Effect of recovery technique and culture media on in vitro maturation of indigenous cattle oocytes of Assam

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ABSTRACT
The study was undertaken to see the effect of recovery techniques and culture media on in-vitro maturation (IVM) of oocytes indigenous cattle of Assam. Oocytes were collected by aspiration and dissection techniques and were graded as A, B, C and D on the basis of their cumulus cell layers surrounding the zona pellucida. Oocytes with A, B and C categories were subjected to IVM using three different media (TCM-199, Ham’s F-12 and Ham’s F-10) with same additives (Sodium pyruvate, Gentamicin, L-glutamine, Porcine FSH, Follicular Fluid and HIOCS). Percentage of oocytes with expansion of cumulus cells was found to be the highest in dissection technique using TCM-199 media in comparison to Ham’s F-12 and Ham’s F-10 using same additives. However, it did not differ significantly between techniques. Incidence of Metaphase-I + Metaphase-II was the highest in oocytes recovered by dissection technique and cultured in TCM-199 with additives when incubated at 38.5°C for 24 hours and it differed significantly between media but there was no significant difference between the recovery techniques. In conclusion, the culture medium TCM-199 along with additives may give better IVM rate of the oocytes recovered by dissection technique from indigenous cattle ovaries of Assam.

Key words: Cattle oocytes, Culture media, In-vitro maturation, Recovery technique.

INTRODUCTION
IVM of oocytes directly influences the overall success and output of assisted reproductive techniques since it allows utilization of active ovaries from slaughtered animals for production of viable embryos from superior breeds. Several works on IVM of cattle oocytes are in record still there is scope of improvement in the area of oocyte maturation using different culture media (Ocana-Quero et al. 1999) in combination with different recovery techniques (Alm et al. 2008). However, very few works have been carried out on IVM of oocytes in indigenous cattle of Assam. Hence, the present study was undertaken with an objective to evaluate the effect of different culture media on IVM of oocytes using different recovery techniques in indigenous cattle of Assam.

MATERIALS AND METHODS
Ovaries from the slaughtered indigenous cattle of Assam were collected from local abattoirs and were transported to the laboratory in a thermosflask containing sterile normal saline with 0.06g Penicillin G per 100 ml at 26–37°C within 2-3 hours of slaughter. In the laboratory the extraneous tissue were removed and ovaries were washed 3-4 times with normal saline containing antibiotic for further processing. For collection of oocytes, TCM-199, Ham’s F-10 and Ham’s F-12 media were used separately in conjugation with BSA (0.0375g), Gentamicin (50µl) and L-glutamine (0.001g) per 10 ml of medium. Media were incubated in carbon-di-oxide (CO₂) incubator for 2 hours at 38.5°C at 5 per cent level of CO₂ with 90-95 per cent humidity before use and were filtered through 0.2µm filter. The cumulus oocyte complexes (COCs) were recovered from the ovarian follicles by using both aspiration and dissection techniques (Alm et al. 2008). Oocytes with A, B and C categories were placed in micro drop at the rate of 10 oocytes per 50 µl of the three different maturation media, TCM-199, Ham’s F-12 and Ham’s F-10 containing same additives (Sodium pyruvate: 0.0009g/ml, Gentamicin: 50µg/ml, L-glutamine: 0.0001g/ml, Porcine FSH: 0.05µg/ml, Follicular Fluid: 1ml/10ml and HIOCS 1ml/10ml, in all the media) which had been equilibrated at 38.5°C in a CO₂ incubator. The oocytes in the droplets were covered with sterile mineral oil and cultured in humidified atmosphere of 5% CO₂ in air at 38.5°C for 24 hours in a CO₂ incubator.

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Following incubation oocytes were examined under stereozoom microscope to observe the expansion of cumulus cells and was made nude and fixed in acetic alcohol (1:3) and stained with 1 per cent lacto-acetic lacmoid stain as per Hussain et al. (2012). The status of IVM of oocytes was then examined at a magnification of 100x and 200x under phase contrast microscope.

Statistical analyses were done using SPSS (Statistical Package for Social sciences) version 16.0.

RESULTS AND DISCUSSION

A total of 702 oocytes were obtained by aspiration (440) and dissection (262) techniques out of which 405 oocytes (aspiration: 204 and dissection: 200) were placed in three different maturation media to evaluate the IVM rates.

The incidence of oocytes with expansion of cumulus cells was found to be the highest in TCM-199 with additives in aspiration and dissection technique and the lowest in Ham’s F-10 using same additives in aspiration and dissection technique (Table 1). The incidence of oocytes with expansion of cumulus cells recorded in the present study both in aspiration and dissection techniques were found higher in comparison to Chauhan et al. (1999) using maturation medium TCM-199 + 10% FBS + EGF 20 ng/ml (64.00%). This may be due to culture of bovine COCs in the presence of growth hormone accelerated the process of cumulus cells expansion (Izadyar et al. 1996). Izadyar et al. (1997) observed that incorporation of 0.05 IU/ml FSH might cause marked expansion of the cumulus cells during IVM of COCs for 24 hours. In the present study, the percentage of oocytes with expanded cumulus cells varied significantly between different media with same additives but there was no significant difference between the techniques (Table 1). The variation in the percentage of oocytes with expansion of cumulus cells in different media might be ascribed to the variation of basic composition in the culture media that were used during IVM of the oocytes.

The rate of Metaphase-I was also found to be the highest in aspiration and dissection technique using TCM-199 and additive medium and lowest in aspiration and dissection technique in Ham’s F-10 using same additives(Table 2). In the present study the incidence of Metaphase-I differed significantly among the media (Table 2) which could be due to difference in efficacy of the media for IVM of oocytes. On the other hand, Barua (1995) observed that the incidence of Metaphase-I did not differ significantly between TCM-199, Ham’s F-12 and m-KRB media. The variation in the incidence of Metaphase-I in different media in the present study might be again due to the difference in basic composition of the media that were used during IVM of oocytes, although using the same additives.

The incidence rate of Metaphase-II was again found to be the highest in TCM-199 medium supplemented with additives following aspiration or dissection technique while the lowest rate in Ham’s F-10 medium supplemented with additives following aspiration or dissection technique (Table 2). The incidence of Metaphase-II recorded in the present study both in aspiration and dissection technique was found lower than that recorded by Martins et al. (2005) in TCM-199 used in combination with 10 per cent OCS and 10ng/ml FSH (74.60%). In the present study, the incidence of Metaphase-II did not differ significantly among the media (Table 2). Barua (1995) also made similar observations. On the other hand, Lorenzo et al. (1993) reported that the incidence of Metaphase-II differed significantly among the media. The discrepancy in the incidence rate of Metaphase-II could be attributed to the difference in composition of basic media that were used during IVM of oocytes as well as in the duration and temperature of incubation.

**TABLE 1: Expansion of cumulus cells of cattle oocytes recovered by aspiration and dissection techniques on in-vitro maturation in different media**

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>Aspiration</th>
<th>Dissection</th>
<th>Chi-square value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of oocytes incubated</td>
<td>Number of cumulus cells showed expansion</td>
<td>Rate of expansion of cumulus cells (%)</td>
</tr>
<tr>
<td>TCM199 + additives</td>
<td>84</td>
<td>73</td>
<td>86.90</td>
</tr>
<tr>
<td>Ham’s F-12 + additives</td>
<td>60</td>
<td>41</td>
<td>68.33</td>
</tr>
<tr>
<td>Ham’s F-10 + additives</td>
<td>60</td>
<td>22</td>
<td>36.66</td>
</tr>
<tr>
<td>Chi-square value</td>
<td>39.86**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**P<0.01, NSNon-significant**
The rate of maturation of Metaphase-I + Metaphase-II was found to be significantly (P<0.01) higher in aspiration and dissection in TCM-199 medium supplemented with additive medium (Table 2). Garcia et al. (1988) also recorded high degree of IVM of oocytes using TCM-199 medium and obtained 85.70 per cent oocyte matured in TCM-199 + FCS while it was 90.60 per cent maturation with TCM-199 + FSH. The incidence of maturation (Metaphase-I + Metaphase-II) in the present study was found to differ significantly (P<0.01) among the media. Rose and Bavister (1992) also made similar observation. The results of present study revealed that the addition of serum + hormone + antibiotic to the medium also enhanced the maturation rate of follicular oocytes in indigenous cattle of Assam. Skinner (1990) postulated that the serum contained a number of known growth factors which had important role in the regulation of oocyte maturation, particularly via cumulus cells. Oocytes matured in vitro in the presence of gonadotrophin and oestradiol resulted in higher maturation rate as compared to medium where no hormones were added (Totey et al. 1992). Thereby, it can be concluded from the present study that TCM-199 with sodium pyruvate, Gentamicin, L-glutamine, Follicular Fluid, pFSH and HIOCS additives may give better performance of IVM of oocytes recovered from indigenous cattle of Assam in comparisons to Ham’s F-12 and Ham’s F-10 media with the same additives in dissection technique.

REFERENCES


