Poster Presentation (PF-17)

The Development of Crude Testicular Cells in In Vitro Culture

Wahono Esthi Prasetyaningtyas[1*], Ni Wayan Kurniani Karja[2], Srihadi Agungpriyono[1], Mokhamad Fahrudin[1]

[1]Department of Anatomy Physiology and Pharmacology, [2]Department of Clinic Reproduction and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University.
Jl. Agatis Kampus IPB Dramaga 16680 INDONESIA
*Corresponding author's email: wah.esthi@gmail.com

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INTRODUCTION

Spermatogenesis is a continuous process in which spermatogonial stem cells (SSC) develop into specific germ cells before terminally differentiating to form spermatozoa. The process is supported by Sertoli cells, which are in close contact with germ cells in the seminiferous tubules. Sertoli cells provide essential hormonal signals, nutrients, and physical support to germ cells for successful spermatogenesis.

The crude testicular cells (CTC) contains many cell types, like Sertoli cell, Leydig cell, spermatogonial stem cell (SSC), spermatocyte and other testicular somatic cells (Shah *et all.* 2016). Testicular cells are believed to secrete various growth factors that induced the spermatogenesis process. The spermatogonial stem cells are unique population of cells in the male testis, which dual function. First self-renewing their population to maintain the number of stem cells, secondary function is differentiating into spermatids in testis (Wang *et al.* 2015).

Spermatogenic cells differentiation needed the similar microenvironment in vivo spermatogenesis. The essential nutrients was collected from healty culture and the culture contained mixed population of cells both the somatic cells and spermatogenic cells. To identification the spermatogenic cells using Periodic Acid Schifft (PAS) staining (Chang *et al.* 2011). The present study examined the development of crude testicular cells using PAS staining.

MATERIALS AND METHODS

Testicular cell isolation. Crude testicular cells were prepared from testicular tissue of 5-day-old male mice. The testes of 5 males were removed from the body and placed in 0.5% Trypsin EDTA solution for 10 minutes (Lacham-Kaplan *et al.* 2006). The animal used was approved by the animal ethics commite of FKH IPB (SKEH Nomor: 089/KEH/SKE/III/2018).

Cell culture. Cultures using DMEM medium was supplemented with 10% FCS, 1% non-essential amino acids, 1 % antibiotic-antimicotic and, and 1 % ITS. The petri dish was coated with gelatin 0.1%. The cells were incubated at 37^0C in 5% CO_2 in air.

Periodic Acid-Schiff (PAS) staining. Fixation in 100% methanol for 10 min at -20^0C, wash with cold PBS 3 times. Stain for 5min with periodic acid, and wash 3× with PBS. Then, stain cells with Schiff's reagent for 5 min, wash 3 times with PBS, counterstain for 90s with hematoxylin solution, and wash 3 times with PBS prior to microscopic examination and imaging. The percentage of positive cells were calculated under a microscope.

RESULT AND DISCUSSION

The culture contained mixed the population of cells with both somatic cells and testicular cells include SSC. The culture of crude testicular cells for 6 days (Fig 1). Growth of this cell can be seen in table below.

Table 1. The development of crude testicular cells in in vitro culture

Treatment	Day 0	Day 3
Concentration	1.86×10^6	4.98×10^7
Viability	96.1%	91.4%
PDT	0.63 hari	

The cells proliferation rate was high in the culture (Table 1, Fig 1), because spermatogenesis is a very productive system in the body. Spermatogenesis is a male germ cell production process that occurs in the seminiferus tubules of the postnatal testes. In mammalian testes, more than 20 million sperm per gram of tissue are produced every day, this high productivity depends on the presence of spermatogonial stem cells (SSCs).

The development of testicular cells in culture can be observed using PAS staining. The morphology of spermatogonia cells was spherical and colored is weakly positive using PAS staining. Another observed cell is a cell with a wide cytoplasm which having many nucleoli that are thought to be Sertoli cells and negative with PAS staining. In addition cells were observed like

fibroblast cells whose nucleus was stained PAS with a weak concentration (Fig 2).

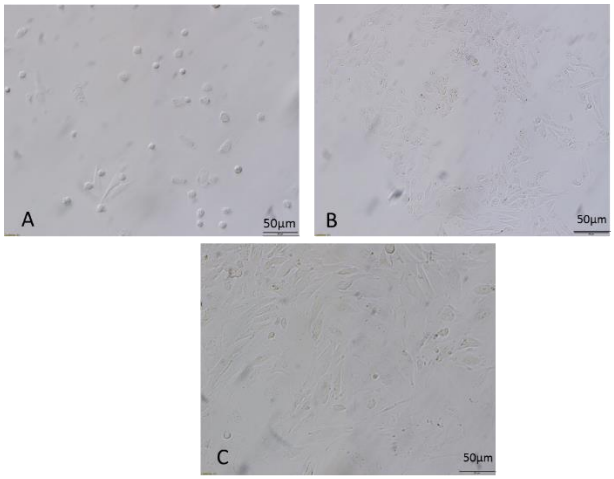

Figure 1. Development of crude testicular cells in in vitro culture 1 day (A), 3 day (B), and 6 day (C)

The development of testicular cells is observed by counting the number of defferent morphological cells and positive staining from day 1, day 3 and day 6. Identification of testicular cells using PAS staining is not specific, to determine cell types using specific immunohistochemistry staining. The percentage of composition of the testicular cells showed in Figure 3.

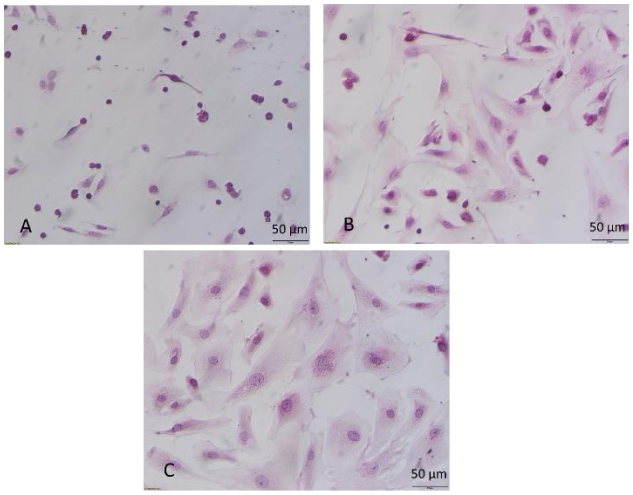

Figure 2. Development of testicular cells using PAS staining 1 day (A), 3 day (B), and 6 day (C)

The number of Sertoli cells like increase on day 1 was 22,1%, day 3 was 34,6% and day 6 was 50,1%. The number of spermatogonia cells like decreased on day 1 was 54.3%, day 3 was 30,4 and day 6 was 18,6%. The fibroblast like cells increase on day 1 was 15,5%, day 3 was 28,8% and day 6 was 26,2. The other cells under 10 % in the population.

The number of spermatogonia cells like decreased because some of these cells are transformed into other cells and some remain as spermatogonia. Spermatogonial stem cells are estimated only 0.03%-0.05% in adult mouse testis (Wang *et al.* 2015). The number of fibroblast like cells was increase, the spermatogonia cells in culture can be differentiated into neuron like cells or fibroblast like cells in vitro culture. The Sertoli like cells had the higher percentage because this cells act as regulators of spermatogenesis.

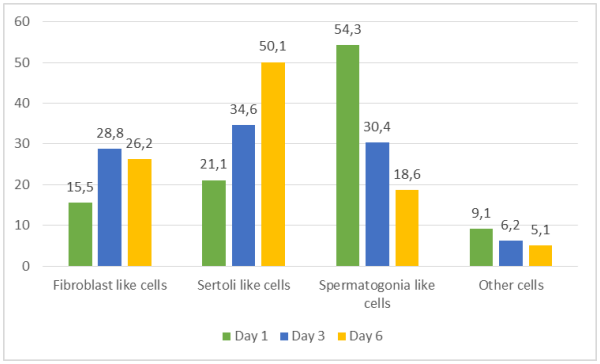

Figure 3. Graph of the composition of testicular cell development

CONCLUSION

Identification of testicular cells using PAS staining is not specific. The percentage of Spermatogonial like cells decreased in vitro cultures.

ACKNOWLEDGMENTS

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