

EFFECT OF FOLLICLE SIZE ON NUMBER AND QUALITY OF OOCYTES IN BOVINES

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ABSTRACT

The 891 follicles from 166 bovine ovaries were categorized as per their size (<4mm, 4 to 8mm and > 8mm) to observe the effect of the follicle size on oocyte number and quality. The number of oocytes recovered from < 4mm sized follicles was significantly higher ($p < 0.01$), when compared to the recovery rate in 4 to 8mm and > 8mm category follicles (50.16, 40.62 and 9.2 per cent). Irrespective of the size of the follicle, the yield of grade I oocytes was significantly higher ($p < 0.01$) when compared to other grades. It was also observed that the quality of oocyte and follicle size had an interactive effect.

Key words: Follicle size, Oocytes, Bovine

Leibfried and First (1979) recorded that follicles of 1 to 3 mm diameter had a higher proportion of oocytes with a compact, complete investment than those from large follicles. Based on the presence or absence and quality of the surrounding cumulus matrix, categorization of follicular oocytes was done by Dalhausen *et al.* (1981) in prepubertal calves and concluded that follicles in < 3mm and 3 to 6mm category contained greater proportion of oocytes with compact or only slightly expanded cumulus cells compared to > 6mm category.

Loneragan *et al.* (1992) observed that significantly more oocytes with many layers of cumulus cells were obtained from follicles > 6mm in diameter (70.2 percent) compared to 2 to 6mm follicles (46.8 percent) in bovine ovaries indicating a clear relationship between follicle size and oocyte quality.

Further, Sarkhel *et al.* (1997) reported that a positive but non-significant correlation was established between follicle size and different grades of caprine oocytes by of which, Grade I oocytes revealed positive and significant correlation. The oocyte recovery per cent from follicles in buffalo ovaries as per the follicle size was 51.05 (3 to 5mm) and 53.96 (>5mm), (Naik *et al.*, 1999). 39.05 per cent of fair and 19.52 per cent of poor oocytes were obtained from 3 to 5mm sized follicles. 40.44 per cent good, 47.05 per cent fair and 12.5 per cent poor quality oocytes were obtained from follicles > 5mm size. Follicles with a low degree of atresia contained a relatively high per cent of COC A, follicles with a high degree of atresia contained higher per cent of COC B and COC C (De Witt *et al.*, 2000). This study was designed to record the number and quality of bovine

oocytes available from different sized follicles seen in ovaries collected from slaughterhouse.

Normal ovaries (166) of crossbred cows, collected from the slaughterhouse were transported to the laboratory in normal saline solution. The ovaries were washed in tap water and then fresh normal saline. The ovaries were categorized according to the stage of estrous cycle and were subjected to further study. The follicles visible on the surface of the ovaries as transparent structures were identified and their diameter was measured using vernier calipers. The follicles were grouped into three categories namely small (<4mm), medium (4 to 8mm) and large (> 8mm) as per method adopted by Selvaraj *et al.* (1992). These measurements were recorded separately for each ovary.

The follicular fluid from each category of follicle was collected through aspiration and it was transferred to separate centrifuge tubes with the Tyrode's Lactate (T.L.) HEPES medium (collection and washing media). The contents were allowed to settle down for 15 minutes and then the supernatant was discarded. The remaining solution was transferred to the petri dish (100x15mm square style with 13mm grid, Falcon) for screening and grading of oocytes under stereozoom microscope (25x). A total of 166 ovaries were utilized to observe the effect of follicle size on the oocyte quality and number, which was statistically analysed as per Snedecor and Cochran (1967).

A total of 447, 362 and 82 oocytes were recovered from follicles belonging to small (<4mm), medium (4 to 8mm) and large (> 8mm) size irrespective of the stage of estrous cycle from 166 ovaries. The number of the oocytes recovered was more from small (< 4mm) which was in accordance to the findings of various authors

reported in bovines, prepubertal calves, buffaloes and goats (Leibfried and First, 1979, Dalhausen *et al.*, 1981, Selvaraj *et al.*, 1992 and Das *et al.*, 1996). In the present study 53.48 per cent, 35.86 per cent and 10.65 per cent of Grade I oocytes were obtained from <4 mm, 4 to 8mm and > 8mm sized follicles. The values recorded for Grade II was 47.33, 42.01 and 10.65 per cent and for Grade III oocytes 41.12, 53.27 and 5.60 per cent. In Grade IV, the oocytes recorded as per the follicle size was found to be 48.81, 46.45 and 4.72 per cent.

Follicles belonging to < 4mm size category yielded significantly more ($p < 0.01$) number of oocytes than 4 to 8 and > 8mm follicles and also the oocytes obtained were of comparatively good quality. Pavlok *et al.* (1992) and Bruck *et al.* (1996), however obtained low proportion of intact cumulus oocyte complex from follicles of this category (< 4mm) in bovines.

It was also observed that 4 to 8mm sized follicles yielded significantly more number of oocytes than > 8mm sized follicles. Chandrahasan (1992) recovered low proportion of intact cumulus oocyte complex from < 2mm follicles compared to 3 to 5mm and 6 to 10 mm range in buffalo ovaries. In the present study a comparatively similar observation was made and was also in accordance with Pavlok *et al.* (1992).

Lonergan *et al.* (1992) obtained good quality oocytes from follicles > 6mm diameter. Whereas, Naik *et al.* (1999) reported that > 5mm size follicles can yield higher number and good quality oocytes. In the present study > 8mm follicles yielded less number of oocytes and comparatively lower quality of oocytes than when compared to other follicle sizes which might be due to the partial or fully expanded cumulus mass and was probably because of loss of connection between cumulus cells and oocyte cytoplasm.

On statistical interpretation of the data, it was identified that irrespective of follicle size, a statistically significant ($p < 0.01$) yield of Grade I oocytes was obtained when compared to the other grades. A combined effect of follicle size and grade on the oocytes obtained was recorded.

The present study demonstrated a clear relationship between the follicle size and oocyte quality. Leibfried and First (1979) reported that the follicles of 1 to 3mm diameter tend to have a high proportion of oocytes possessing a compact, complete investment and follicle > 3mm diameter have expanded cumulus cells. However, follicles of < 4mm diameter possessed high compact complete investment than follicles of 4 to 8mm diameter which confirm the findings of Nebar and Threfall (1993) who observed that as the follicle size increased the per cent of cumulus oocyte complex investment decreased.

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