Isolate Leydig Cells at Different Development Stages and Comparison of Function and Morphology

Huajian Tian [1, 2, a], Qihao Zhang [1, 2, b,*]

[1]College of Life Science and Technology, Jinan University, Guangzhou 510632, China

[2]Institute of Biomedicine, Jinan University, Guangzhou 510632, China

*Corresponding Author: Qihao Zhang; Phone: +86-20-85222601

[a]36640340@qq.com, [b]tqhzhang@jnu.edu.cn

Abstract

Leydig cells，situated in the testicular interstitium，produce the steroid hormone in males. There are four main cell types are present in Leydig cell linage. In each stage, Leydig cells have different shape and express different maker protein. To study more about the function of Leydig cells in different development stages, we used Precoll Density Gradient Centrifugation to isolate primary Leydig cells from SD rats at different postnatal day(PND). 3β-HSD staining was used to identify the purity of Leydig cells. Radioactive immunity assay was used to detect the synthesis ability of steroid hormone of Leydig cells. We compared the morphology of Leydig cells in SD rats at different PNDs by HE staining. The above study provide us further understanding of Leydig cells.

Keywords

Leydig cells; Development stages; Progesterone; Testosterone.

1. Introduction

Leydig cells are the testosterone-producing cells of the testis[1]. The undifferentiated mesenchymal-like stem cells that present in the interstitial compartment of the testis develops into Leydig cells [2]. There are four distinct stages of Leydig cell development: stem Leydig cells (SLCs), progenitor Leydig cells (PLCs), immature Leydig cells (ILCs) and adult Leydig cells (ALCs)[3]. The SLCs usually form after postnatal day(PND) 7. They are undifferentiated cells that are capable of indefinite self renewal. Most SLCs differentiate to PLCs. These spindle-shaped cells are luteinizing hormone (LH) receptor positive, have high mitotic activity, and produce little testosterone but rather testosterone metabolites.The PLCs give rise to immature Leydig cells (ILCs) which are round, contain large amounts of smooth endoplasmic reticulum, and only produce some testosterone but very high levels of progesterone[4]. A single division of these cells differentiate to adult Leydig cells (ALCs), which are terminally matured cells with ability of producing high levels of testosterone[5].

2. Experimental detail

2.1 Materials

PND21 SD male rats; PND35 SD male rats;PND56 SD male rats.

2.2 Reagent formulation

PBS buffer

Chemicals	Amount
NaCl	16 g
KCl	0.4 g
KH2PHO4	0.4 g
Na2HPO4	7.62 g
Ultra-pure water volume to	2000 mL

D-buffer

Chemicals	Amount
M199	1 pack
Na2HCO3	0.71 g
HEPES	2,1 g
BSA	1 g
SBTI	25 mg
ddH2O	900ml

Percoll buffer

Chemicals	Amount
HBSS	100ml
Na2HCO3	0.35 g
HEPES	2,1 g
BSA	2.5 g
SBTI	25 mg
ddH2O	800ml

Leydig cells Medium(LCM)

Chemicals	Amount
DMEM/F12	1 pack
Na2HCO3	1.2 g
BSA	1 g
ddH2O	800ml

2.3 Experimental Procedure

A: Isolation of Leydig cells

Rats were euthanasia, take testes from SD rats, wash in PBS and remove the membrane.

Put testes into 5ml D-buffer in a clean tube, add 5ml collagenase IV.

Water bath the tube at 37℃ to digest the tissue for 10mins.

End digestion by add D-buffer to the tube.

Filter the mixed liquor through mesh 200 into a clean tube, discard the residue.

Centrifuge at 250g 10mins, discard the supernatant.

Dilute the Percoll with Percoll buffer and add into the tube, resuspend the sediment.

Frozen centrifuge 13000rpm for 40mins.

Transfer the middle layer to a clean tube, dilute with D-buffer.

Centrifuge at 250g for 10mins, discard the supernatant.

Add LCM into the tube and resuspend the cell sedimentation, plant cells in petri dish.

B: 3β-HSD staining to identify the purity of Leydig cells

Add a drop of cell suspension on slide glass.

Dry the slide glass at room temperature.

Add 3β-HSD staining solution on slide glass, avoiding light for 20mins.

Exam the positive rate by using fluorescence microscope.

C: Steroid hormone detected by radioimmunoassay

Progesterone (P) or testosterone(T) levels of PLC, ILC, ALC were measured by I125 RIA Kits (Beijing North Institute of Biological Technology, Beijing, China) according to the manufacturer's instruction. All samples were measured in duplicate in one assay.

D: HE staining

1. Testes were embedded in paraffin and cut to sections (5μm)

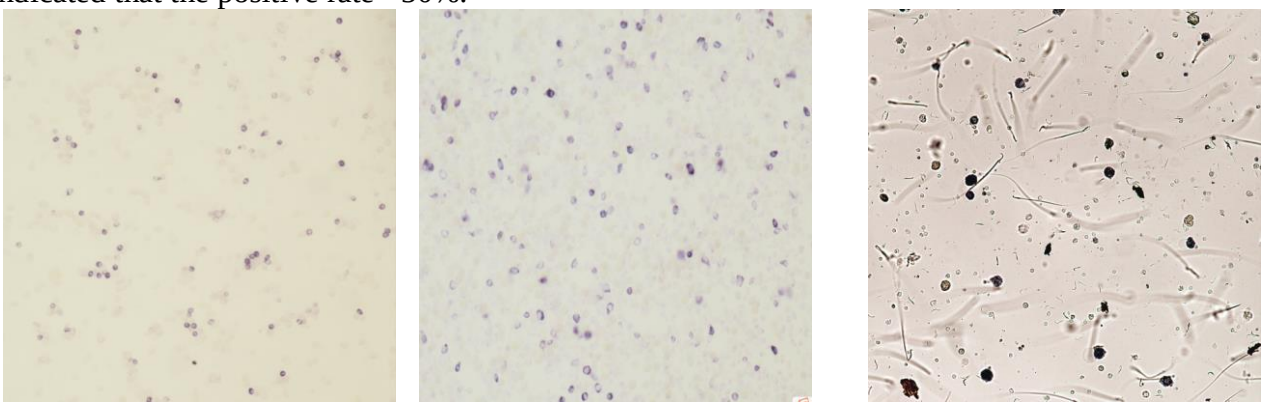
2.Tissue sections were stained with henmatoxylin and eosin for histological observation.

2.4 Statistical analyses

All data are presented as mean±SD and statistically significant were determined by one-way ANOVA. The differences were considered significant if the P value was less than 0.05.

3. Results and discussion

3β-HSD staining result showed the positive rate of the Leydig cells we isolated. The positive cells were stained to deep purple. From Fig.1, we can calculate the purity of PLC, ILC, ALC. The result indicated that the positive rate> 50%.



PLC (PND21) ILC (PND35) PND56 (ALC)

Fig.1 3β-HSD staining of PLC, ILC, ALC (200x)

We used radioimmunoassay to detect the level of testosterone, progesterone of PLC, ILC, ALC. The result showed that PLC only can produce progesterone. Testosterone began to be produced in ILC and ALC have high ability to produce Testosterone.

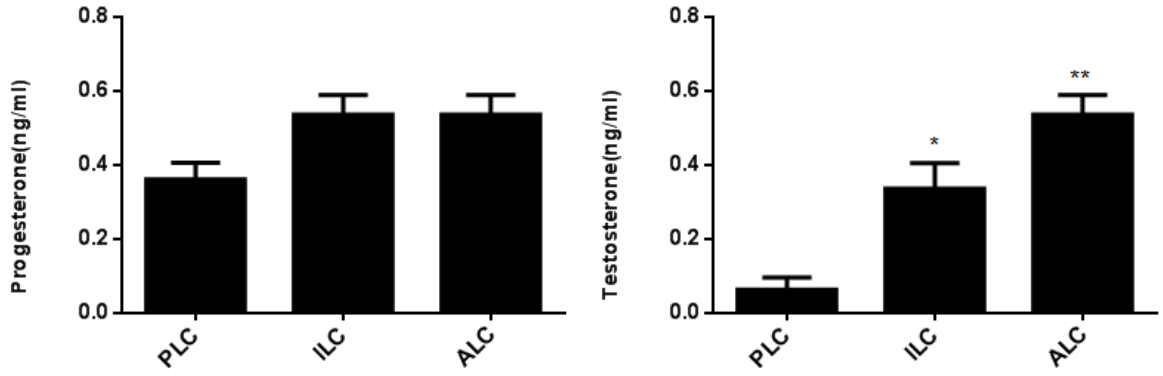
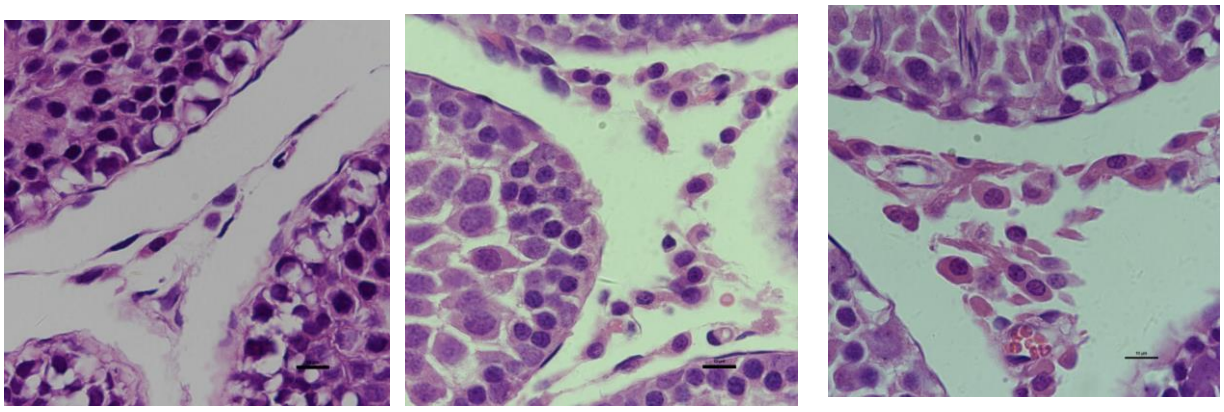


Fig.2 Level of progesterone and testosterone of PLC, ILC, ALC. n= 3.
*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the control group.

Through testis tissue HE staining, we can observe the morphology of Leydig cells at different stages. The result showed clearly that PLCs were long and spindle shape. ILCs became oval and still long. ALCs were bigger and round.



PLC (PND21) ILC (PND35) PND56 (ALC)

Fig.3 HE staining of PLC, ILC, ALC (1000x)

4. Conclusion

In this study, we successfully isolated the PLCs, ILCs, ALCs from PND21, PND35, PND56 SD rats respectively. The results of 3β-HSD staining showed that the purity of Leydig cells we isolated was over 50%. After we cultured three days, we detected the testosterone and progesterone. The results indicated that progesterone can be produced in different development stages and PLCs lacked of ablity to produce testosterone. The ability of synthesis steroid hormone was different in these three development stages. The morphology of Leydig cells was observed by HE staining. The results showed that PLCs, ILCs and ALCs have different shape of their own, in conclude, Leydig cells in different stages have different function and morphological characteristics.

Acknowledgements

The work was supported by grants of the National Natural Science Foundation of China (No. 31271607, 81373313), the Science & Technology Major Project of Guangdong province (No. 2012A080201010, 2013B051000063) and the Guangdong Province Higher Vocational Colleges & School Pearl River Scholar Funded Scheme.

References

[1] Haider S G. Cell biology of leydig cells in the testis[J]. Int Rev Cytol, 2004, 233: 181-241.

[2] Chen H, Irizarry R A, Luo L, et al. Leydig cell gene expression: Effects of age and caloric restriction[J]. Exp Gerontol, 2004, 39(1): 31-43.

[3] Chen H, Ge R S, Zirkin B R. Leydig cells: From stem cells to aging[J]. Mol Cell Endocrinol, 2009, 306(1-2): 9-16.

[4] Zhang Y, Ge R, Hardy M P. Androgen-forming stem leydig cells: Identification, function and therapeutic potential[J]. Dis Markers, 2008, 24(4-5): 277-286.

[5] Midzak A S, Chen H, Papadopoulos V, et al. Leydig cell aging and the mechanisms of reduced testosterone synthesis[J]. Mol Cell Endocrinol, 2009, 299(1): 23-31.