Research Article

STUDY ON In vitro MATURATION OF LOCAL BUFFALO OOCYTES

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Abstract

The present work was conducted to study the in vitro maturation buffalo follicular oocytes. Buffalo ovaries were collected from local abattoir within four hours of slaughter and transported immediately to the laboratory. Follicular oocytes were recovered by aspiration methods. Quality oocytes ovary as it yielded higher number of good quality oocytes 34, 16, 18 oocytes/ovary, type A, B and C. There was a significant higher (P˂ 0.05) number of grade A (34) oocytes comparative with grade B (16) and C (18) oocytes. Experimental maturation media revealed that tissue culture medium - 199 (TCM-199) resulted in significantly better maturation rate (44). The present study concluded the practical, economical and scientific value of establishing a biological bank for oocytes. The possibility of obtaining collection in all grade oocytes by aspiration method from samples slaughterhouse and the possibility maturation oocytes in laboratory.

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1. Introduction

Bubalis bubalis play a prominent role in rural livestock production, particularly in Iraqi contributing high quality animal protein, both milk and meat. However, the production of milk in buffalo is lower than its potential (Bilal et al., 2006). Reproductive efficiency is the primary factor affecting productivity and is hampered in female buffalo by (i) Inherent late maturity, (ii) Poor estrous expression in summer, (iii) Distinct seasonal reproductive patterns and (iv) Prolonged intercalving intervals (Barakat et al., 2012). Reproductive efficiency can be improved by introducing embryos produced in vitro using the fruits of now matured artificial insemination industry and many oocytes being wasted in the slaughterhouse. It is remarkable that viable embryos can be produced from ovarian oocytes collected hours after death of animals at the abattoir. Oocytes may be matured cultured in vitro (Das et al., 1996). Therefore, securing a plentiful and economical source of zygotes is central to capitalizing in buffalo on many new genetic technologies currently available in both research and commercial settings. Very little work has been done previously on the development of an in vitro system for buffalo (Totey et al., 1992). In vitro maturation (IVM) procedure performed on oocytes obtained from slaughter -house derived ovaries have recently provided a practical means for producing large number of bovine zygotes at

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low cost for research and commercial settings (Hansen, 2006).

2. Materials and Methods

Collection of the ovaries

Fifty ovaries from sexually mature buffaloes were collected within 30 minutes after slaughter in the abattoir, 12 ovaries was neglected because ovarian disease. They were transported within 2 hrs of slaughterhouse to the laboratory in physiological normal saline (0.9 % NaCl) containing antibiotics (100 μg/ml streptomycin sulfate and 100 IU/ml penicillin) and preserved in cool boxes at 4 - 8 °C Upon arrival at the laboratory foreign tissue was removed from the ovaries. Then, the ovaries were washed with 70 % ethanol to control contamination, rinsed 3 times in normal saline (0.9 % NaCl), and finally dried with sterilized paper towels (Warriach and Chohan, 2004).

Harvesting and Evaluation of Follicular Oocytes

Oocytes were collected by aspiration method 50 ovaries use. Oocytes were aspirated from 5 to 18 mm follicles using a different size - gauge needle attached to a 5 ml sterile syringe containing 2 ml media with 100 μg/ml streptomycin sulfate, 100 IU/ml penicillin and 100 IU/ml Nystatin in a glass petridishes. Oocytes were searched using a stereo zoom microscope. Furthermore, Oocytes were picked up with micropipette under stereomicroscope and transferred into another dish containing media Khandoker et al. (2001). The oocytes with intact layers of cumulus cells and homogenous cytoplasm were selected for the study, as shown in Figure - 1 (Warriach and Chohan, 2004).

Figure - 1: A) follicle measurement and B) Follicle aspiration

The COCs were graded according to the morphology of the cumulus cells and nucleus, COCs were classified into 4 grades as described by Nandi et al. (2003). They are: Grade - I: Compact cumulus oocytes complexes with unexpanded cumulus mass having ≥ 5 layers of cumulus cells and homogenous evenly granular ooplasm as Figure – 2 A. Grade - II: COCs similar to Grade - I but with 2 - 4 layers of cumulus cells as Figure – 2 B. Grade - III: Oocytes with partially denuded or completely devoid of cumulus cells and having an irregular dark cytoplasm as Figure - 2C. Grade - IV: Oocytes with highly expanded or scattered cumulus cells and an irregular dark ooplasm. The oocytes of Grades I and II were used for in vitro maturation.
Figure - 2: A grade (A) Oocyte, B grade (B) Oocyte, C grade (C) Oocyte (10 X)

Oocytes Maturation (IVM)

Ten ml of Maturation medium with Supplement was equilibrated for two hours in CO₂ incubator before oocytes added (Jamil et al., 2007). Ninety oocytes recovery, all Oocytes must be rinsed 2 - 3 times in same Maturation media with 100 IU/ml penicillin, 100 mg/ml streptomycin and 100 IU/ml. Nystatin before oocytes added in Maturation medium, Maturation media used with supplemented containing 100 IU/Ml Penicillin, 100 mg/ml Streptomycin and 100 IU/ml Nystatin. The culture dishes were placed in a CO₂ incubator (95 % relative humidity, 5 % CO₂ at 38.5 °C) for 24 hrs. Maturation of the oocytes was evaluated after 24 - 28 hrs of culture to access the degree of cumulus cell expansion under a stereo zoom microscope and also the appearance first polar body as good indicator for maturation oocyte, number of matured oocytes were counted and recorded using the methods described by Kobayashi et al. (1994). The Figure - 3 shows the first polar body.

Figure - 3: Matured oocyte with first polar body

3. Results

Recovery of Buffalo Follicular Oocytes

A total of 68 oocytes were recovered by aspiration of follicles from 50 ovaries obtained from an abattoir is given in Table - 1. Oocytes were categorized into three different grades (A, B and C) according to their cumulus investment and ooplasm homogeneity. Higher number of good quality oocytes 34, 16, 18 oocytes or ovary, type A, B and C. There were a significant higher (P˂ 0.05) number of grade A (34) oocytes comparative with grade B (16) and C (18) oocytes.

Effect of TCM -199 on IVM of Oocytes

The IVM rates of oocytes were 44 % for TCM - 199. These media significantly (P < 0.01) for IVM. As addition of TCM - 199 higher maturation rates was assessed according on the cumulus expansion and the appearance first polar body. There was a significant difference (P < 0.01) between number of culture and maturation oocytes (Table - 2).
Table - 1: Influence of recovery method on quality of buffalo follicular oocytes types obtained for in vitro maturation

<table>
<thead>
<tr>
<th>Number of Ovary</th>
<th>Number of oocytes collection</th>
<th>Oocytes surrounded completely by cumulus cell</th>
<th>Oocytes surrounded partially by cumulus cell</th>
<th>Oocytes no surrounded by cumulus cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>68</td>
<td>34</td>
<td>16</td>
<td>18</td>
</tr>
</tbody>
</table>

X² = 8.59, df = 2, significant p<0.05

Table - 2: The effect TCM - 199 on buffalo follicular oocytes types obtained for in vitro maturation

<table>
<thead>
<tr>
<th>Maturation media</th>
<th>Number of ovary</th>
<th>Oocytes incubated</th>
<th>Oocytes matured</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCM-199</td>
<td>50</td>
<td>50</td>
<td>22</td>
<td>44 %</td>
</tr>
</tbody>
</table>

X² = 10.89, df = 1, significant p<0.01

4. Discussion

The quantity and the quality of oocytes recovered per ovary are important considerations in the production of IVM-IVF embryos. The overall recovery of good quality COCs with the method in the present study was 1.36 COCs/ovary this result which agrees with different studies have reported 0.4 - 3.85 good COCs/ovary in buffalo (Jainudeen et al., 1993). The influence of season on oocyte quality has been largely considered, as a factor of high impact. We have studied the effect of seasonality on the quality of oocytes and consequently their maturation rate in buffaloes. Our results showed that there are significant differences between the four seasons that consequently affected the quality and the yield of oocytes. This variation is due to the different methods used for COC recovery, seasonal effects, and variation in the reproductive status of the slaughtered buffaloes. However, contributing to the lower recovery of good quality oocytes (Totey et al.,1993). In comparison to a stock of 50,000 primordial follicles in cattle ovaries at the time of puberty (Donald, 1975).

The number of these follicles in buffaloes has been reported to be only 12,000 - 19,000 Danell (1987) as compared to temperate cattle (10 COCs/ovary) (Das et al.,1996). The number of good COCs/ovary in buffalo is lower, which may be due to an inherently smaller number of primordial follicles and a higher frequency of atresia in buffalo (Madan et al.,1994). The present study shows that the low number COC recovery rate from slaughterhouse ovaries by aspiration method, which agrees with earlier findings reporting greater numbers of COCs/ovary with the slicing method than with aspiration (Singh and Majumdar, 1992). Lower COC recovery via the aspiration method might have been because oocytes were recovered from selected follicles (2 - 3 mm) on the ovarian surface and were limited in number. Jainudeen et al. (1993) reported that oocytes were recovered via aspiration from 55 % of follicles, as compared to the slicing method, which recovered oocytes from 78 % of follicles from buffalo ovaries. It was observed in the present study that the percent of maturation (44 %) in maturated media. Buffalo oocytes showed significant variation (p<0.01) between the number of culture oocytes and the number of matured oocytes in TCM - 199 this result which an agreement with Yosef et al. (2013) who reported the percentage values (89.1 ± 3.5 %) of maturation buffalo oocytes in TCM-199 medium.

Fukuda et al. (1990) reported 74 per cent maturation rate for bovine oocytes cultured in TCM-199 supplemented with 10 % bovine estrus serum (BES). Totey et al. (1993) also reported a maturation rate of 76 % when oocytes were matured in TCM-199 supplemented with hormones and BES. These results are
disagreement with Waheed et al. (2016) reported percentage values (74.82%) of maturation buffalo oocyte in TCM-199 medium. Previous studies on buffalo oocytes maturation in vitro have shown TCM-199 to be better over Hams F-10 (Hammam et al., 2010). The beneficial effect of TCM-199 on IVM may be related to some factors in its composition such as essential amino acids and glutamine that stimulate DNA and RNA synthesis and enhance cell division (Mahmoud and El-Naby, 2013). Therefore, development of a suitable culture system for IVM of oocytes is a major component of in vitro embryo production procedures. In this study, when media supplementation (TCM-199), a significant difference for maturation rate was media. Moreover, the addition of serum and hormones to TCM-199 was for supporting maturation of buffalo follicular oocytes. Oocytes matured in TCM-199 with serum and hormones had a significantly higher (P < 0.05) maturation rate have been used for in vitro maturation of follicular oocytes. However, higher maturation rates in TCM-199 have been reported previously (Pawshe et al., 1996; Smetanina et al., 2000).

5. References


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