IV. RESULTS

4.1 Effect of frequency of OPU on the number of follicles available for aspiration, follicles aspirated and oocytes recovered.

Twice a week aspiration frequency resulted in a significantly higher number of small, medium, large (Plate 3) and total follicles (Plate 4) recorded per buffalo per week when compared to once a week aspiration (Table 1). Compared to aspiration once per week, a significantly higher number of follicles were aspirated and oocytes retrieved per buffalo per week on twice a week aspiration. However, aspiration frequency did not significantly affect the oocyte recovery rate.

4.2 Effect of frequency of OPU on oocyte quality and in vitro embryo production

Aspiration twice a week resulted in a significantly higher number of grade A (Plate 5), B (Plate 6) and C oocytes (Plate 7) recovery when compared to aspiration once a week. However, the aspiration frequency did not significantly affect the grade D oocytes (Plate 8) recovery and transferable embryo production rate per buffalo per week (Table 2). Twice aspiration per week resulted in significantly higher number of culturable oocytes (grade A and B), cleavage rate (Plate 15) and 8-cell (Plate 16), 16-cell (Plate 17) and transferable embryos [morulae (Plate 18) and blastocysts (Plate 19,20,21)] per buffalo per week when compared to aspiration once per week.

4.3 Effect of breeding season on the number of follicles available for aspiration, follicles aspirated and oocytes recovered.

Aspiration once a week during peak breeding season resulted in significantly higher number of medium, large, total follicles recorded and follicles aspirated per week per buffalo when compared to low breeding season (Table 3). However, the breeding season did not significantly influence the number of small follicles, oocyte recovery and the oocyte recovery rate. Aspiration twice a week during peak breeding season resulted in a significantly higher number of small, medium follicles, total follicles recorded, follicles aspirated and oocytes recovered per week per buffalo
when compared to low breeding season. However, breeding season did not significantly affect the number of large follicles, and oocyte recovery rate.

Aspiration twice a week during peak breeding season resulted in a significantly higher number of small, medium, total follicles recorded, follicles aspirated and oocytes recovered compared to aspiration once a week. However, during low breeding season, aspiration twice a week resulted in significantly higher number of small, medium, total follicles recorded, follicles aspirated and oocytes recovered compared to aspiration once a week.

Maximum oocytes (2.75 oocytes per buffalo per week) were recovered when OPU was conducted twice a week during peak breeding season.

4.4 Effect of breeding season on quality of oocytes and *in vitro* embryo production

When the aspiration was performed once a week, the breeding season did not significantly affect the different grades of oocytes recovered, cleavage rate, number of embryos progressing to 8 cell, 16 cell, transferable embryos and transferable embryo production rate (Table 4). Aspiration twice a week during peak breeding season resulted in a significantly higher number grade B oocytes, culturable oocytes and transferable embryos compared to those observed in low breeding season. However, season did not significantly affect the number of grade A, C, D oocytes, cleavage rate, 8 cell, and 16-cell and transferable embryo production rate.

During peak breeding season aspiration twice a week resulted in a significantly higher number of grade A, B, C oocytes, culturable oocytes, cleavage rate, 16 cell and transferable embryos when compared to aspiration once a week. During low breeding season aspiration twice a week resulted in a significantly higher number of culturable oocytes, 16 cell and transferable stage embryos compared to aspiration once a week.
Maximum transferable embryos (0.6 embryos per buffalo per week) were produced in vitro when OPU was conducted twice a week during peak breeding season.

4.5 Effect breeding season on the number of follicles available for aspiration, follicles aspirated and oocytes recovered in superstimulated buffaloes
The breeding season did not significantly influence the number of small, medium and large follicles, follicles aspirated, oocyte recovered and oocyte recovery rate in PMSG superstimulated donors. However, significantly higher numbers of total follicles were recorded during peak breeding season as compared to low breeding season (Table 5). Maximum oocytes (3.20 oocytes per buffalo per session) were recovered when OPU was conducted during peak breeding season in PMSG superstimulated buffaloes.

4.6 Effect of breeding season on oocyte quality and in vitro embryo production in PMSG superstimulated buffaloes
The breeding season did not significantly influence the different grades of oocytes recovered, oocytes cultured, cleavage rate, 8 cell, 16 cell, transferable embryos and embryo production rate in PMSG superstimulated buffaloes (Table 6). Maximum transferable embryos (0.7 embryos per buffalo per session) were produced in vitro when OPU was conducted in superstimulated buffaloes.

4.7 Effect of oocyte source on oocyte recovery and in vitro embryo development
The total number of oocytes recovered per ovary was significantly higher in slaughterhouse ovaries and OPU in PMSG-superstimulated ovaries when compared to OPU in non-superstimulated ovaries per session (Table 7). However, the total number of oocytes recovered per ovary was significantly higher in once a week than twice a week OPU in non-superstimulated ovaries per session. The number of culturable oocytes recovered per ovary per session was significantly higher in PMSG-
superstimulated ovaries than in non-superstimulated ovaries by OPU and manual aspiration in slaughterhouse ovaries. The number of culturable oocytes recovered per ovary was significantly higher in slaughterhouse ovaries than in non-superstimulated ovaries by OPU. The OPU once a week in non-superstimulated ovaries resulted a significantly higher number of culturable oocytes than twice a week. The oocyte source did not significantly affect the percentage of embryos progressing to 8 cell, 16 cell and to transferable embryos. However, significantly higher percentage of OPU derived oocytes from non stimulated ovaries aspirated once a week progressed to 2 cell embryos.

4.8 Effect of cryoprotectants with different exposure time on post-thaw survivability of vitrified embryos

The percentage of morulae that progressed to blastocyst stage on vitrification with ethylene glycol exposure time of 2 and 4 min did not differ significantly from non-vitrified controls (Table 8). The morulae that progressed to hatched blastocyst were significantly lower on vitrification with EG exposure time of 2 and 6 min than non-vitrified control. However, morulae progressing to hatched blastocyst stage did not differ significantly on vitrification with EG exposure time of 4 min from non-vitrified control. The percentage of blastocyst progressing to expanded blastocyst and hatched blastocyst stages on vitrification with EG exposure time of 4 min did not differ significantly from non-vitrified control. The percentage of morulae progressing to blastocyst and hatched blastocyst stages on vitrification with glycerol+ethylene glycol (G+EG) exposure time of 2 min did not differ significantly from non-vitrified controls. The percentage of blastocyst progressing to expanded blastocyst and hatched blastocyst stages on vitrification with G+EG exposure time of 4 min did not differ significantly from non-vitrified control.
The percentage of morulae progressing to blastocyst and hatched blastocyst stages on vitrification with ethylene glycol and dimethyl sulfoxide (EG+DMSO) exposure time of 2 and 4 min did not differ significantly from non-vitrified control. The percentage of blastocyst progressed to expanded blastocyst and hatched blastocyst stages on vitrification with EG+DMSO exposure time 2, 4 or 6 min did not differ significantly from non-vitrified control.

4.9 Effect cytochalasin-B in the vitrification media on post thaw survivability of vitrified embryos

The percentage of tight morulae progressing to hatched blastocyst stage on vitrification with cytochalasin-B was significantly higher than in control (Table 9). However, the percentage of tight morulae, blastocyst and expanded blastocysts progressing to hatching blastocyst stage on vitrification with or without cytochalasin-B did not differ significantly.

4.10 Effect of BCB staining test on in vitro maturation and in vitro fertilization of oocytes.

The in vitro maturation rate of BCB (−) oocytes was significantly lower compared to that observed in BCB (+) (Plate 22), control and holding control group oocytes (Table 10). However, no significant difference in the total fertilization rates among the different groups was observed. The BCB (+) group oocytes showed significantly higher percentage of 2 pronuclei than BCB (−) oocytes. There was no significant difference in the number of 2 pronuclei oocytes between BCB (−), control and holding control group. The polyspermy (Plate 14) rate was significantly higher in BCB (−) group oocytes than that of BCB (+) group oocytes, but no significant difference was observed between the BCB (−), control and holding control group.
oocytes. No significant difference in the asynchronous fertilization rates among the different groups was observed.

4.11 Effect of BCB staining test on embryonic development

The blastocyst production rate was significantly higher in the BCB (+) group oocytes compared to BCB (–), control and holding control group (Table 11). The cleavage rate was significantly higher in BCB (+) group oocytes as compared to BCB (-) and holding control group. However, no significant difference in the cleavage rate between BCB (–), control and holding control groups was observed. The blastocyst production rate was significantly higher in control and holding control group compared to BCB (–) group.

4.12 In vitro developmental competence of oocytes collected from ovaries at different morphofunctional states

No significant difference among different groups of ovarian categories in terms of in vitro maturation rates was observed (Table 12). The cleavage rate of oocytes collected from ovaries with CL and DF (group 2) was significantly lower than those collected from ovaries with CH and NO-DF (group 1) and ovaries with CL and NO-DF (group 3). The embryos that progressed to 8-cell, 16-cell and transferable embryos were significantly higher in group 1 than those obtained from ovaries with RCL and DF (group 4) and from ovaries with out any luteal like structures and with small follicles (group 5). However, the embryos progressing to 16-cell and transferable stage were significantly lower in group 2 and group 4 ovaries than group 1 and group 3 ovaries. Whereas, in group 5, the cleavage rate, 8 cell, 16 cell and transferable embryo production rates were significantly lower than the other groups.

4.13 Effect of taurine, cysteamine and melatonin on in vitro maturation, fertilization and embryo production

When comparison was made between all the antioxidants tested, it was observed that significantly higher maturation rates were obtained with cysteamine at 50µM level and melatonin at 10 µM and 50 µM levels when compared to control and melatonin at 100µM levels (Table 13). Cysteamine at 50µM level and melatonin at 10
µM resulted in significantly higher fertilization rates compared to that of control. Cysteamine and melatonin at the all the dose levels tested significantly improved cleavage rates compared to control. With respect to the yield of transferable embryos, taurine at 1 mM dose and cysteamine and melatonin tested at all dose levels (cysteamine:50, 100µM and Melatonin: 10, 50, 100µM) had shown significantly better results when compared to control group.