Isolation and Culture of Ovine and Bubaline Small and Large Pre-antral Follicles: Effect of Cyclicity and Presence of a Dominant Follicle

S Nandi1, V Girish Kumar2, HS Ramesh2, BM Manjunatha2 and PSP Gupta1

1National Institute of Animal Nutrition and Physiology (NIANP), ICAR, Adgadoli; 2Karnataka Veterinary, Animal and Fishery Sciences University (KVAFSU), Bidar Bangalore Campus, Hebbal, Bangalore, India

Contents

Studies were conducted to examine the effects of the cyclicity and the presence of a dominant follicle (DF) in ovary on the recovery and in vitro growth of pre-antral follicles (PFs) in sheep and buffalo. Small pre-antral follicles (SPFs, 100–250 μm) and large pre-antral follicles (LPFs, 250–450 μm) were isolated from slaughterhouse ovaries in the breeding seasons by a mechanical and enzymatic method. The sheep and buffalo PFs were cultured in vitro for 6 and 15 days, respectively, and examined for their growth, survival and antrum formation rates and growth rates of oocytes in cultured pre-antral follicles. The follicles of the sheep and buffalo were recovered and cultured simultaneously within replicates. The recovery rates (number per ovary) of both SPFs and LPFs were significantly (p < 0.05) higher in cyclic ewes (SPFs: 22.0 ± 3.3 vs 12.1 ± 2.6 and LPFs: 16.0 ± 3.6 vs 9.2 ± 1.8) and buffaloes (SPFs: 9.2 ± 1.3 vs 4.1 ± 1.0 and LPFs: 10.3 ± 2.7 vs 5.4 ± 0.7) compared with those recovered from acyclic ones. Presence of a DF in ovary significantly (p < 0.05) reduced the recovery rates of LPFs in ewes (9.06 ± 2.7 vs 16.4 ± 3.8) but had no effect in buffalo. Cyclicity of animals or follicular dominance had no effects on in vitro growth, survival and antrum formation rates and growth rates of oocytes in cultured PFs of SPFs and LPFs in both sheep and buffalo. The in vitro growth, survival and antrum formation rates of LPFs and growth rates of oocytes in cultured LPFs were significantly (p < 0.05) higher than those observed in SPFs in both sheep and buffalo. The overall recovery and growth rates of the PFs were lower in buffaloes compared with ewes.

Introduction

The recent advancement in reproductive biotechnologies, namely in vitro fertilization, embryo transfer, transgenesis, embryo sexing, nuclear transfer and cloning cannot be widely applied to domestic species because of the shortage of suitable oocytes to manipulate in vitro. Superovulation and in vitro maturation of oocytes from the antral follicles are not sufficient enough to allow the rapid progress in these technologies. Domestic animals are born with thousands of pre-antral follicles, of which only 0.01% ovulates in the reproductive life (Santos et al. 2006). New approaches to increase the oocyte yield cannot be widely applied to domestic species because of the shortage of suitable oocytes to manipulate in vitro. Initial studies were carried out using the mouse as a model and resulted in the development of various culture systems for in vitro growth of PFs (Eppig et al. 1996) and birth of live pup from fertilized oocytes recovered from in vitro grown PFs (Eppig and O’Brien 1996). Embryo production from oocytes derived from in vitro grown PFs was reported only in pigs (Wu et al. 2001) and buffalo (Gupta et al. 2008). Offspring could not be produced using embryos generated with oocytes from PFs in any domestic animals so far. The growth rate of PFs was less in domestic animals compared with that reported in rodents (Smitz and Cortvrindt 2002). The time requirement for the PFs to develop to antral stage varied between species (Telfer 1996; Vanderhurk et al. 1997). Various factors influence the recovery and growth of the PFs in culture (Smitz and Cortvrindt 2002). The morphological criteria of ovaries for recovery of PFs were demonstrated recently in cattle (Choi et al. 2006). We hypothesized that the biological factors like the cyclicity (presence of corpus haemorrhagicum or corpus luteum or corpus albicans) and the presence of the dominant follicle (DF) in the ovary influence the recovery and growth rates of PFs in vitro.

The aim of this study was to assess, in two mono-ovulatory and seasonal breeders (sheep and buffalo), the effects of cyclicity and presence of a DF on the recovery rates of small pre-antral follicles (SPFs) and large pre-antral follicles (LPFs) and on the ability of SPFs and LPFs to survive and grow in vitro. In the present study, a comparative reproductive physiology in terms of ovarian pre-antral follicle isolation and growth in culture in sheep and buffalo was investigated.

Materials and Methods

Media and chemicals were obtained from Sigma Chemical (St Louis, MO, USA), unless otherwise stated. Ovaries collected during the breeding seasons (November to February) from matured (2–3 years of age as determined by dental formula), healthy, non-pregnant slaughtered buffaloes (Murrah breed, Bubalus bubalis) and sheep (Bennur breed, Ovis aries) were brought to the laboratory in warm (32–35°C) normal saline (0.9% NaCl) containing 50 μg/ml gentamicin within 1 h of slaughter.

Aims and replications of experiments

The aim of the experiment 1 was to investigate the effect of cyclicity on recovery, growth, survival and antrum formation rates of SPFs and LPFs and growth rates of
Isolation and Culture of Ovine and Bubaline SPFs and LPFs

75

... oocytes in cultured PFs in sheep and buffalo. Eighty-eight buffalo ovaries and 66 sheep ovaries were used. Experiment was replicated 20 times.

The aim of the experiment 2 was to examine the effect of the presence of a DF in ovary on the recovery, growth, survival and antrum formation rates of SPFs and LPFs and the growth rates of oocytes in cultured PFs in sheep and buffalo. Seventy-two buffalo ovaries and 60 sheep ovaries were used. Experiment was also replicated 20 times.

Recovery and culture of pre-antral follicles

In experiment 1, ewes or buffaloes with paired ovaries in which at least one ovary containing a corpus haemorrhagicum or a mature functional corpus luteum or a regressing corpus luteum or a corpus albicans (recently regressed corpus luteum characterized by increasing cicatization and shrinkage of the cicatricial core with an amorphous lutein zone surrounding the central plug of scar tissue and deposition of brown pigment, Lunn et al. 2002; Stocco et al. 2007) were considered as cyclic. Acyclicity in animals selected in this study was due to missed cycle or due to endocrine imbalance and not due to an effect of age or disease. Paired ovaries having only corpora albicains resembling as a scar on the surface of the ovary as the only remnants of past ovarian functional activity were not considered.

In experiment 2, the paired ovaries were collected from the slaughtered individual sheep and buffalo and divided into two groups on the basis of the following criteria: (i) ovaries with a mature functional corpus luteum or a regressing corpus luteum, many follicles of varying diameter and with DF; (ii) ovaries with a corpus haemorrhagicum or a mature functional corpus luteum, many follicles of varying diameter and without DF. The DF was characterized as the one, which has more than 5 mm (sheep) and 10 mm (buffalo) diameter and exceeding the diameter of other subordinate follicles (Baruselli et al. 1997; Evans 2003). Haemorrhagic and morphologically atretic dormant follicles, identified macroscopically (Kruijp and Dieleman 1998), were not considered. The DFs were dissected out and evaluated for their atretic and non-atretic status. The degree of translucency, visualization of the follicle wall and the integrity of the membrana granulosa, as seen under a stereoscopic microscope, were used to distinguish between non-atretic and atretic isolated follicles (Moor et al. 1978; Rajesha et al. 2002). Only ovaries with growing, healthy and non-atretic DFs were considered in this experiment.

A combined mechanical and enzymatic method (Gupta et al. 2007) recently developed in our laboratory was used to isolate PFs from buffalo and sheep ovaries. Thin cortical pieces were suspended in trypsin (1%) (Himedia Lab. Pvt. Ltd, Mumbai, India) prepared in normal saline, taken in 15-ml screw cap tubes and incubated at 37°C for 5 min (sheep) and 10 min (buffalo). The cortical pieces were teased with the needles (26G) and the scalpel blade under the zoom stereomicroscope and the PFs were separated from the ovarian stroma. The PFs were washed in the isolation and washing medium containing minimum essential medium (MEM) supplemented with bovine serum albumin (BSA, 0.3%), glutamine (2 mm), sodium pyruvate (0.23 mm), hypoxanthine (2 mm) and gentamicin (50 μg/ml). The diameter of PFs was measured using a pre-calibrated micrometer. A follicle with an oocyte, surrounded by granulosa cells and limited by an intact basement membrane, was considered the morphologically normal (Gupta et al. 2007). Only SPFs (100–250 μm) and LPFs (250–450 μm) with normal appearances and without visible signs of degeneration were selected for culture. The isolated sheep and buffalo PFs (two to three in a group) were transferred in 100-μl droplets of culture media under the liquid paraffin oil into a 35-mm petri dish and cultured in a CO₂ incubator (38.5°C, 5% CO₂ in air, 90–95% relative humidity) for 6 days (Ceccon et al. 1999) and 15 days (Gupta et al. 2002), respectively. Only viable PFs as measured by trypan blue staining technique (Gupta et al. 2002) were used for culture. The composition of small and large ovine and bubaline pre-antral follicle culture media was MEM supplemented with BSA (0.3%), glutamine (2 mm), sodium pyruvate (0.23 mm), hypoxanthine (2 mm), insulin–selenium–transferin (1%) and gentamicin (50 μg/ml) and FSH-P (7 μg/ml; biological potency = 7 U/ml; F2293; LH ≤ 1%). The medium was replenished on every alternate day. The initial and final diameter of the PFs and the oocytes was measured using the micrometry set fitted on the microscope. Morphological criteria like layers of compact granulosa cells around the oocyte, normal/abnormal follicular outline, the presence/absence of dark patches within membrana granulosa and signs of degeneration (if any) for the assessment of PFs under the stereozoom microscope and inverted microscope were used for in vitro pre-antral follicle growth determination (Gupta et al. 2002). The follicles after isolation and culture were checked for the vitality using the trypan blue staining technique (Gupta et al. 2002). The final diameter of the follicles was recorded together with the presence and absence of an antral cavity (a visible translucent area within the granulosa cell mass). The growth, survival rates of pre-antral follicles, antrum formation rate and growth rate of oocytes were calculated as follows:

1. Growth rate of follicle (μm/day) = [Final diameter (μm) of follicle as observed by day 6 (sheep) or day 15 (buffalo) of culture – Initial diameter (μm) of follicle]/six (sheep) or 15 (buffalo).
2. Survival rate (%) = [No. of follicles not got stained (survived)/Viable follicle cultured] × 100.
3. Antrum formation rate (%) = [No. of follicles with antrum after day 6 (sheep) or day 15 (buffalo) of culture]/Initial no. of follicles) × 100.
4. Growth rate of oocyte (μm/day) = [Final diameter (μm) of oocyte as observed by day 6 (sheep) or day 15 (buffalo) of culture – Initial diameter (μm) of oocyte]/six (sheep) or 15 (buffalo).

Statistical analysis

The effect of each parameter on the recovery number and follicular features (growth/survival) were evaluated by a prospective, randomized study. Significant
differences in experiment 1 between the mean recovery rates, growth rates and survival rates of PFs were analyzed by ANOVA and the respective means were compared using Tukey’s test. Significant differences in experiment 2 between the mean recovery rates, growth rates and survival rates of PFs within a pair of ovaries were compared by paired t-test. The percentage values were transformed by arcsine square root before analysis. Difference was considered to be significant at p < 0.05.

The computer assisted statistical software package (Graph Pad Prism, San Diego, CA, USA) was used for analyzing the data.

**Results**

**Experiment 1**

The recovery rates of both SPFs (ewes: 22.0 ± 3.3 vs 12.1 ± 2.6; buffalo: 9.2 ± 1.3 vs 4.1 ± 1.0) and LPFs (ewes: 16.0 ± 3.6 vs 9.2 ± 1.8; buffalo: 10.3 ± 2.7 vs 5.4 ± 0.7) were significantly (p < 0.05) higher in cyclic ewes and buffaloes compared with acyclic ones (Tables 1 and 2). No significant differences in growth, survival and antrum formation rates and growth rates of oocytes (in cultured pre-antral follicles) of both SPFs and LPFs retrieved from ovaries of cyclic and acyclic ewes and buffaloes were observed (Tables 1 and 2).

**Experiment 2**

The recovery rates of LPFs were significantly (p < 0.05) higher (16.4 ± 3.8 vs 9.06 ± 2.7) from ovine ovaries without DF than that retrieved from ovaries with DF (Table 3). No significant difference was observed in SPF recovery rates from ovine ovaries with and without DF. Similarly, no significant differences in recovery rates of SPFs and LPFs collected from ovaries with and without DF were observed in buffalo (Table 4). No significant differences in growth, survival and antrum formation rates and growth rates of oocytes (in cultured pre-antral follicles) of both SPFs and LPFs retrieved from ovine ovaries were observed (Tables 3 and 4).

### Table 1. Effect of cyclicity on recovery, *in vitro* growth, survival and antrum formation rates of ovine pre-antral follicles (PFs) and growth rates of oocytes in cultured PFs

<table>
<thead>
<tr>
<th>Reproductive status</th>
<th>Recovery rates (no. of PFs per ovary)</th>
<th>Growth rates of PFs (µm/day)</th>
<th>Survival rates of PFs (%)</th>
<th>Antrum formation rates (%)</th>
<th>Growth rates (µm/day) of oocytes in cultured PFs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPF</td>
<td>LPF</td>
<td>SPF</td>
<td>LPF</td>
<td>SPF</td>
</tr>
<tr>
<td>Cyclic</td>
<td>22.0 ± 3.3^a</td>
<td>16.0 ± 3.6^a</td>
<td>2.9 ± 0.6^c</td>
<td>10.6 ± 2.1^d</td>
<td>77.7 ± 12.6^e</td>
</tr>
<tr>
<td>Acyclic</td>
<td>12.1 ± 2.6^b</td>
<td>9.2 ± 1.3^b</td>
<td>3.0 ± 1.0^b</td>
<td>9.4 ± 2.4^d</td>
<td>76.5 ± 9.4^c</td>
</tr>
</tbody>
</table>

Values are mean ± SEM based on 20 replicates per treatment with two to three pre-antral follicles per plot. SPF, small pre-antral follicle; LPF, large pre-antral follicle. ^a,b^Values in the same column with different superscripts differ significantly (p < 0.05). ^c,d^Values in the same row with different superscripts differ significantly (p < 0.05).

### Table 2. Effect of cyclicity on recovery, *in vitro* growth, survival and antrum formation rates of bubaline pre-antral follicles (PFs) and growth rates of oocytes in cultured PFs

<table>
<thead>
<tr>
<th>Reproductive status</th>
<th>Recovery rates (no. of PFs per ovary)</th>
<th>Growth rates of PFs (µm/day)</th>
<th>Survival rates of PFs (%)</th>
<th>Antrum formation rates (%)</th>
<th>Growth rates (µm/day) of oocytes in cultured PFs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPF</td>
<td>LPF</td>
<td>SPF</td>
<td>LPF</td>
<td>SPF</td>
</tr>
<tr>
<td>Cyclic</td>
<td>9.2 ± 1.3^a</td>
<td>10.3 ± 2.7^a</td>
<td>1.1 ± 0.3^c</td>
<td>2.5 ± 0.8^d</td>
<td>65.3 ± 6.4^e</td>
</tr>
<tr>
<td>Acyclic</td>
<td>4.1 ± 1.0^b</td>
<td>5.4 ± 0.7^b</td>
<td>0.7 ± 0.1^c</td>
<td>2.1 ± 0.4^d</td>
<td>68.7 ± 5.6^c</td>
</tr>
</tbody>
</table>

Values are mean ± SEM based on 20 replicates per treatment with two to three pre-antral follicles per plot. SPF, small pre-antral follicle; LPF, large pre-antral follicle. ^a,b^Values in the same column with different superscripts differ significantly (p < 0.05). ^c,d^Values in the same row with different superscripts differ significantly (p < 0.05).

### Table 3. Effect of presence of a dominant follicle (DF) on recovery, *in vitro* growth, survival and antrum formation rates of ovine pre-antral follicles (PFs), and growth rates of oocytes in cultured PFs

<table>
<thead>
<tr>
<th>Ovaries</th>
<th>Recovery rates (no. of PFs per ovary)</th>
<th>Growth rates of PFs (µm/day)</th>
<th>Survival rates of PFs (%)</th>
<th>Antrum formation rates (%)</th>
<th>Growth rates (µm/day) of oocytes in cultured PFs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPF</td>
<td>LPF</td>
<td>SPF</td>
<td>LPF</td>
<td>SPF</td>
</tr>
<tr>
<td>With DF</td>
<td>20.6 ± 2.7^e</td>
<td>9.06 ± 2.7^f</td>
<td>3.2 ± 1.2^c</td>
<td>11.8 ± 3.2^d</td>
<td>75.7 ± 10.4^c</td>
</tr>
<tr>
<td>Without DF</td>
<td>18.4 ± 2.9</td>
<td>16.4 ± 3.8^b</td>
<td>3.0 ± 0.8^b</td>
<td>10.6 ± 2.6^d</td>
<td>73.4 ± 6.3^c</td>
</tr>
</tbody>
</table>

Values are mean ± SEM based on 20 replicates per treatment with two to three pre-antral follicles per plot. SPF, small pre-antral follicle; LPF, large pre-antral follicle. ^e,f^Values in the same column with different superscripts differ significantly (p < 0.05). ^c,d^Values in the same row with different superscripts differ significantly (p < 0.05).
and bubaline ovaries with and without DF were observed (Tables 3 and 4).

Overall, the recovery, growth, survival and antrum formation rates and growth rates of oocytes (in cultured pre-antral follicles) of ovine PFs were higher than those observed in buffaloes. The growth rates of LPFs were significantly (p < 0.05) higher than those of SPF from both ewes and buffaloes (Tables 1–4). The recovery rates of LPFs were significantly (p < 0.05) higher than those of SPF from ovine ovaries without DF. The recovery rates of LPFs and SPF were similar in acyclic ewes. Like cyclic ewes, the growth rates of LPFs were significantly (p < 0.05) higher than those of SPF from ovaries of acyclic ewes. The survival rates after culture were similar in SPF and LPF retrieved from ovaries of cyclic and acyclic ewes.

**Discussion**

The months of October to March were considered as the peak breeding season for buffalo and the reminder of the year as the low breeding season (Shukla et al. 1973). Breeds of sheep in southern India (location of study) were reported to be seasonally anoestrous from March to April and September to October (Kaushish, 1994). Hence, the present study was conducted in breeding seasons of sheep and buffalo to nullify any seasonal effects.

In the present study, lower number of PFs could be isolated in buffalo than those in sheep, which might be due to the comparatively smaller germ cell reserve in buffalo ovaries (Danell 1987). The germ cell reserve in sheep ovary was reported to be 805 000 (Smith et al. 1993; Sawyer et al. 2002) compared with 12 000–19 000 in buffalo (Samad and Nasseri 1979). It is also likely that the lower yield might be due to more fibrous nature of buffalo ovaries (Gupta et al. 2001). The recovery rate of sheep PFs in the present study was comparable with an earlier study (Jun et al. 2004). In contrast, Amorim et al. (2000) isolated 4735 ovine PFs per ovary. The difference may be due to different methods of isolation of PFs adopted. The growth rates of buffalo PFs were comparable with that reported in the earlier studies (Gupta et al. 2002; Santos et al. 2006), however, the survival rates of PFs were higher in the present study compared with those observed by Santos et al. (2006). This difference may be due to different follicle diameter used or other factors such as breeds and nutritional status of donor.

Various attempts had been made to obtain development of mammalian PFs in vitro. Pre-antral follicle isolation has been standardized for many domestic animals, namely cattle (Hulshof et al. 1995), sheep (Cecconi et al. 1999), goats (Chelikani et al. 1998), buffalo (Gupta et al. 2001) and pigs (Morbeck et al. 1993). Follicular antrum formation was accomplished in the cultured PFs in cattle (Cetin et al. 2000), sheep (Cecconi et al. 1999), goat (Huanmin and Yong 2000) and pig (Wu et al. 2002). The success in producing offspring by using the oocytes retrieved from the in vitro grown PFs was accomplished only in mice (Eppig and Schroeder 1989).

Maintenance of viability was the major problem in the culture of follicles (Gupta et al. 2002). Trypan blue dye exclusion test of follicles had been used as a quick and easy assessment of viability during isolation and culture of follicles (Gupta et al. 2002). It was earlier reported that ovine pre-antral follicular architecture could not be maintained following 6 days of culture (Cecconi et al. 1999). Buffalo PFs were found to develop maximum from day 6 to day 10 in culture (Nandi et al. 2005). Hence, the PFs were cultured for 6 and 15 days in sheep and buffalo, respectively, in the present study. Antrum formation of the LPFs in both sheep and buffalo had been accomplished in the present study, suggesting that the additives used support the survival and growth in vitro of pre-antral follicles. The follicular growth of buffalo follicles in culture in the present study was lower than that observed in sheep which might be due to differences in criteria for follicle size classification and species difference.

In most domestic animals, the later stages of follicle development occurs in a wave-like pattern during oestrous cycles. A follicle wave is the organized development of a cohort of gonadotrophin-dependent follicles, all of which initially increase in size, but most of which subsequently regress and die by atresia (subordinate follicles). The number of remaining (dominant) follicles is specific to the species and is indicative of litter size. Follicle waves develop during both luteal and follicular phases and it is the DF(s) of the last follicular wave that ovulates. However, in sheep and goats, DFs from the last two follicle waves can ovulate (Evans 2003). It was reported that the ovaries with corpus luteum yielded greater numbers of PFs than those without corpus luteum in cattle (Gao et al. 2001) and buffalo (Gupta et al. 2002). In contrast, Gao et al. (2004) and Rodrigues et al. (1998) did not observe any

---

### Table 4. Effect of presence of a dominant follicle (DF) on recovery, in vitro growth, survival and antrum formation rates of bubaline pre-antral follicles (PFs) and growth rates of oocytes in cultured PFs

<table>
<thead>
<tr>
<th>Ovaries</th>
<th>Recovery rates (no. of PFs per ovary)</th>
<th>Growth rates of PFs (μm/day)</th>
<th>Survival rates of PFs (%)</th>
<th>Antrum formation rates (%)</th>
<th>Growth rates (μm/day) of oocytes in cultured PFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovaries</td>
<td>SPF</td>
<td>LPF</td>
<td>SPF</td>
<td>LPF</td>
<td>SPF</td>
</tr>
<tr>
<td>With DF</td>
<td>9.6 ± 2.1</td>
<td>11.2 ± 3.1</td>
<td>1.0 ± 0.2</td>
<td>2.4 ± 0.6</td>
<td>64.1 ± 6.7</td>
</tr>
<tr>
<td>Without DF</td>
<td>8.7 ± 2.5</td>
<td>9.3 ± 2.6</td>
<td>0.9 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>66.3 ± 7.1</td>
</tr>
<tr>
<td>Values in the same row with different superscripts differ significantly (p &lt; 0.05).</td>
<td><strong>a,b</strong></td>
<td><strong>a,b</strong></td>
<td><strong>a,b</strong></td>
<td><strong>a,b</strong></td>
<td><strong>a,b</strong></td>
</tr>
</tbody>
</table>

© 2009 The Authors. Journal compilation © 2009 Blackwell Verlag
difference in the number of PFs recovered from the cattle and goat ovaries with or without corpus luteum. It is obvious that, in cyclic animals, the recruitment of follicles would be more than that observed in acyclic ones (Mcgee and Hsueh 2000; Macklon et al. 2006). The recruitment of SPFs is directly related to both cyclicity and the stage of follicle development within oestrous cycles. The recovery and the physiological growth rate of PFs are strongly linked to both cyclicity and stage of the oestrous cycle. Although each growing follicle may initially have an equal potential to reach full maturation, only those follicles that happen to be at a more advanced stage of maturation gain gonadotropin dependence and continue to grow (Webb et al. 2004). This process is referred to as cyclic, gonadotropin-dependent or ‘secondary’ recruitment, as opposed to the initial gonadotropin-independent ‘primary’ recruitment of primordial follicles (Mcgee and Hsueh 2000). Pre-antral follicles were reported to be gonadotropin responsive (Mcgee and Hsueh 2000) and FSH affected the rate of development of PFs in vivo in a large monovalutory species that has a prolonged period of pre-antral follicle development (Campbell et al. 2004). During antral development, future DFs indirectly affect the growth of subordinate follicles through their secretion of oestrogens and inhibins. DFs might produce factors that act directly on subordinate follicles from the growing cohort to impair their development (Sears et al. 1996). DF, while depressing the growth of its neighbour, is not inducing irreversible atresia in the subordinate follicle (Sears et al. 2002). Our result was in accordance to an earlier report where a decreased number of PFs in cystic ovaries were reported, which indicated a relationship between the recovery of PFs and regulation of folliculogenesis (Choi et al. 2006).

In conclusion, more number of PFs could be retrieved from cyclic ewes and buffaloes compared with their acyclic counterparts. DF reduced the recovery rates of ovine PFs but not in bubaline pre-antral follicles. Cyclicity and follicular dominance had no effect on growth, survival and antrum formation rates of pre-antral follicles and growth of oocytes in cultured PFs in both sheep and buffalo.

Acknowledgements

The authors thank the Director, National Institute of Animal Nutrition and Physiology, Bangalore for providing the necessary facilities.

References


