±0.31	6.22^{b}±0.32	5.66±0.25	1.33±0.11	0.98±0.11	2.32±0.12

Values in the parentheses indicate the number of observation; Means with different superscripts within the column differ significantly (P<0.05)

Table 2. Dynamics (mean±SE) in buffalo ovaries in presence or absence of corpus lutetium (CL)

Class (No. ovaries)	Length (cm)	Width (cm)	Weight (g)	No. follicles	No. follicle aspirated	Collected COCs per ovary		
						Normal	Abnormal	Total
Without CL (93)	2.15^{b}±0.04	1.56^{b}±0.04	2.73^{b}±0.12	7.84^{a}±0.21	6.78^{a}±0.18	1.71^{a}±0.08	0.92^{a}±0.09	2.63^{a}±0.12
With CL (43)	2.38^{a}±0.06	1.73^{a}±0.06	3.64^{a}±0.18	4.35^{b}±0.31	4.09^{b}±0.26	0.86^{b}±0.12	0.95^{a}±0.13	1.81^{b}±0.17

Values in the parentheses indicate the number of observation; Means with different superscripts within the column differ significantly (P<0.05)

A number of 916 follicles were recorded on the surface of the ovaries and 806 follicles were aspirated from the surface of both (right and left) ovaries and among them 385 were obtained with a mean of 5.66±0.25 per ovary from right and 421 from left ovaries with a mean of 6.19±0.24 per ovary (Table 1). The collected COCs were higher in left ovaries (2.42±0.14 per ovary) compared to right ovaries (2.32±0.12 per ovary]. When the COCs were classified as normal and abnormal groups, the highest numbers of normal COCs were found in left than that of right ovary, which supports the previous result of Islam et al. (2007) performed in goat.

In other cases, from a total of 806 aspirated follicles, 630 were obtained from ovaries without CL (Follicular phase) and 176 from ovaries with CL (Luteal phase). The significantly higher ($p<0.05$) number of follicles were aspirated per ovary in ovaries without CL (6.78±0.18) than in CL containing ovaries (4.09±0.26) (Table 2). The causes of higher number of follicles found in ovaries with CL than those of CL containing group were understood well as it fits the endocrinological explanation. It is known that all female mammals are born with a large number of follicles which rapidly decline as puberty approaches; but whether this early losses represent a mechanism of physiological wastage or not, is not definitely known. Follicle growth initiation is one of the most important and least understood aspects of ovarian biology and represents a major challenge for experimental study. Changes in the local micro environment (i.e. pH, hormonal concentration, etc.) might occur during evolving of primary follicular stage may affect follicular dynamics and quality in the ovaries

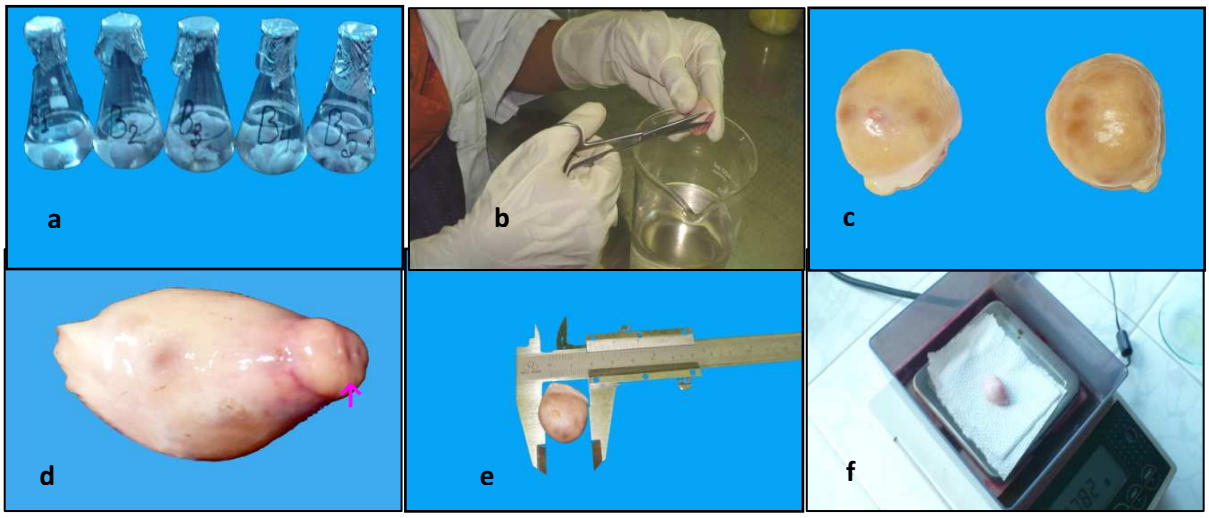

Figure 1. *a) collection of buffalo ovaries in physiological saline b) trimming of ovary c) ovaries without corpus luteum d) ovary with corpus luteum (arrow indicates the CL) e) measuring the length (cm) and diameter (cm) of the ovary f) weighing of the ovary*

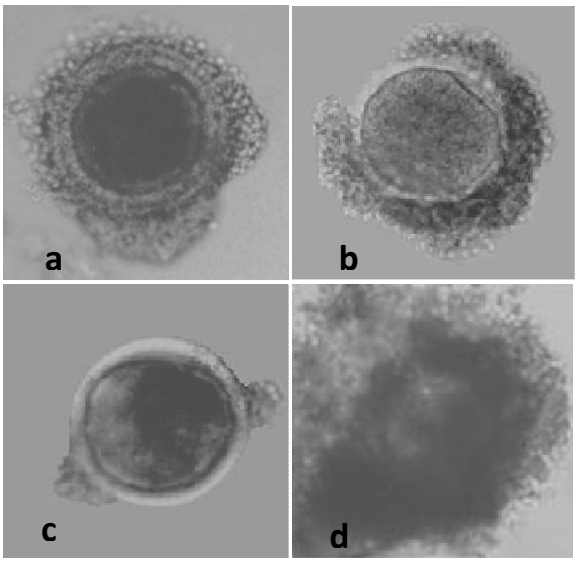

Figure 2. *Representative photograph showing different grades of cumulus-oocyte-complexes (COCs). a) Grade-A COCs are completely surrounded by cumulus cells; b) Grade-B COCs are partially denuded; c) Grade-C oocytes are mostly denuded and d) Grade-D COCs are surrounded by expanded cumulus cells.*

Higher numbers of COC were found in ovaries without CL (2.63±0.12) than ovaries with CL (1.81±0.17). Again, when the COCs were classified in normal and abnormal groups, the significantly higher ($P<0.05$) number of normal COCs was found in ovaries without CL than those ovaries with CL with the mean of 1.71±0.08 and 0.86±0.12 follicles per ovary respectively and the reverse trend was found in abnormal group (0.92±0.09 and 0.95±0.13 follicles per ovary respectively). The follicular growth is inhibited while atresia is increased in presence of CL in the ovary (Hafez, 1993). These might be the physiological explanation for lower numbers of COC in the ovaries having CL. In other way, due to the absence of CL, the negative effect of progesterone on anterior pituitary might not be functional in this ovary. So, the highest number of COCs in this category other than CL functional group explains the role of hormonal balance on buffalo folliculogenesis. Similarly, the higher number of normal COCs in without CL ovaries may be due to the hormonal effect of CL. This result supports the previous report of Hafez (1993) on the role of progesterone on buffalo follicular degeneration.

The female buffalo destined slaughtering were usually less reproductive performer and most of them might be non-cyclic. So, there had been the possibility to get more non-cyclic ovaries from the slaughterhouse during random sampling.

Conclusion

The present findings revealed that ovaries without corpus luteum have higher number and higher grade cumulus-oocyte-complexes than those with corpus luteum.

Acknowledgement

We gratefully acknowledge the Ministry of Science and Information and Communication Technology (MOISCT) for financial support to do this work.

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