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Morphological Features of Porcine Oviductal Epithelial Cells and Cumulus-Oocyte Complex

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ABSTRACT

Porcine oviductal epithelial cells (POEC) and cumulus-oocyte complexes (COCs) were observed using inverted microscopy and scanning electron microscopy. At follicular phase, POEC contained a great number of long ciliated cells whereas those at luteal phase consisted mostly of round shaped non-ciliated cells with short microvilli on the apical surface. This change in morphological features of POEC seemed to serve well on their functions as oocyte transporters at follicular phase. As for COCs, they were morphologically classified into 4 types based on the accumulation and arrangement of cumulus cells around the oocytes. These were intact cumulus cell layer, single cumulus cell layer, partial cumulus cell layer and completely denuded oocytes at the percentages of composition of 19.87%, 18.13 %, 28.88 % and 33.12 %, respectively. Cumulus cells attached on the surface of oocytes were teardrop-like shape having the conical ends pointed towards the oocytes membrane surface while free-floating cumulus cells in the follicular fluid and those at the outer layers were round in shape. These POEC and COCs were high potential feeder cells and could be further cultured for *in vitro* fertilization use.

Key words: cumulus-oocyte complexes, morphology, porcine oviductal epithelial cells

INTRODUCTION

Cells in the mammalian female reproductive system, i.e., oviductal epithelial cells and cumulus cells have direct effect and interactions which contribute to the success of fertilization. They are, therefore, high potential cellular materials to be used as cultured feeder-cells for gamete development and *in vitro* fertilization (White *et al*., 1989; Nagai and Moor, 1990; Kitiyanant *et al.*, 1989, 1993, 1995; Park and Sirard, 1996; Vatzias and Hargen, 1999; Romar *et al*., 2001, 2003). Since the reproductive organs of pig are not used as human food, they are readily available and can be collected from the slaughter house for research work. Kitiyanat *et al*. (1993) reported the use of porcine oviductal cells to support *in vitro* bovine embryo development. It is also known that there are some materials in the reproductive tract of animals that produce several offspring at one time, i.e., pig, rodents, could better facilitate the growth and development of embryo in other types of animal in culture. These cells not only help the sperm capacitation and acrosome reaction but also assist the penetration of oocytes and hence, gave higher percentage of *in vitro* fertilization (White *et al.*, 1989; Anderson and Killian, 1994; Hyttel *et al*., 1997; Park and Sirard, 1996; Romar *et al*., 2001, 2002). In addition, these cells are very useful in determining the toxic effect of environment

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which could be accumulated and lead to the abnormal development of the embryo. The results of these effects could be clearly seen and evaluated than using the experimental animals and therefore, ethically acceptable.

To be able to make the best use of these cells, attempts are made to thoroughly study the characteristics of them. Unfortunately, basic knowledge on their morphological aspects of these cells are still unknown. This work was aimsed to discern the morphological features of porcine oviductal epithelial cells during the estrous cycle and those of cumulus oocytes complex using inverted microscopy and scanning electron microscopy (SEM).

MATERIALS AND METHODS

POEC and COCs collection and preparation

Oviducts and ovaries of Large White pigs were obtained from slaughter house at Nakorn Pathom Province. They were removed within 30 minutes after being slaughtered and transported to the laboratory within 1 hour in a thermos containing 0.9% normal saline.

The oviduct from follicular phases (estrous cycle, day 15) and luteal phases (estrous cycle, day 1-2) were trimmed free from fat and connective tissues and rinsed 3 times in 0.1 M phosphate buffer (pH 7.2). They were cut in small size (2-3 mm) and prepared for SEM observation.

For COCs collection, selected healthy follicles of 2-6 mm in diameter were aspirated using a 5 ml disposable syringe with 18-gauge needle containing 0.9% normal saline and placed in petri dishes. Follicular content was observed under a stereomicroscope and COCs were collected using a pipette of narrow pore size (200 μm). After aspiration, COCs were washed 3 times in TALP-HEPES supplemented with 10% heat treated fetal calf serum (HTFCS) and 50 mg/ml gentamycin, then observed under an inverted microscope and prepared for SEM observation.

Preparation for scanning electron microscopy

Samples were pre-fixed in 2.5% glutaraldehyde in 0.1 M phosphate-buffer (pH 7.2) for 2 h and post-fixed in 1% osmium tetroxide in the same buffer for 24 h. They were then dehydrated in a graded series of ethanol (30, 50, 70, 80, 90% and absolute ethanol) and dried in a critical point dryer machine (CPD). All samples were mounted on stubs with conductive carbon tape, coated with gold particle at 20 nm thick in an ion sputtering, observed and examined under SEM (CamScan Analytical, Maxim 2000S) operating at 10 kV.

RESULTS

Ultrastructures of POEC

SEM of porcine ampullary oviduct (PAO) showed two different cells types, ciliated and non-ciliated cells. The porcine ampullary oviductal epithelium at the follicular phase contained numerous ciliated cells and some non-ciliated cells. The cilia consistently projected themselves above the apex of the non-ciliated cells (Figure 1A, B and C). At high magnification, non-ciliated cells were clearly seen as spherical shape cells with numerous short microvilli (Figure 1D).

At luteal phase, the number of ciliated cells decreased while non-ciliated cells increased (Figure 2A). At high magnification, the apical surfaces of the non-ciliated cells were round in shape with numerous small microvilli (Figure 2B).

Ultrastructures of COCs

From twenty collected ovaries, 921 oocytes were isolated resulting in the average of 46 oocytes per ovary. Inverted microscopic observation of COCs showed the oocytes from follicular fluid were round in shape and 120-145 μm in size. They were surrounded with zona pellucida and several layers of cumulus cells (Figure 3A, C). The SEM gave distinct surface appearances of both oocytes and cumulus cells (Figure 3B, D, F, H). Cumulus

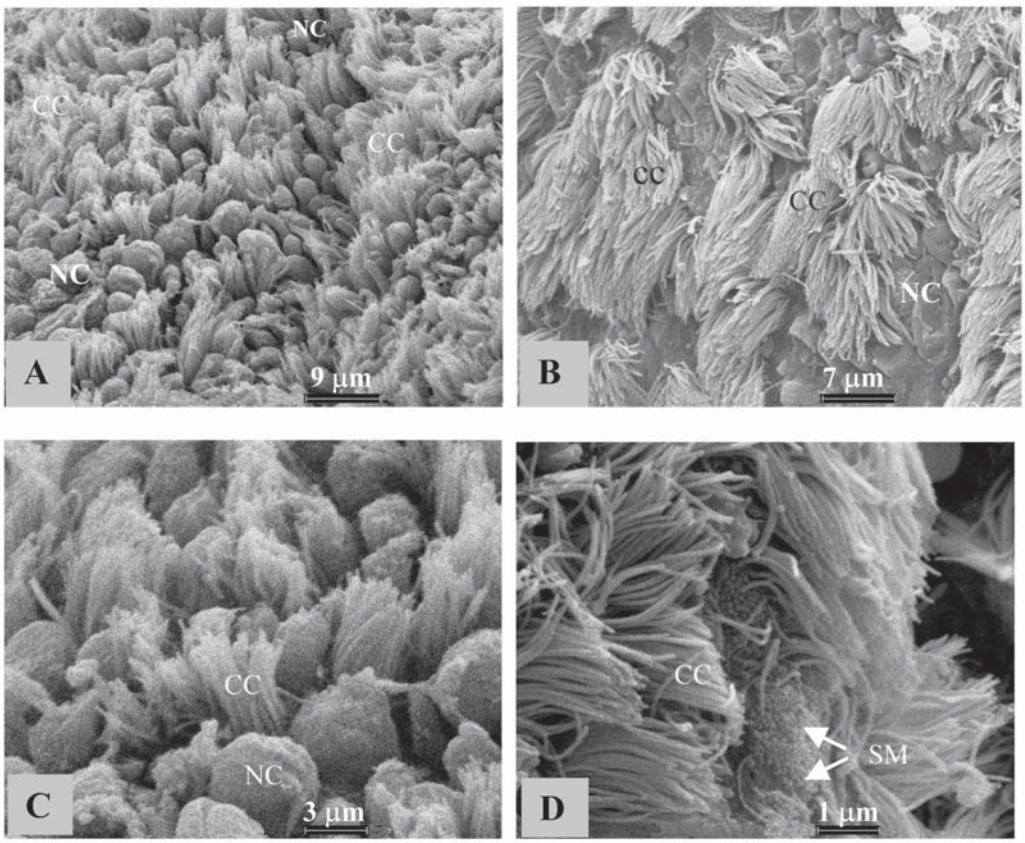

Figure 1 Scanning electron micrographs of POEC at follicular phase, (A, B) showing numerous ciliated cells (CC) and non-ciliated cells (NC). At high magnification (C,D) showing non-ciliated cells (NC) of spherical shape having short microvilli (SM) on the apical surfaces.

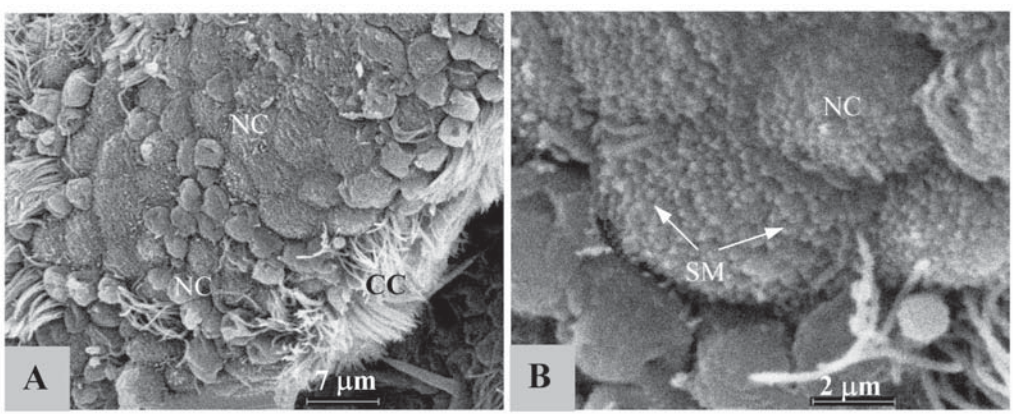

Figure 2 Scanning electron micrographs of POEC at luteal phase showing (A) numerous non-ciliated cells (NC) small number of ciliated cells (CC). At high magnification (B) showing round shape non-ciliated cells (NC) with short microvilli (SM) at the apical surface.

cells were also round in shape and contained no microvilli on the surface membrane. Based on the surrounded cumulus cells of the oocytes, COCs were classified into 4 types as follows:-

Types I- Intact cumulus cells layer- The oocytes were at early development stage having several compact layers of cumulus cell. They were found at secondary follicle part (Figure 3 A,B).

Types II- Single cumulus cell layer- Oocytes were found with one or incomplete two layers of cumulus cell (Figure 3 C,D).

Type III- Partial cumulus cell layer-Oocytes were partially covered with some cumulus cells. The cumulus cells were loosely attached to the zona pellucida. Cytoplasm became fainted in color (Figure 3 E,F).

Type IV- Completely denuded oocyte - Oocytes were completely free from cumulus cells. These oocytes were from follicle atresia. Cytoplasm was pale in color (Figure 3 G,H).

It was found that 33.12% of these COCs was completely denuded oocyte, 28.88% was partial cumulus cell layer type while those of single cumulus cell layer and intact cumulus cell layer types were found at only 19.87% and 18.13 %, respectively (Table 1).

Cumulus cells attached to the oocyte surface were not round in shape like those in the follicular fluid but conformed to a teardrop-like structure having the conical end pointed towards the oocyte surface membrane (Figure 4). The remaining cumulus cells were all round in shape and could be found as a single cell or as a monolayer surrounding the oocyte (Figure 5).

DISCUSSION

Two types of mammalian oviductal epithelial cell, i. e., non-ciliated and ciliated cell, were present in porcine ampullary oviduct (PAO) at both follicular and luteal phases. Ciliated cells in ampulla, however, were found at increased number at the follicular phase than at the luteal phase. This finding agreed with that of bovine oviductal epithelial cells as reported by Songthaveesin (1998). Although the alternation of population of two types of cell in both phases were similar in these two different species, the morphology of the epithelial cells were distinctly different. In bovine, oviductal epithelial cells form a "wormlike" structure (Xu *et al*., 1992) while those in porcine were small and round in shape.

Since ampullary oviduct was the site of fertilization, it was found to contain more synthetic secretion than the whole oviduct (Murray, 1992). In the ampullary oviduct at follicular phase, the high number of ciliated cells corresponded to the transporting of ovulated oocytes. At luteal phase, numerous non-ciliated cells with short microvilli at the apical surface also corresponded to the secretory substance for nutritional support of embryonic development (Hole and Koos, 1994). It has been suggested that the cycle of long ciliated and non-ciliated cells population as seen in the mammalian oviduct depends on the levels of circulating estrogen and progesterone (Verhange and Jaffe, 1986). Furthermore, POEC are used as co-culture *in vitro* to support oocytes maturation and increase normal fertilization, sperm capacitation and early embryonic development (White *et al*., 1989; Nagai and Moor, 1990; Kitiyanant *et al.*, 1993; Park and Sirard, 1996; Vatzias and Hargen, 1999; Romar *et al*., 2001, 2003). Nagai and Moor (1990) also suggested that glycoproteins secreted from non-ciliated oviductal epithelial cells could bind to the porcine spermatozoa and reduce the incidence of polyspermy. Further studies should be carried out to elucidate the characterization of protein synthesis from non-ciliated POEC during luteal phase.

COCs were collectively characterized into 4 types based on their accumulation and arrangement of cumulus cells around the oocytes. The co-existences of these 4 types of COC were found in all follicular fluid samples collected, even from the similar follicle sizes. However, these 4

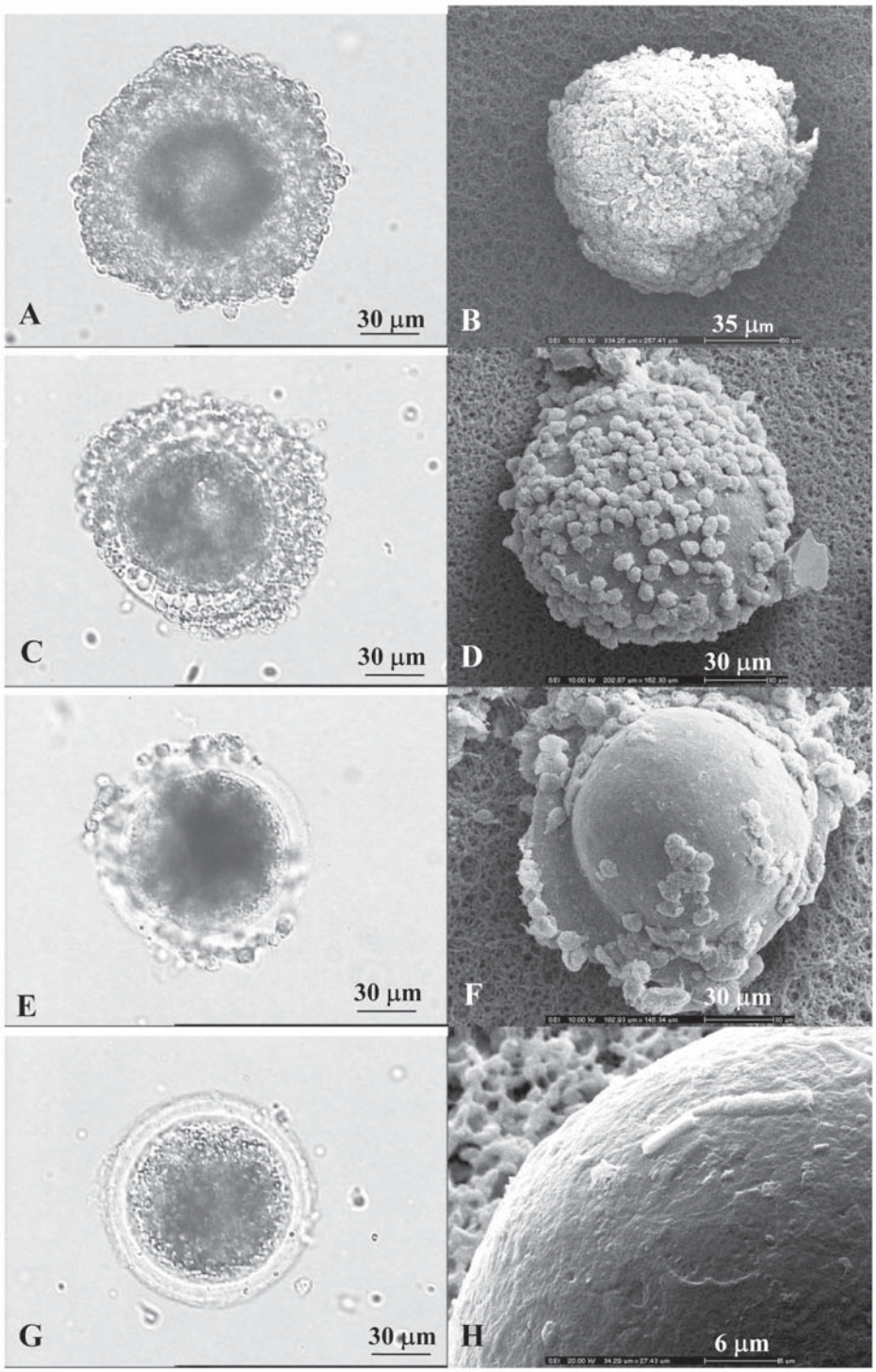

Figure 3 Micrographs and scanning electron micrographs of COCs, (A,B) intact cumulus cell layer, (C, D) single cumulus cell layer, (E,F) partial cumulus cell layer, (G,H) completely denuded oocyte.

Table 1 Classification of COCs based on the types of cumulus cells surrounding oocyte.

Types	No. of COCs	(%)
Intact cumulus cell layer	183	19.87
Single cumulus cell layer	167	18.13
Partial cumulus cell layer	266	28.88
Completely denuded oocyte	305	33.12
Total	921	100.00

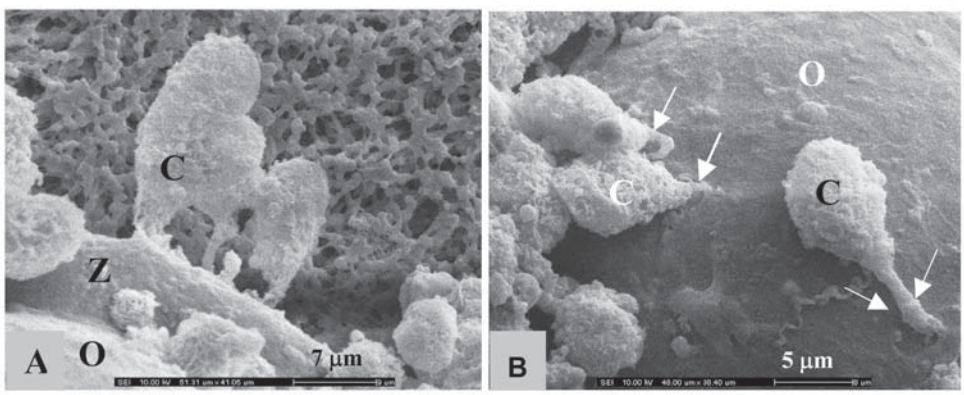

Figure 4 Scanning electron micrographs of COCs, (A) teardrop-like structure of cumulus cells attached to the oocytes surface (cumulus cells: C, zona pellucida: Z, oocytes :O), (B) showing the conical end (arrow) pointed towards the oocyte membrane.

types of COC could be selectively used for different experimental purposes. The partial cumulus cell layer type and the completely denuded oocyte are considered more mature in their natural stage of development and ready for sperm penetration. As for culturing oocyte cells to reach the maturation, Mori *et al*. (2000) found that intact cumulus cell layer type and single cumulus cell layer type had higher potential to become matured oocytes. These types of COC were successfully cultured in the artificial medium supplemented with follicular stimulating hormone (FSH) and leutinizing hormone (LH) using cell samples from rat (Magnusson, 1980), sheep (Staigmiller and Moor, 1984), bovine and swamp buffalo (Kitiyanant *et al.*, 1989, 1995).

Cumulus cells are known to transmit low molecular weight substances, i. e., ion nucleotides and amino acids to oocytes in the young non-reproductive females (Dekel and Beers, 1980). These substances are collectively called oocytes maturation inhibiting factor (or meiosis arresting factor) which arrest the oocyte development at the diplotene stage of prophase I, thereby preventing the primary oocytes from progressing to secondary oocytes (Eppig, 1993). Further study on these molecular secretion from cumulus cells and the oviductal epithelial cells in culture could render us more information on the use of these cells for fertilization control.

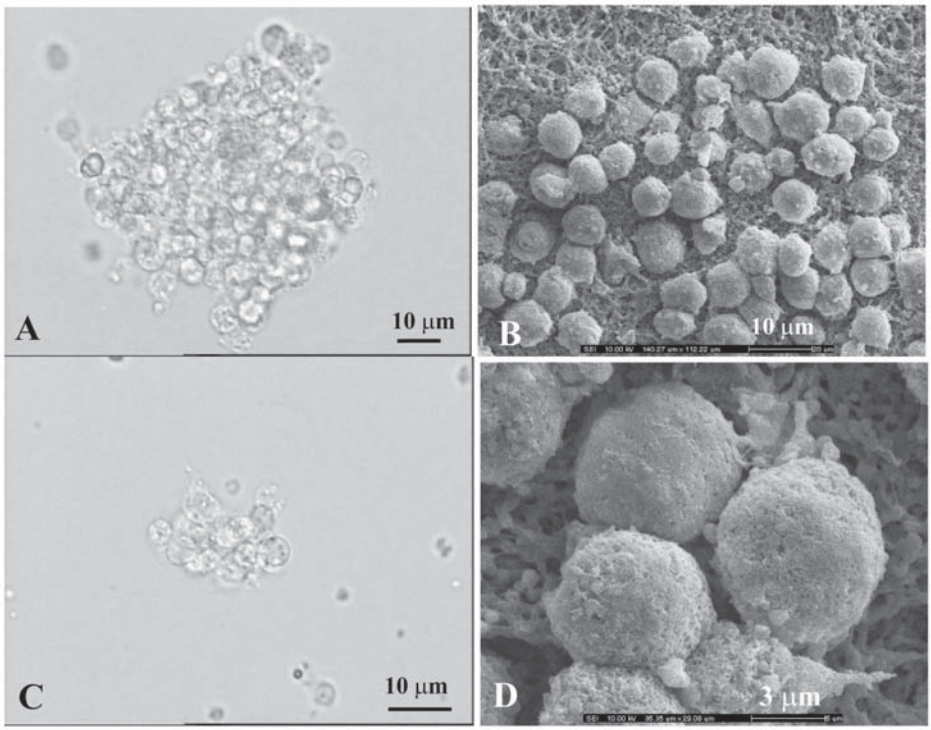

Figure 5 Micrographs (A,C) and scanning electron micrographs (B,D) showing the round shape cumulus cells in follicular fluid (free-floating cumulus).

CONCLUSION

These findings indicated that POEC changed both the morphological features and the population of cell types during the estrus cycle. At follicular phase, POEC contained the greater number of long ciliated cells than at luteal phase. The luteal phase, however, was filled up with numerous round shaped non-ciliated cells having short microvilli on the apical surface.

COCs could be collected from the antral follicle of porcine. They were classified according to the surrounding cumulus cells into 4 types, i. e., intact cumulus cell layer, single cumulus cell layer, partial cumulus cell layer, and completely denuded oocytes at the percentage composition of 19.87, 18.13, 28.88 and 33.12%, respectively. The first two types of COC could be further developed into matured eggs in culture while the last two types were too advanced in their developmental stages and became deteriorated in culture. The first layer of cumulus cells attached to the oocyte membrane were teardrop-like in shape having the conical ends pointed towards the surface membrane while free-floating cumulus cells in the follicular fluid were round in shape and were found both as single cell or forming a monolayer. These cumulus cells could be used as feeder cells for *in vitro* fertilization. Their biochemical compositions and secretion are being further investigated for the best use of them.

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