

# Evaluation on the phagocytosis of apoptotic spermatogenic cells by Sertoli cells *in vitro* through detecting lipid droplet formation by Oil Red O staining

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## Abstract

During spermatogenesis, more than half of the differentiating spermatogenic cells undergo apoptosis before they mature into spermatozoa. Ultrastructure studies showed that the formation of lipid droplets in Sertoli cells was associated with phagocytosis of residual bodies and apoptotic germ cells by Sertoli cells. Here, a relationship between the phagocytosis of apoptotic spermatogenic cells and lipid droplet formation in Sertoli cells was studied *in vitro* by Oil Red O (ORO) staining. The results confirmed that the formation of lipid droplets was a result of phagocytosis of apoptotic spermatogenic cells in Sertoli cells. By comparing phagocytosis of apoptotic spermatogenic cells and thymocytes by Sertoli cells to that by macrophages, we demonstrated that the lipid droplets accumulation in phagocytes depended on phagocytosed apoptotic cell type, but not phagocyte type. However, the size of lipid droplets was related to the type of phagocytes. By this approach, we found that Sertoli cells at different postnatal stages of development had a similar phagocytic ability. These results suggested that the detection of lipid droplets by ORO staining was a practical method to evaluate the phagocytic functions of Sertoli cells *in vitro*. This approach could also be considered as an *in vitro* model to study the lipid formation, metabolism, and function in Sertoli cells.

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## Introduction

Spermatogenesis is a complex process of cellular differentiation by which a spermatogonial stem cell is gradually transformed into a highly differentiated haploid spermatozoon. In this process, spontaneous apoptotic death of spermatogenic cells was observed during postnatal first wave spermatogenesis and adult life (Allan *et al.* 1992, Packer *et al.* 1995, Blanco-Rodriguez & Martinez-Garcia 1996). As an intrinsic homeostasis, it had been estimated that 25–75% expected sperm yield were lost during spermatogenesis (Oakberg 1956, Huckins 1978, Johnson *et al.* 1983, Dym *et al.* 1994). In later stage of spermiogenesis, the cytoplasmic portions of elongated spermatids are shed and formed residual bodies during extrusion of differentiated sperm into the lumen of the seminiferous tubule (Kerr & de Kretser 1974, Chemes 1986, Jegou 1991). As the only somatic cell type in seminiferous epithelium, Sertoli cells play critical roles to endocytose and degrade residual bodies and apoptotic spermatogenic cells

(Russell & Clermont 1977, Chemes 1986, Pineau 1991, Miething 1992). Although the effect of phagocytosis on testis function has not been intensely analyzed, one report showed that the phagocytic elimination of apoptotic cells was necessary for healthy spermatogenic cells to proceed through spermatogenesis (Ren & Savill 1998).

In the early years, the evaluations on phagocytosis by Sertoli cells were based on the observations of lysosomes and lipid inclusions in testis section by electron microscope. In rat, the number of lysosomes in Sertoli cells showed cyclic variations during the cycle of the spermatogenesis. A few lysosomes were presented in Sertoli cells at stages I–IV of the seminiferous epithelium and progressively increased to be numerous at stages VI–IX, the time of spermiation and forming residual bodies. After spermiation, the number of lysosomes rapidly decreased (Kerr & De Kretser 1975, Chemes 1986, Ueno & Mori 1990). Lipid inclusions in Sertoli cells also showed a cyclic variation based on the stages of seminiferous epithelium (Kerr *et al.* 1984, Ueno &

Mori 1990). However, the timing of cyclic appearance of lipids was different from that of lysosomes. Maximum lipid contents in Sertoli cells appeared at stages IX–XIV of the spermatogenic cycle, then lipid numbers were dramatically declined at stages I–III and remained low level at stages IV–VIII.

In recent years, an *in vitro* model to investigate the phagocytosis of germ cells by Sertoli cells was established (Shiratsuchi *et al.* 1997, 1999). Using this method, apoptotic spermatogenic cells labeled with fluorescence were co-cultured with primary isolated Sertoli cells, and the mechanisms of the phagocytosis by Sertoli cells were studied. One problem with this *in vitro* model was a complication to distinguish germ cells phagocytosed by Sertoli cells from those adhered to the Sertoli cells (Shiratsuchi *et al.* 1997), which made difficult to evaluate the phagocytic function of Sertoli cells.

Here, we introduce a new *in vitro* approach that is applicable to evaluate the phagocytosis of apoptotic spermatogenic cells by Sertoli cells through detecting lipid droplets using Oil Red O (ORO) staining. Using this method, we investigated the phagocytic function of Sertoli cells at different developmental stages postnatal, and the lipid formation after the phagocytosis of apoptotic germ cells and thymocytes by Sertoli cells and macrophages respectively.

## Materials and Methods

### Animals

All C57BL/6 mice used in this study were obtained from the Laboratorial Animal Center of Beijing University (Beijing, China) and maintained in a temperature- and humidity-controlled room on a 12 h light:12 h darkness cycle. They had free access to food and water. All the measures taken for the mice were in accordance with approved guidelines (*Guideline for the Care and Use of Laboratory Animals*) established by the Chinese Council on Animal Care.

### Isolation of Sertoli cells and preparation of apoptotic spermatogenic cells

The procedure for the isolation of Sertoli and spermatogenic cells was based on previous description (Cheng *et al.* 1986) with a modification. Briefly, mice were anesthetized with CO<sub>2</sub> and then killed by cervical dislocation. Decapsulated testes were incubated with 0.5 mg/ml collagenase (Sigma) at room temperature for 15 min with gentle oscillation, and then were filtered through 80 µm copper meshes to eliminate interstitial cells. Seminiferous tubules were resuspended in the collagenase at room temperature for 20 min to remove myoid cells. The tubules were then incubated with 1 mg/ml hyaluronidase (Sigma) for 25 min with gentle oscillation and pipetting. The cells were washed three

times with F12/Dulbecco's modified Eagle's medium (DMEM; Gibco), and plated on culture dish in F12/DMEM supplemented with sodium bicarbonate (1.2 mg/ml), penicillin (100 U/ml) and streptomycin (100 µg/ml), and 10% fetal calf serum (Gibco). The cells were maintained in a humidified atmosphere of 95% air:5% CO<sub>2</sub> (v:v) at 32 °C for 48 h. Thereafter, the spermatogenic cells suspending in medium were collected and cultured for another 2 days to induce spontaneous apoptosis as previous description (Shiratsuchi *et al.* 1997). The Sertoli cells attaching on the dish were treated with a hypotonic solution (20 mM Tris, pH 7.4) for 2 min to remove the spermatogenic cells adhering to the Sertoli cells. Twenty-four hours later, the Sertoli cells were detached with trypsin and seeded in 24-well tissue-culture plate at  $5 \times 10^4$  cells/well and cultured for an additional 24 h for phagocytosis assay. The purity of Sertoli cells was determined by immunofluorescence staining of Wilms' tumor nuclear protein 1 (WT1, a marker of Sertoli cells).

### Isolation and culture of macrophages

The procedure of macrophage preparation was performed based on a previous approach (Chong *et al.* 2005). Briefly, resident peritoneal macrophages were collected from mouse peritoneal cavities by lavage with 5 ml cold PBS containing 2% fetal calf serum. The cells were cultured in DMEM supplemented with 10% fetal calf serum on tissue-culture dishes in a humidified atmosphere of 95% air:5% CO<sub>2</sub> (v:v) at 37 °C. After 2 h, suspending cells were removed by washing with PBS. Adherent cells were then liberated and replated out at  $5 \times 10^4$  cells/well in a 24-well culture plate.

### Preparation of apoptotic thymocytes

The protocol for obtaining apoptotic thymocytes was based on a previous method (Scott *et al.* 2001). Briefly, the thymocytes were harvested from the thymus of 3-week-old C57BL/6 mice, and treated with 2 µM dexamethasone (Sigma) for 4 h to induce apoptosis. The apoptotic cells were washed with PBS, and resuspended in DMEM containing 10% fetal calf serum for phagocytosis assay.

### Phagocytosis of apoptotic cells

The procedure of phagocytosis of apoptotic cells by Sertoli cells and macrophages was performed based on previous protocol (Shiratsuchi *et al.* 1997) with modifications. The apoptotic spermatogenic cells or thymocytes ( $5 \times 10^3$ ) were added to the Sertoli cells or macrophages cultured in 24-well plates. The co-cultures were maintained at 32 °C for Sertoli cells and 37 °C for macrophages until ORO staining. Single-cultured Sertoli cells and macrophages were used as controls at each

assay point. Total 100 phagocytic cells from three repeat wells were analyzed for each point. The mean value was presented in the results.

### **ORO staining**

The co-cultures were washed in PBS by pipetting for removing suspended apoptotic cells. The phagocytic cells were fixed with 10% formalin for 40 min. After a wash with PBS, the cells were stained with ORO (Sigma) solution (ORO-saturated solution in isopropanol:water, 3:2) for 15 min as previous description (Lillie & Fullmer 1976). Then, the cells were washed with 70% alcohol for 5 s to remove background staining. Finally, the cells were rinsed in tap water, counterstained with Harris hematoxylin (10 s), and mounted in glycerol–PBS (9:1) for observation.

### **Morphometric procedures**

The amount of lipid droplets by ORO staining in Sertoli cells was quantified using a microscope image analyzer (Yali'en, Beijing, China). The area of Sertoli cell nucleus was used as a reference to that of lipid droplets. The area ratio of lipid droplets to nucleus in Sertoli cells was used to evaluate quantitatively lipids. The morphometric data were analyzed statistically with Student's *t*-test.

## **Results**

### **Lipid droplet formation in Sertoli cells after phagocytosis of apoptotic spermatogenic cells**

Sertoli cell is phagocytic and helps to remove the residual bodies and degenerating germ cells by phagocytosis *in vivo*. Such activities lead to the formation of lipid droplets that are located throughout the cytoplasm of Sertoli cell. In order to investigate whether the lipid droplets in Sertoli cells could be a criterion to evaluate the phagocytic activities of Sertoli cell *in vitro*, a study on the time course for the lipid droplet formation during co-culture of primary Sertoli cells and degenerating germ cells was carried out. Sertoli and spermatogenic cells were isolated from the testes of 21-day-old C57BL/6 mice. The purity of Sertoli cells was 95% based on the immunofluorescence staining of WT1 (data not shown). The spermatogenic cell population was a mixture of germ cells at different differentiation stages. The spermatogenic cells were cultured for 48 h to remove contaminated somatic cells that adhered to the culture dish and induce apoptosis of the germ cells. The spermatogenic cells with a viability of 80% as determined by Trypan blue staining were subjected to a phagocytosis assay through co-cultured with the Sertoli cells. In order to determine the time course of the lipid droplet formation, ORO staining was performed at 0, 6, 12, 24, and 48 h after co-culture of

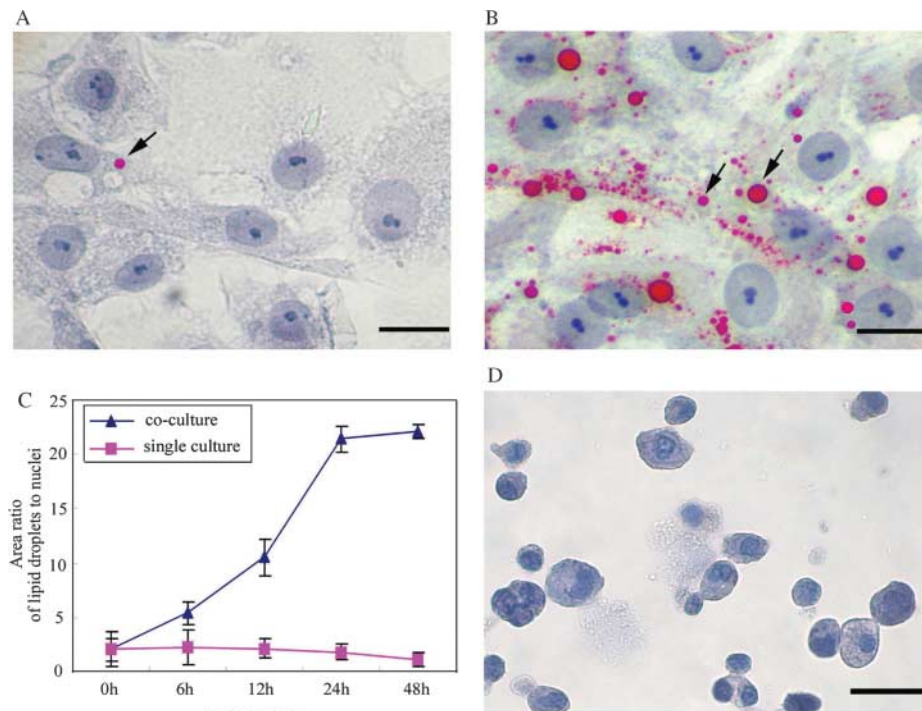
Sertoli and spermatogenic cells. At each time point, single-cultured Sertoli cells were stained as negative controls. In order to analyze lipid droplet formation in Sertoli cells in a quantitative manner, the ratios of lipid droplets area to nucleus area of Sertoli cell were determined by image analyzer. The results were presented in Fig. 1. At 0 h, the ratio was low (Fig. 1A and C). The lipid droplet formation dramatically increased after 12 h of the co-culture of Sertoli and germ cells and reached a plateau at 24 h (Fig. 1B and C). In contrast, the ratio in single-cultured Sertoli cells remained a low level in all time points and showed a slight decrease as the culture continued. Furthermore, the fact that apoptotic spermatogenic cells were not stained by ORO (Fig. 1D) excluded that the lipid droplets were from the germ cells adhered to the Sertoli cells. Taken together, we speculated that the formation of lipid droplets in Sertoli cells *in vitro* resulted from the phagocytosis of apoptotic spermatogenic cells.

### **Comparison of lipid droplet formation in Sertoli cells and macrophages after phagocytosis of different apoptotic cells**

It is well-known that macrophages and Sertoli cells can phagocytose apoptotic thymocytes and apoptotic germ cells respectively. In this study, using immunofluorescence-labeled apoptotic cells, we confirmed that the apoptotic spermatogenic cells could also be phagocytosed by macrophages and Sertoli cells (data not shown). However, bacteria (*Escherichia coli*) or yeast (*Saccharomyces cerevisiae*) inactivated by heating, common agents used to evaluate phagocytotic activity of macrophages, were not phagocytosed by Sertoli cells (not shown), suggesting a difference of Sertoli cells and macrophages in their phagocytic targets.

In order to analyze whether the formation of lipid droplets after phagocytosis was a cell-type (phagocytic and apoptotic cells) specific phenomenon or a common phenomenon, a comparison study on the lipid droplet formation in the Sertoli cells that phagocytosed apoptotic spermatogenic cells or thymocytes and in the macrophages that phagocytosed apoptotic spermatogenic cells or thymocytes was carried out respectively (Fig. 2A). Abundant lipid droplets were observed in both Sertoli cells and macrophages after phagocytosis of apoptotic spermatogenic cells, although the size of the lipid droplets showed a significant difference in these two kinds of phagocytes (Fig. 2B, lanes 1 and 3). By contrast, only a few lipid droplets were seen in the Sertoli cells and macrophages co-cultured with the apoptotic thymocytes (Fig. 2B, lanes 2 and 4). A timing of lipid droplet formation in Sertoli cells and macrophages co-cultured with apoptotic spermatogenic cells was examined. Abundant lipid droplets appeared in the macrophages as early as 6 h after co-culture with germ cells (Fig. 2B, lane 3 and Fig. 3), whereas, we did not





**Figure 1** Formation of lipid droplets after phagocytosis of apoptotic germ cells by Sertoli cells. Co-culture of Sertoli and apoptotic germ cells and single-culture of Sertoli cells were performed synchronously. Lipid droplet formation was detected at 0, 6, 12, 24, and 48 h during the culture by ORO staining. (A) Only a few lipid droplets (arrow) were observed at 0 h. (B) Abundant lipid droplets (arrow) were observed at 24 h. (C) Area ratios of lipid droplets to nucleus in co-culture of Sertoli and spermatogenic cells (—▲—) and single-culture of Sertoli cells (—■—) at different time points were measured. Each datum was from the calculation of 100 cells in three repeat wells. (D) No lipid droplet was observed in degenerating germ cells by ORO staining. Bar = 20  $\mu$ m.

observe abundant lipid droplets in the Sertoli cells until 12 h after co-culture with apoptotic germ cells (Fig. 2B, lane 1 and Fig. 3). This result might reflect more phagocytic activities of the macrophages than those of the Sertoli cells and difference in the rates of processing of phagocytosed components in the two cell types.

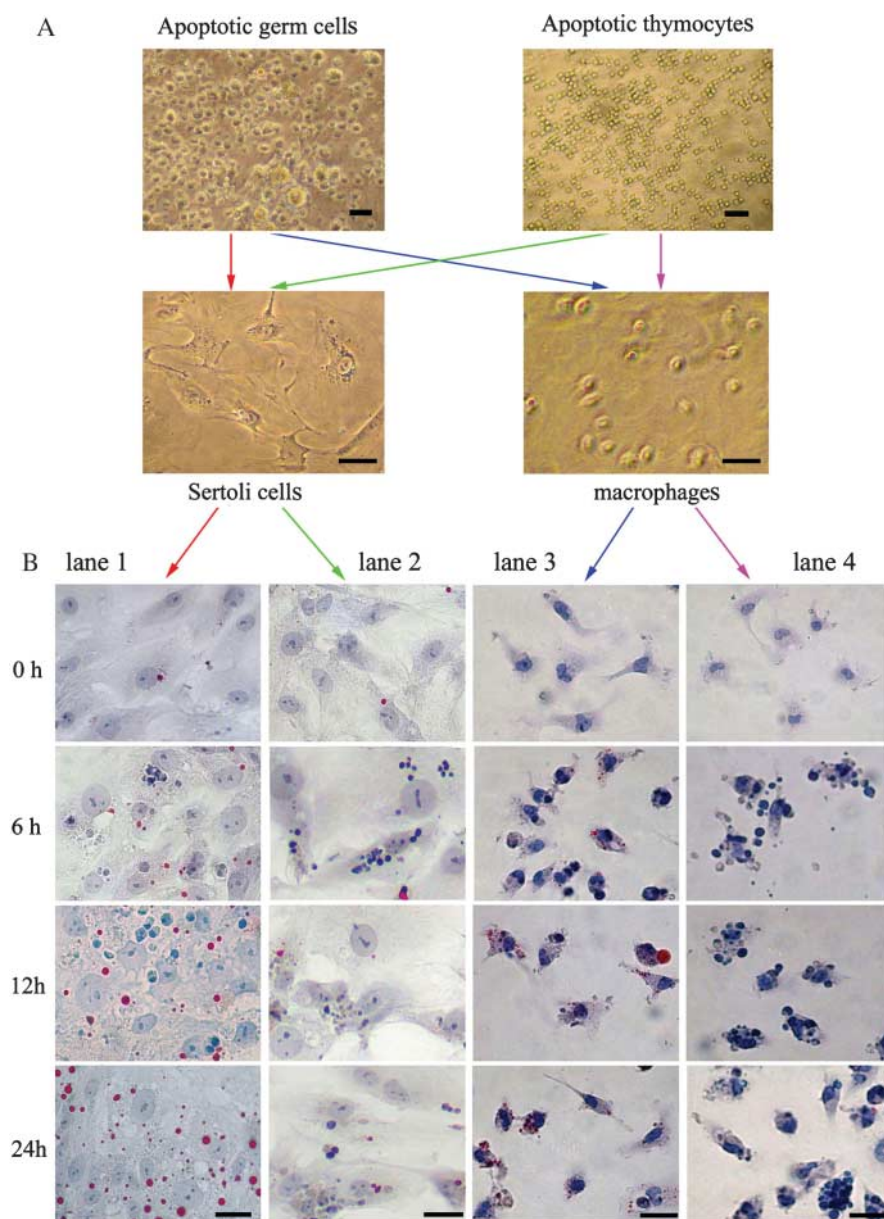
Although the number of lipid droplets in both Sertoli cells and macrophages co-cultured with the apoptotic spermatogenic cells did not show a significant difference at 24 h (Fig. 3A), the size of lipid droplets was greatly varied in these two kinds of phagocytes. We divided the lipid droplets into three types based on their diameter under light microscope (magnification  $\times 400$ ): large ( $\geq 3 \mu$ m in diameter), medium (1–3  $\mu$ m), and small ( $\leq 1 \mu$ m). As shown in Fig. 2B, we could see many large lipid droplets in the Sertoli cells (lane 1), but a few in the macrophages (lane 3). The comparison of the percentage of the three types of lipid droplets in Sertoli cells and macrophages at 24 h after co-culture with apoptotic spermatogenic cells was shown in Fig. 3B. Most of the lipid droplets in the macrophages were detected as small. Whereas in the Sertoli cells, about 16.7% lipid droplets were large, and 26.2% were medium. This result might reflect different ways of lipid metabolism in Sertoli cells and macrophages. Differences in cell size between Sertoli cells and macrophages might also create physical constraints that influence the size and number of droplets per cell that could be accommodated in the respective cytoplasm.

### Phagocytic ability of Sertoli cells in different developing stages postnatal

It was expected that the different functions would be expressed by mature adult Sertoli cells when compared with prepuberty immature, proliferating Sertoli cells, but there is a surprising lack of definitive comparative studies in the literature. Using ORO staining in this study, we analyzed the lipid-droplet formation in Sertoli cells isolated from mice of different ages with an effort to evaluate the phagocytic capacity of Sertoli cells in different developing stages postnatally. To this aim, the Sertoli cells were isolated from the testes of 1-, 3-, 5-, and 8-week-old mice and co-cultured with apoptotic spermatogenic cells from 3-week-old mice. At 24 h after co-culture, the Sertoli cells were stained using ORO, and the lipid droplets were analyzed. To our surprise, almost equal amount of lipid droplets was detected in the Sertoli cells from mice at different postnatal ages after phagocytosis of apoptotic germ cells (Fig. 4), suggesting a similar phagocytic capacity of Sertoli cells at different postnatal stages of development.

### Discussion

Spermatogenesis is a complex process, in which phagocytic clearance of apoptotic spermatogenic cells and residual bodies by Sertoli cell is necessary for healthy spermatogenic cells to proceed through spermatogenesis, although the mechanism remains to be

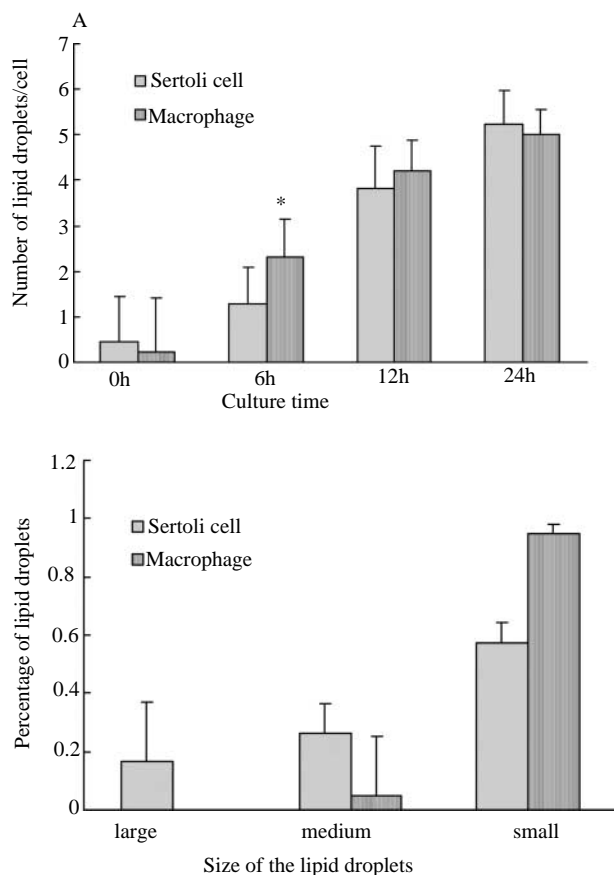


**Figure 2** Comparison of lipid droplet formation in Sertoli cells and macrophages after phagocytosing apoptotic germ cells and thymocytes. (A) Designs of the experiment and the characteristics of cells. (B) Lipid droplets were stained by ORO in the Sertoli cells after co-culture with apoptotic germ cells (lane 1) and apoptotic thymocytes (lane 2), and in the macrophages after co-culture with apoptotic germ cells (lane 3) and apoptotic thymocytes (lane 4). At 0, 6, 12, 24 h during co-culture, apoptotic cells were removed and phagocytes were stained by ORO and hematoxylin. Abundant lipid droplets were observed at 6 h after co-culture of macrophages and apoptotic germ cells and 12 h after co-culture of Sertoli and apoptotic germ cells. However, only a few lipid droplets appeared in Sertoli cells and macrophages after co-culture with apoptotic thymocytes at any time points. Bar = 20  $\mu$ m.

clarified. The methods for *in vitro* investigation of the phagocytosis of apoptotic spermatogenic cells by Sertoli cells face a problem that it is complicated to discriminate the germ cells adhered to Sertoli cells from that phagocytosed by Sertoli cells. In this study, we created a practical method of detecting lipid droplets in Sertoli cells by ORO staining to analyze the phagocytosis of apoptotic germ cells by Sertoli cells *in vitro*.

Previous studies on testis sections by electron microscope disclosed that stage-dependent formation of lipid droplets in Sertoli cells was associated with phagocytosis of residual bodies (Kerr & de Kretser 1974, Chemes 1986, Ueno & Mori 1990, Jegou *et al.* 1991) and apoptotic germ cells (Braun 1998, Pentikainen *et al.*

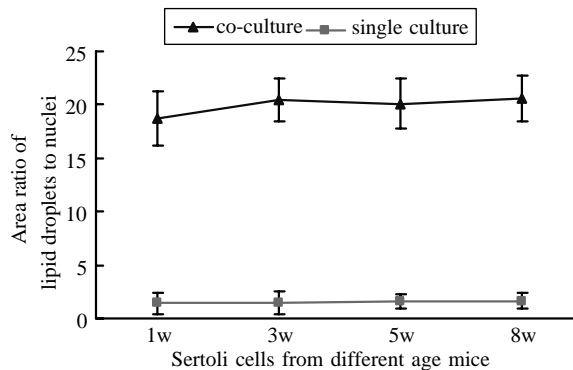
2003, Sinha Hikim *et al.* 2003). The occurrence of spermatogenic cell apoptosis at various stages of differentiation has been reported (Allan *et al.* 1992, Shikone *et al.* 1994, Brinkworth *et al.* 1995, Callard *et al.* 1995), but only a limited number of apoptotic spermatogenic cells were detected when testis sections were examined histochemically. This was probably due to rapid elimination of apoptotic cells by phagocytosis. Ultrastructure studies with rodent testis sections showed that Sertoli cells could phagocytose degenerating spermatogenic cells (Russell & Clermont 1977, Chemes 1986, Pineau *et al.* 1991, Miething 1992, Kerr *et al.* 1993). Studies *in vitro* also confirmed that rat Sertoli cell in primary cultures could phagocytose apoptotic



**Figure 3** The number (A) and size (B) of lipid droplets in Sertoli cells and macrophages after phagocytosis of apoptotic spermatogenic cells. (A) The lipid droplet numbers at 6 h after co-culture of macrophages and apoptotic spermatogenic cells were higher than that after co-culture of Sertoli and apoptotic spermatogenic cells ( $P < 0.01$ ). A similar numbers of the lipid droplets at 12 and 24 h of co-culture were shown in two phagocytes. (B) The size of the lipid droplets showed a significant difference in the Sertoli cells and macrophages. In Sertoli cells, 16.7% of the lipid droplets were large ( $\geq 3 \mu\text{m}$  in diameter) and 26.2% of them were medium (1–3  $\mu\text{m}$ ). In contrast, almost no large and a few medium lipid droplets were observed in macrophages, and 95% of the lipid droplets in macrophages belonged to small size ( $\leq 1 \mu\text{m}$ ). Each datum was from the calculation of 100 cells in three repeat wells.

spermatogenic cells (Shiratsuchi *et al.* 1997, 1999). Considering these previous observations, we might conclude that the formation of lipid droplets in our present study was a result of phagocytosis of spermatogenic cells. Detection of lipid droplet formation by ORO staining could be a practical method to analyze phagocytosis of apoptotic germ cells by Sertoli cells *in vitro*. Adhesion problem in the evaluation on phagocytic function of Sertoli cells in previous studies could be overcome by this approach. It should be noted that a quantitative relationship between measures of phagocytic activity and amount of lipid droplets has not been established in this system.

Various kinds of phagocytic cells can engulf different target cells in different tissues. We asked whether the



**Figure 4** Comparison of lipid droplet formation in Sertoli cells at different postnatal stages of development. Sertoli cells isolated from 1, 3, 5, and 8-week-old mice postnatal were co-cultured with apoptotic spermatogenic cells from 3-week-old mice. After 24 h, spermatogenic cells were removed and Sertoli cells were stained by ORO and hematoxylin. At each time point, single-cultured Sertoli cells were stained as a control. Area ratios of lipid droplets to nucleus in Sertoli cells were measured. Almost equal amount of lipid droplets were showed at different stages of Sertoli cells at 24 h after co-cultured with apoptotic germ cells. Each datum was from the calculation of 100 cells in three repeat wells.

formation of lipid droplets could be a common criterion for the evaluation of phagocytosis by other phagocytes. Then we tried to answer the question using macrophages, a well-known phagocytic cell type widely existed in many kinds of tissue and immune system (Ellis *et al.* 1991, Francavilla *et al.* 2002). The results demonstrated that the apoptotic cell-type phagocytosed by phagocytes was related to the formation of lipid droplets. We proposed that the internal membrane system of apoptotic cells determined the formation of lipid droplets. Compared to thymocytes, spermatogenic cells might contain more internal membranes, including rich lipids, such as mitochondria and Golgi apparatus. The mechanism of lipid droplet formation after phagocytosis has been explained in two different ways. Kerr *et al.* (1984) thought that an accumulation of lipid droplets after phagocytosis of residual bodies represented the resynthesis of lipids by Sertoli cells, whereas Chemes (1986) proposed that the lipid droplets were last remnants of digested residual bodies. This *in vitro* system may provide a practical approach to confirm the way of the lipid droplet formation by blocking the pathway to synthesize lipid in Sertoli cells.

A previous study showed that Sertoli cells from 20-day-old rats *in vitro* were capable of phagocytosing all types of spermatogenic cells undergoing apoptosis during spermatogenesis (Shiratsuchi *et al.* 1999). During postnatal development of testis, Sertoli cells undergo a radical switch from an immature, proliferative state to a mature, non-proliferative state at around the onset of puberty (Sharpe *et al.* 2003). Adult Sertoli cells perform dual phagocytic functions, namely phagocytosing apoptotic spermatogenic cells and residual bodies from elongated spermatids. A comparison study on the phagocytic ability



of Sertoli cells in different development stages has not been reported. Our present study demonstrates for the first time that Sertoli cells from different age mice have a similar ability in the phagocytosis of apoptotic spermatogenic cells. Lipid droplets were first reported as the Sertoli cell component by the original discoverer of the cell (Sertoli 1865), the amount of the lipid droplets varies considerably between stages of spermatogenesis, as well as between species. It has been hypothesized that lipid droplets in Sertoli cell are evidence to recycle lipids from the residual bodies and degenerated germ cells (Russell 1993). This study provided a direct evidence to support this hypothesis.

Accumulated lipid droplets in Sertoli cells can be used for nourishing germ cells. It has been reported that Sertoli cell lipids were absorbed into spermatids during certain stages of spermatogenesis, in which the spermatids are embedded in Sertoli cell cytoplasm (Lynch & Scott 1951, Lacy 1962). Other studies reported a transfer of these lipid inclusions from the Sertoli cells to primary spermatocytes (Kerr & De Kretser 1975). Several recent studies showed that the accumulation of excess lipid droplets in Sertoli cells resulted in impaired spermatogenesis in mice deficient in genes related to lipid metabolism or transfer such as the nuclear receptors, retinoid X receptor- $\beta$  (RXR $\beta$ ) (Mascres *et al.* 2004), liver X receptor- $\beta$  (LXR $\beta$ ) (Robertson *et al.* 2005), transcriptional intermediary factor 2/glucocorticoid receptor-interacting protein 1 (TIF2/GRIP1), a nuclear receptor co-activator (Gehin *et al.* 2002), Cnot7, a regulator of RXR $\beta$  (Nakamura *et al.* 2004), ATP-binding cassette transporter 1 (ABCA1), a transporter that shuttled excess cholesterol and phospholipids out of the cells (Selva *et al.* 2004), and multifunctional protein 2 (MFP-2), which was associated with peroxisomal  $\beta$ -oxidation (Huyghe *et al.* 2006). Thus, a balance of the metabolism of lipids in Sertoli cell is critical to maintain normal spermatogenesis. The question of lipid metabolism in this cell type remains open. The *in vitro* model in this study could be useful to investigate the cycle of lipid formation in Sertoli cells and lipid function in regulating spermatogenesis.

In summary, a practical *in vitro* method has been developed to investigate phagocytic function of Sertoli cells through detecting the lipid droplet formation by ORO staining. Using this approach, we demonstrated that the lipid droplet formation depended on the apoptotic cell type, but not the phagocyte type. The results of the present study showed a similar phagocytic ability of the Sertoli cells at different developing stages postnatally. This approach could also be a practical model to study the lipid formation, metabolism, and function in Sertoli cells.

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