**Supplementary data**

In the present study, kisspeptin gene expression studies were performed on follicular cells and oocytes collected from *in vivo* developed follicles and their assumed equivalent, *in vitro* cultured preantral follicles. Different stages of follicles developed *in vivo* were collected from ovaries. These were designated preantral follicles (250–400 µm diameter) with no signs of antrum formation, early antral follicles with initiation of antrum development (400–500 mm in diameter), antral follicles (500–700 mm in diameter), large antral follicles (700 mm or more in diameter). These follicles were grouped as *in vivo* developed follicles and were isolated mechanically using two 26-gauge needles that were fitted to 1-ml syringe barrels and a surgical blade from pooled ovarian cortical slices (1 mm3) under a stereoscopic zoom microscope (SMZ 2T; Nikon Corporation, Tokyo, Japan). On routine isolation an average of 5–10 preantral, 5–10 early antral, 3–5 antral, and 1–2 large antral follicles could be obtained. The assumed *in vitro* cultured equivalent stages for the above-mentioned *in vivo* developed follicles of various stages are given in Table S1 below.

**Table S1**

|  |  |  |
| --- | --- | --- |
| S. no. | *In vivo* developed stage | Assumed equivalent morphologically corresponding *in vitro* stage |
| 1 | Preantral follicles (250–400 µm diameter) with no signs of antrum formation | Preantral follicles (250–400 µm diameter) exposed to culture medium for 15 min |
| 2 | Early antral follicles with initiation of antrum development (400–500 mm in diameter) | Preantral follicles cultured for 2 days |
| 3 | Antral follicles (500–700 mm in diameter) | Preantral follicles cultured for 4 days |
| 4 | Large antral follicles (700 mm and above in diameter) | Preantral follicles cultured for 6 days |
| 5 | COCs from Graafian follicles(collected by aspiration) | COCs collected from preantral follicles cultured for 6 days |

Follicular cells and oocytes were collected separately from the follicles mentioned in Table S1 (S. nos. 1–4) were subjected to gene expression studies. The COCs as mentioned in S. no. 5 were subjected to *in vitro* maturation for 24 h; the oocytes and cumulus cells at the end were used to study the gene expression profile.