*Journal of Chemical Health Risks*

Journal of Chemical Health Risks (2017) 7(2), 113–118

**ORIGINAL ARTICLE**

# Ovarian Expression of Sox2 during Mouse Estrous Cycle

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*(Received: 7 January 2017 Accepted: 16 March 2017)*

**KEYWORDS**

Ovarian Tissue;
Estrous Cycle;
Sox2;
Mouse

**ABSTRACT:** The transcriptional factor Sox2 regulates the expression of some of the developmental genes, which are essential for the maintenance of pluripotency of stem cells. Sox2 also expresses in the female gamete during folliculogenesis, but its role remains ambiguous. The aim of this study was to investigate the expression of Sox2 in the mice ovarian tissue during different stage of estrous cycle. Adult National Medical Research Institute (NMRI) mice were considered as pro-estrous, estrous, met-estrous and di-estrous based on the cell type of the vaginal smear. Immunohistochemical staining of Sox2 marker was performed in mice ovarian tissue. Immunohistochemical staining revealed the expression of Sox2 in the cytoplasm of corpus luteum cells, stromal cells and oocyte. Our results suggest that adult mice ovaries accommodate cells carrying stem cell features.

## INTRODUCTION

Sox2, a member of the Sox HMG box family of transcription factors (SRY-related HMG box gene 2), regulates stemness and pluripotency in embryonic stem cells and plays important roles during early embryogenesis [1]. Sox2 expresses during early embryogenesis in the morula, inner cell mass, epiblast, and germ cells. This suggests a role for it in the maintenance of pluripotentiality [2]. Sox2 are required in the lineage leading to epiblast formation, whereas, in its absence trophectoderm is formed. However, Sox2 alone is required for extraembryonic ectoderm [3].

More recently, expression of Sox2 was detected in cytoplasm of ovarian somatic cells. Sox2 also expresses in the cytoplasm of stromal cells during folliculogenesis [3] but its role remains ambiguous. Furthermore, Sox2 commutes between the cytoplasm and nucleus of oocyte at early embryogenesis period. Presence of Sox2 protein in the cytoplasm of growing oocytes led to stop acting of it in the nucleus and implying the role of it in post fertilization period [3].

The expression of Sox2 demonstrates its potential role for the control of specific gene expression in embryonic stem cells. Oct4 was expressed in uterus and ovaries of mouse during reproductive cycles [4, 5].

This study aimed to investigate the expression pattern of Sox2 at protein level during the different phases of

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the mouse normal estrous cycle (i.e., proestrous, estrous, metestrous and diestrous phases) in ovarian tissue.

## MATERIALS AND METHODS

### *Animals and experimental design*

This study was performed in accordance with the guidelines of the "Care and Use of Laboratory Animals." Experimental protocols were reviewed and approved by the Ethical Committee of the School of Biology, Damghan University, Damghan, Iran, ensuring compliance with the Declaration of Helsinki as revised in Tokyo 2004.

Adult virgin female mice from the NMRI (National Medical Research Institute) (6-8 wk old; n=12), were purchased from Pasteur Institute, Iran. The animals were housed in an animal house under controlled humidity and temperature conditions in a 12 h/12 h light/darkness cycle with free access to food and water. The animals were classified as proestrous, estrous, metestrous, and diestrous based on vaginal cytology (three mice per group). The stage of estrous was determined by cytological evaluation of vaginal smears as described previously [6]. In brief, vaginal smears were prepared by inserting the tip of a plastic pipette filled with 10 µL of PBS into the vagina and repeatedly pipetting and flushing. The samples were fixed in 100% methanol and stained with methylene blue. The smears were then evaluated using a light microscope under 10× and 40× objective lenses (Nikon, Japan).

Three types of cells were recognized. The round and nucleated cells were the epithelial cells, the irregular ones without nuclei were the cornified cells, and the small round cells were the leukocytes. After that, the mice were sacrificed and their ovaries were removed.

### *Histological studies*

The excised ovaries were collected and fixed in 4% paraformaldehyde for 12 h at 4 °C. Subsequently, the samples were dehydrated in an ethanol series of ascending concentrations, cleared in xylene, and embedded in paraffin wax. Then, 5 µm-thick transverse sections of tissue were obtained and stained with hematoxylin–eosin (H&E). Tissue images were attained with a camera coupled to a light microscope (Nikon, Japan).

### *Immunohistochemistry*

The embedded paraffin sections of ovarian tissues (5 µm thicknesses) were mounted on poly-L-lysine-coated slides, deparaffinized in xylene, and then hydrated in descending grades of ethanol. These sections were washed with distilled water for 6 min. Afterward, the sections were boiled in deionized water supplemented with 0.01 M sodium citrate antigen retrieval solution (pH = 6) using a microwave oven at 720, 360, and 180 W in 3 min intervals. After cooling, the sections were washed with Tris buffer saline containing 25% Triton-X100 for 5 min and then treated with 10% normal goat serum in PBS for 1 h to prevent non-specific staining. Subsequently, the sections were incubated overnight at 4°C with primary antibodies for Sox2 (anti-Sox2 antibody, Abcam, ab97959) diluted in PBS and 2% Tween in compliance with the manufacturers' instructions. For the negative control, the primary antibody was omitted, and the samples were washed thrice with PBS buffer at 5 min intervals. The sections were incubated with goat anti-rabbit IgG fluorescein-conjugated secondary antibodies (FITC, AP132F, CHEMOCON) at 37°C for 1 h and then washed with PBS for 5 min. Finally, the sections were mounted with Antifade Vectashield mounting medium containing 4′,6-diamidino-2-phenylindole and visualized under a fluorescent microscope (Nikon Eclipse, E600, Japan). Analysis was performed independently by investigators blinded to the identity of the slides.

## RESULTS

### *Vaginal cytology*

The cytological appearances of the cells recovered from mouse vaginal washing corresponded to the four stages of the estrous cycle are shown in Figure 1. At the proestrous stage, the vaginal opening was reddish pink, swollen, and wet. The vaginal smears showed

few cornified epithelial cells and few leukocytes with dominant nucleated epithelial cells (Figure 1a). At the estrous phase, the vaginal opening became lighter pink, less wet, and less engorged, and the cytological appearance of the vaginal smear showed predominantly anucleated cornified epithelial cells (Figure 1b). At the metaestrous stage, the vaginal opening with pale and unswollen appearance was not open. Furthermore, equal proportions of leukocytes and cornified and nucleated epithelial cells were detected in the vaginal smears (Figure 1c). At the diestrous stage, the vaginal orifice was small and closed without swelling. Furthermore, a high proportion of leukocytes, some nucleated epithelial cells, and mucus were observed in the vaginal smears at this stage (Figure 1d).

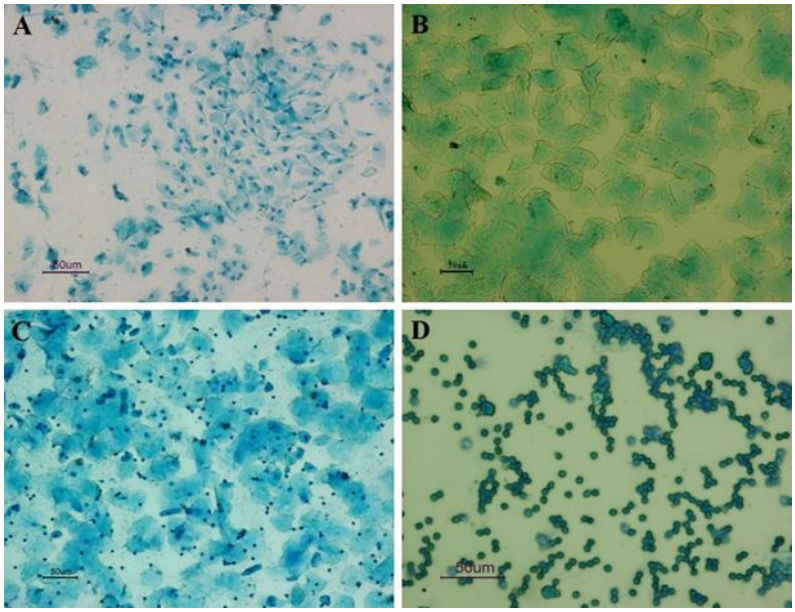


**Figure 1.** Photomicrographs of stained vaginal smears from mice at different phase of estrous cycle (A) Proestrous, mostly comprising of nucleated epithelial cells; (B) Estrus, with predominantly anucleated cornified cells; (C) Metestrous, containing of, leukocytes, cornified, and nucleated epithelial cells; and (D) Diestrus, comprising largely of leucocytes.

***Histology study***

Histological findings of the H&E-stained ovarian sections at each stage of estrous cycle are shown in (Figure 2). Ovarian sections at the proestrous stage showed degenerated corpora luteum with obvious luteal cell vacuolation. Moreover, several tertiary follicles were noted at this stage. In estrous phase, several noticeable Graafian (preovulatory) follicles with freely floating oocytes were observed. In the metestrous samples, newly formed corpora lutea were detected. These bodies were characterized by small cells with eosinophilic cytoplasm and without cytoplasmic vacuolation. At the diestrous stage, the corpora lutea appeared in maximum size. Small follicles were also present at this stage.

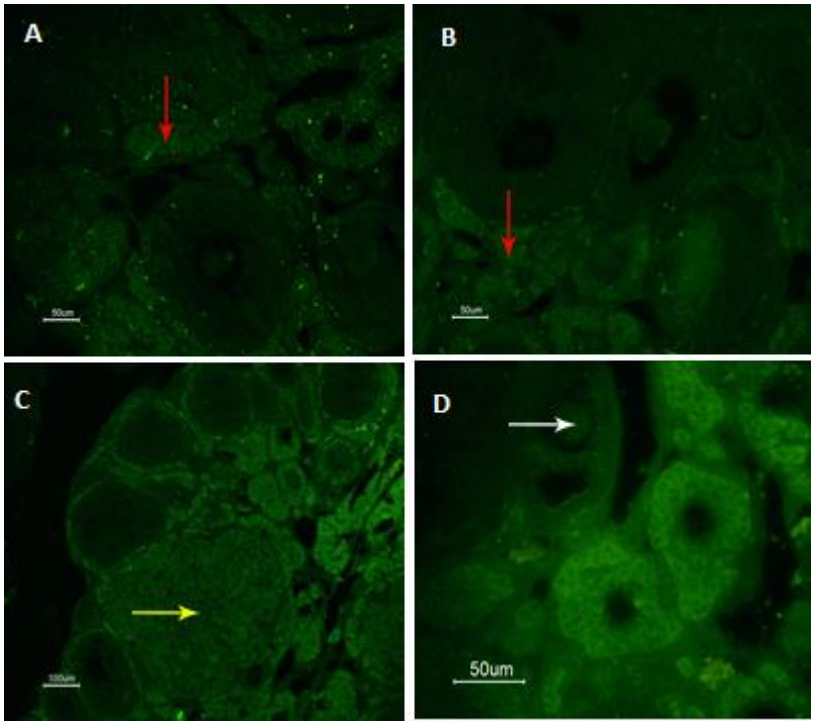


**Figure 2.** H&E stained cross section of ovary from mice at different phase of estrous phase: (A), Estrous; (B), Diestrous; (C) Proestrous and (D), Metestrous. PF, Primary Follicle; SF, Secondary Follicle; GF, Graffian Follicle; CL, Corpus luteum.

### *Immunohistochemical study*

Immunohistochemical results showed that Sox2-specific staining was detectable in the cytoplasm of oocyte. In addition, the Sox2 proteins were ubiquitously expressed in the cytoplasm of luteal cells. Furthermore, the cytoplasm of the ovarian stromal cells in all stages of estrous cycle was immunopositive for the Sox2 proteins (Figure 3).

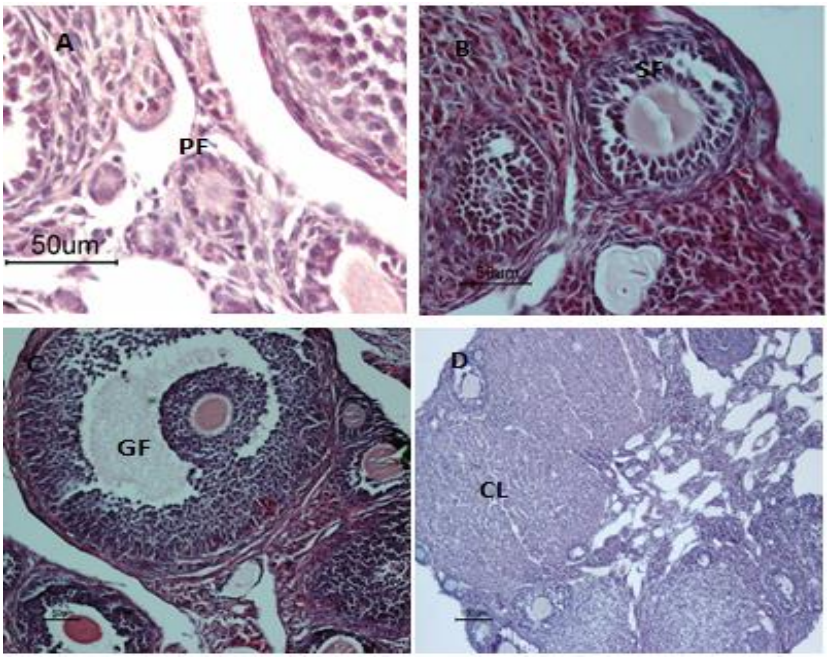


**Figure 3.** Expression of Sox2 in the ovarian tissue section from mice at different stage of sterous cycle. (A) Proestrous; B, Estrous; C, Metestrous; D, Diestrous; oocyte (white arrow), Luteal Cells (yellow arrow), stromal cells (red arrow).

## DISCUSSION

Sox2 is a key regulator for maintaining the pluripotency and self-renewal of embryonic stem cells and contributes to the reprogramming of differentiated somatic cells back to a pluripotent stem cell state [1].

The present study revealed expression of Sox2 at different stages of the estrous cycle in the cytoplasm of stromal cells and oocytes of adult mice. This finding is consistent with previous results [1, 3, 4]. The presence of Sox2 in the cytoplasm of growing oocytes suggests that the protein prevents the cells from acting in the nucleus. Meanwhile, after fertilization, the maternal Sox2 actively enters the nucleus by the two-cell stage to participate in the regulation of embryonic development before implantation [3]. Therefore, the presence of Sox2 in the ovaries of adult mice corresponds to the insurance production of maternal Sox2 during early embryonic development [1, 3, 7-10]. The expression of Sox2 indicates its potential role for the control of specific gene expression in embryonic stem cells. As such, Sox2 has a role in regulating transcriptional programs to maintain embryonic stem cell pluripotency [8, 11, 12]. This observation is consistent with the results of the previous study [4, 5], showing a similar pattern of Oct4 expression in the cytoplasm of stromal cells and luteal cells of the corpus luteum. Considering the multiple roles of Oct4 in oogenesis and folliculogenesis, it was speculated that Oct4 interacts with Sox2 to regulate oogenesis and folliculogenesis [4, 5]. It was suggested that stress conditions can induce expression of Oct4 in somatic cells cytoplasm [13].

## CONCLUSIONS

The hormonal secretion by interstitial, hilus, and corpus luteum cells may be considered as a stress that leads to the cytoplasmic expression of Sox2. Furthermore, it seems that some aspects of hormone synthesis pathways regulate by cytoplasmic expression of Sox2 [13].

## ACKNOWLEDGMENTS

This research project was funded by Damghan University. We are grateful to Mr. Abutaleb Koosha for his technical assistance.

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