

Effects of Single Transplantation and Multiple Transplantation of Human Umbilical Cord Mesenchymal Stem Cells on the Recovery of Ovarian Function in the Treatment of Premature Ovarian Failure in Mice

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Research

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Abstract

Background: Previous studies have reported that transplantation of mesenchymal stem cells (MSCs) from many human tissues can improve ovarian dysfunction. However, the therapeutic effects of single injection of MSCs and multiple injections of MSCs on premature ovarian failure (POF) have not been reported yet. In this study, we used long-term follow-up to study the effect of human umbilical cord mesenchymal stem cell (hUC-MSCs) on the functional recovery of mouse POF models.

Methods: In this study, we used a mouse model of premature ovarian failure induced by the combination of 120mg/kg cyclophosphamide and 30mg/kg busulfan. Enzyme-linked immunosorbent assay (ELISA) was used to detect estradiol (E2) and follicle stimulating hormone (FSH) levels in mouse serum. Evaluate ovarian function by counting follicles, ovarian weight, number of proliferating cells, anti-Mullerian hormone (AMH) and oocytes.

Results: Our study shows that hUC-MSCs have obvious therapeutic effect for the POF mice model, and treatment effect of multiple transplantation is better than single transplantation. Mesenchymal stem cells were detected in follicular granulosa cells with tracer of the ovarian tissue freezing slice, which show hUC-MSCs to selectively migration and stay in damaged tissues. Genome Array screened a number of differentially expressed genes, the hUC-MSCs can change the level of expression of certain genes in the ovarian tissue, thus affecting ovarian function, and laid the foundation for us to further explore the mechanism of hUC-MSCs treatment of premature ovarian failure.

Conclusion: This study demonstrated that hUC-MSCs transplantation significantly restored ovarian function after chemotherapy-induced damage.

Full Text

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Figures

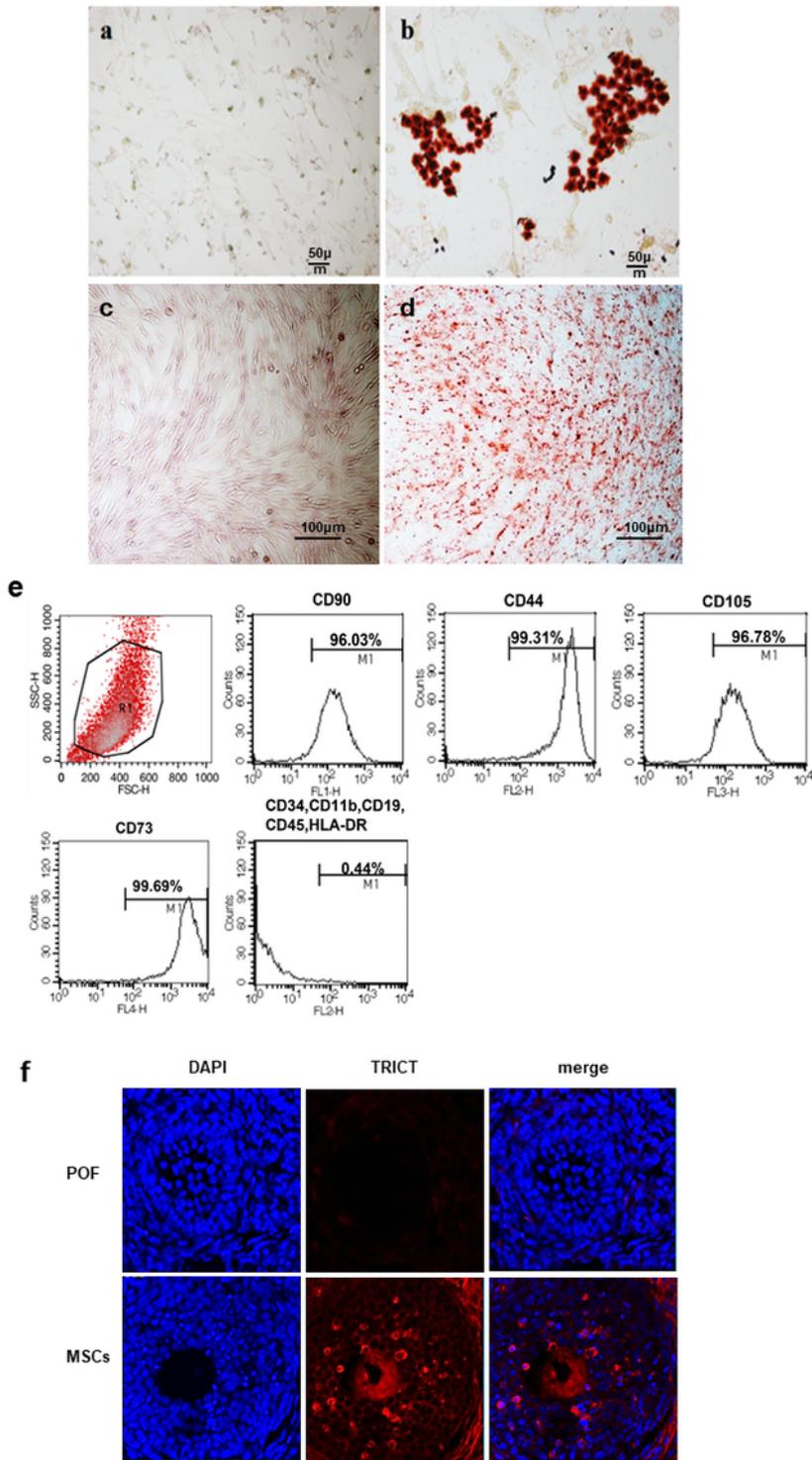


Figure 1

The characterization and differentiation of hUC-MSCs. (a,b) The hUC-MSCs differentiate into osteoblasts, scale bar = 50 μ m. (c,d) adipocytes, scale bar = 100 μ m. (e) Flow cytometric analysis of hUC-MSCs. CD44, CD73, CD90, and CD105 were positive and CD34, CD11b, CD19, CD45 and HLA-DR were negative. (f) Human nuclei antigen was negative in POF murine ovarian section, the recipient ovaries after hUC-MSCs transplantation were expressed human nuclei antigen.

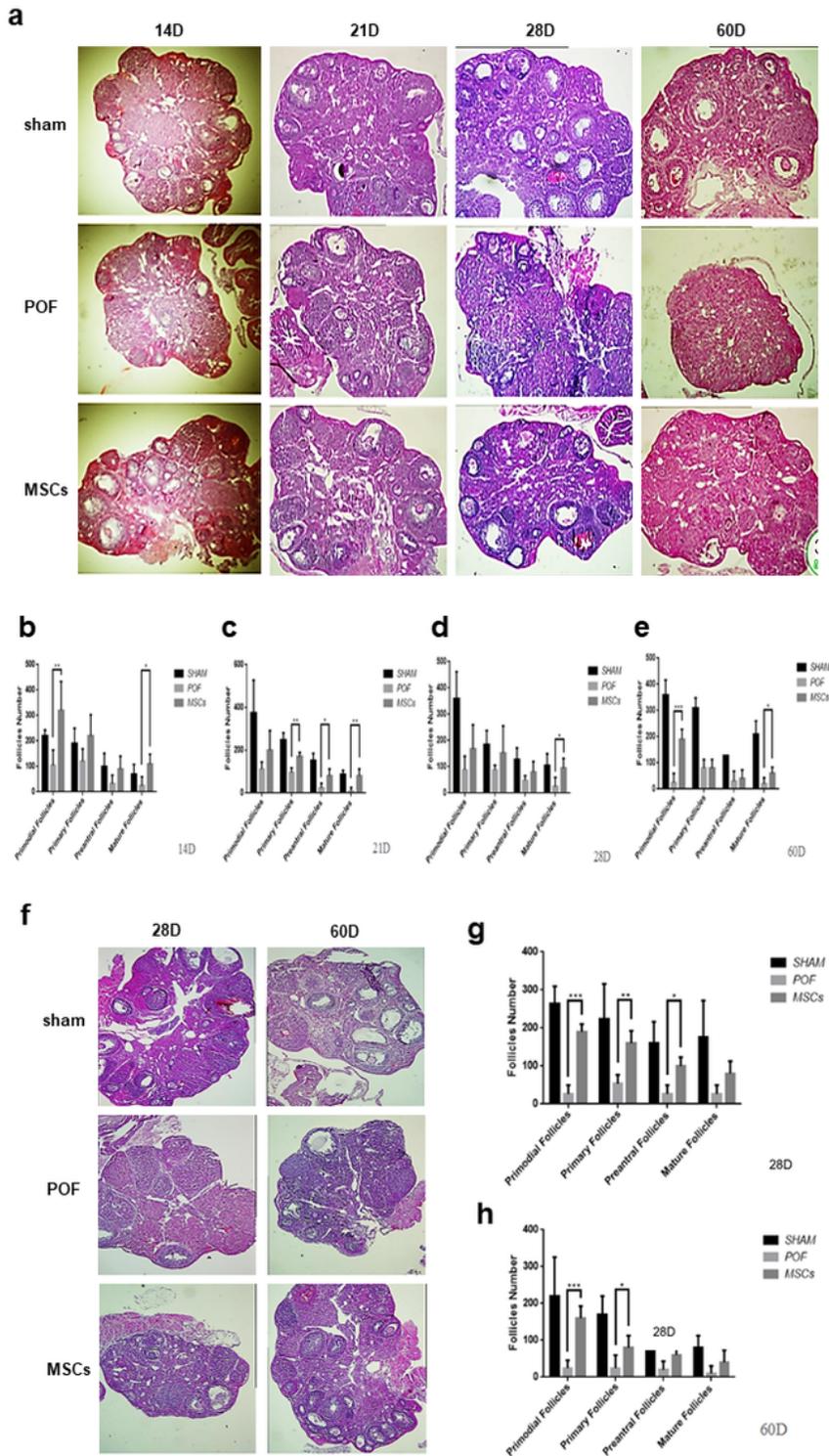


Figure 2

The effects of hUC-MSC transplantation on ovarian morphology and follicular development in the POF mouse. (a) Representative H&E micrographs of ovary sections from the once intravenous group over 60 days showing all stages of follicles in Sham group, stroma, and atretic primordial or primary follicles in POF group, primordial as well as primary and large antral follicles in MSCs group. Original magnification: 100 \times . (b) Follicle counts at all stages on the 14th day. (c) Follicle counts at all stages on the 21th day. (d)

Follicle counts at all stages on the 28th day. (e) Follicle counts at all stages on the 60th day. (f) Ovarian sections of the multiple intravenous injection group. Original magnification: 100×. (g) Morphological follicle count of mouse ovaries, the follicle counts at all stages on the 28th day. (h) Morphological follicle count of mouse ovaries, the follicle counts at all stages on the 60th day. * $P < 0.05$; * $P < 0.01$; *** $P < 0.001$.

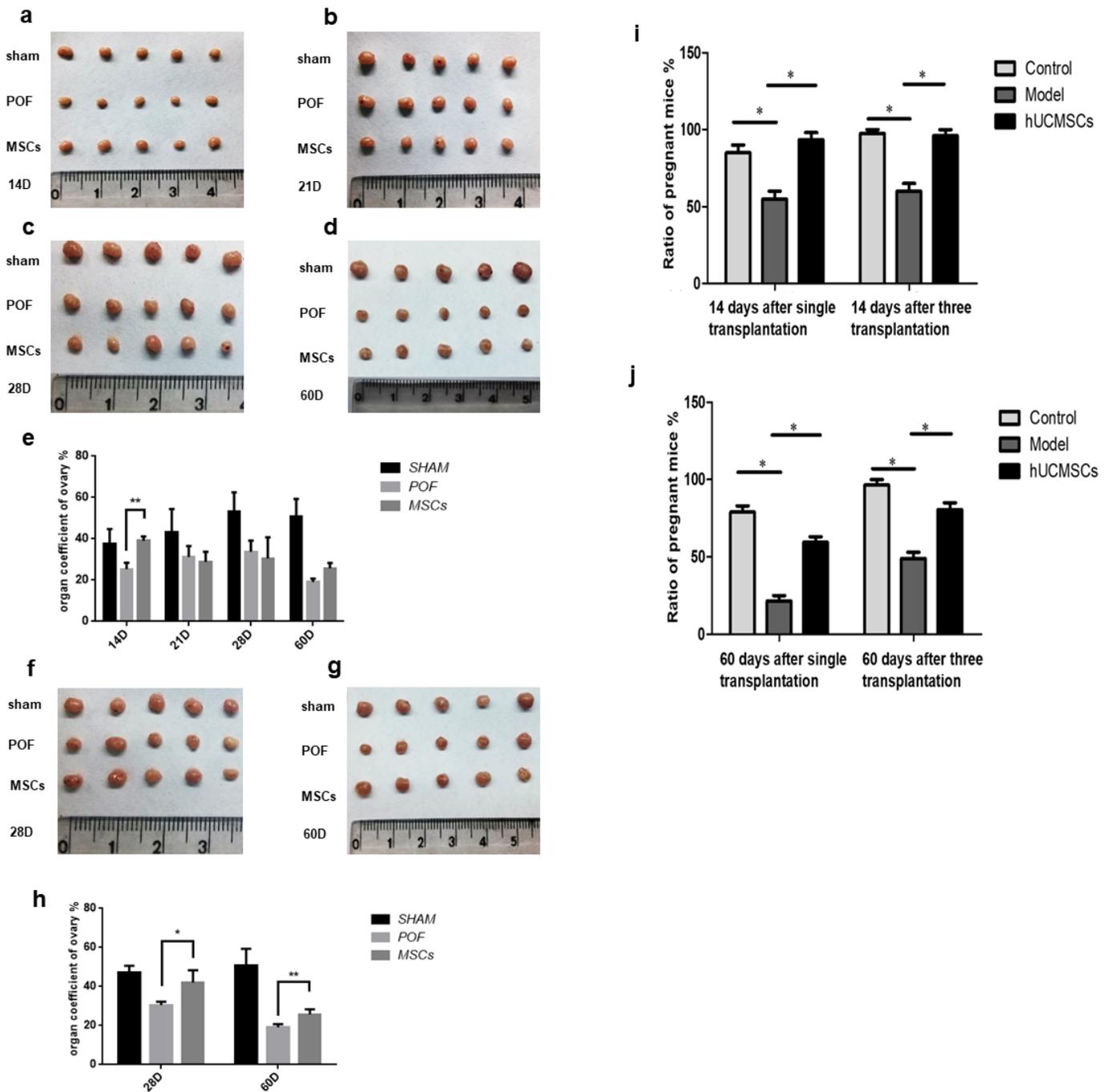


Figure 3

Changes of ovary weight in the once intravenous group and the multiple intravenous group over 60 days. (a, b, c, d) In the once intravenous group, ovarian volume of mice were remarkably increased at 7, 14, 21, 28 days post-induction, respectively. (f, g) The multiple intravenous group showed significantly increased of ovarian volume at 28, 60 days post-induction, respectively. (e, h) The histogram represents the results

of statistical analysis of ovarian weight by single and multiple injections, respectively. Organ coefficient = organ weight/weight (i, j) Changes in mouse fertility. *P<0.05; **P<0.01.

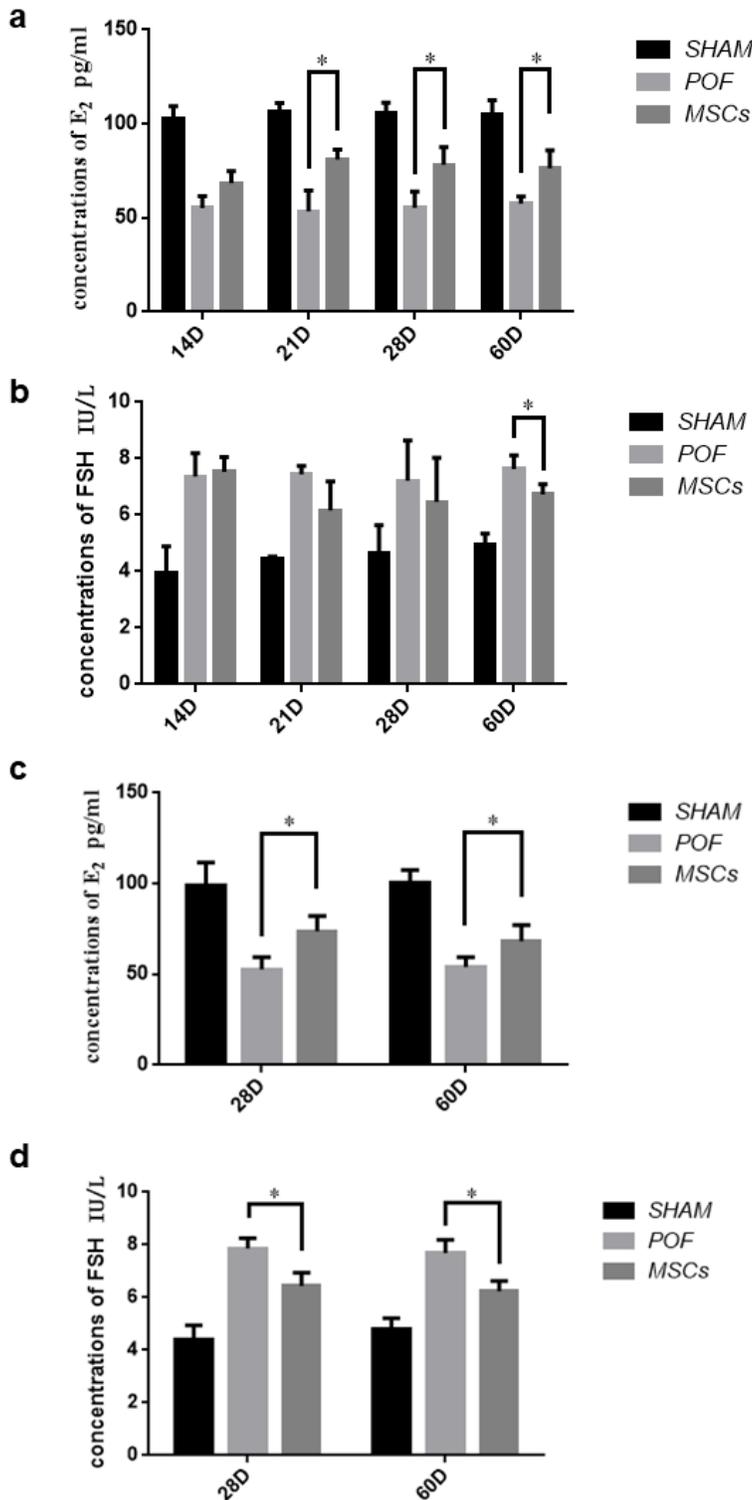


Figure 4

The effect of hUC-MSCs on the endocrine function of POF mice. (a) The hormone levels of E₂ from the once intravenous group and the multiple intravenous group. Comparing the POF and MSCs group, the level of E₂ had a significant increase at 21, 28, 60 days post-induction (P < 0.05). (b) The level of FSH

from the once intravenous group and the multiple intravenous group. It had an obvious difference at 60D. (c) In the multiple group, the levels of E2 were significant differences at 28, 60 days post-induction. (d) In the multiple group, the levels of FSH were significant differences at 28, 60 days post-induction, *P<0.05.

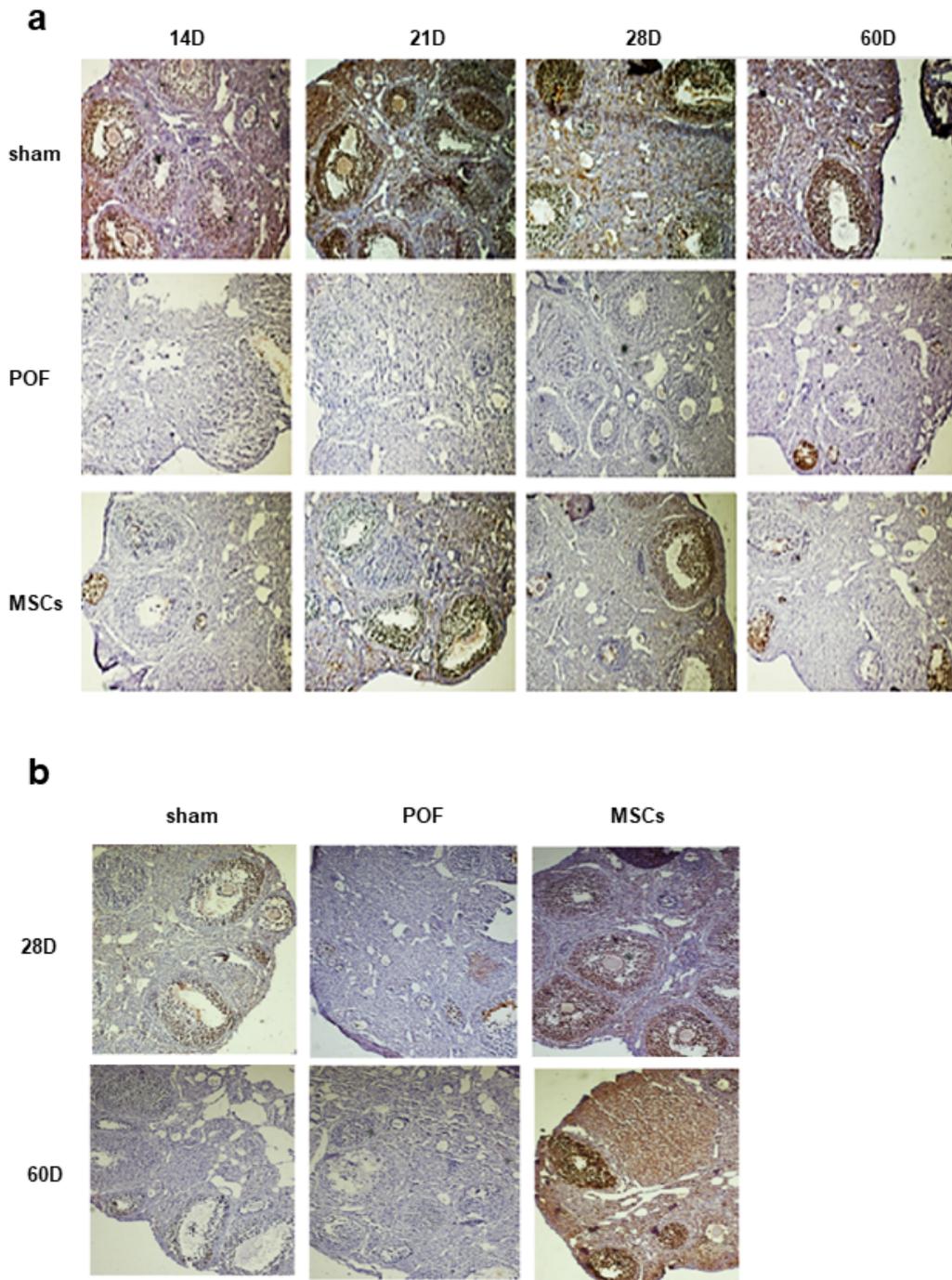


Figure 5

The ovarian reserve capacity analysis. Immunohistochemistry for AMH in once intravenous group (a) and the multiple intravenous group (b). Strong expression of AMH were seen in Sham group at each time

point, granulosa cells were negative in POF group, AMH expression reappeared in ovaries from MSCs group. Original magnification: 100×.

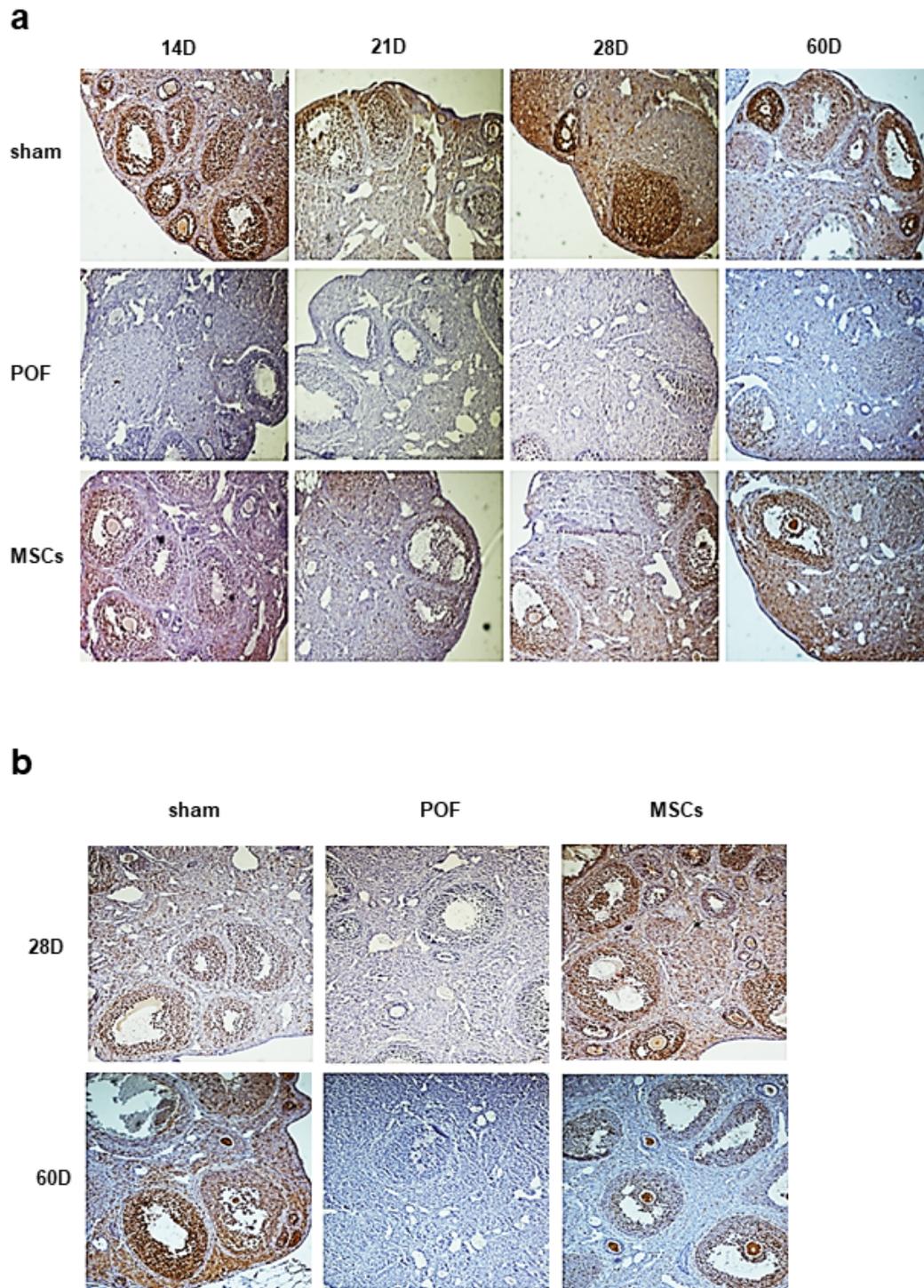


Figure 6

The comparison of ovarian proliferation potential in each group. Immunohistochemistry for KI67 in once intravenous group (a) and the multiple intravenous group (b). Sham mice follicle highly expressed KI67,

POF mice follicle lacked KI67 signal, the expression were observed in MSCs group. Original magnification: 400×.

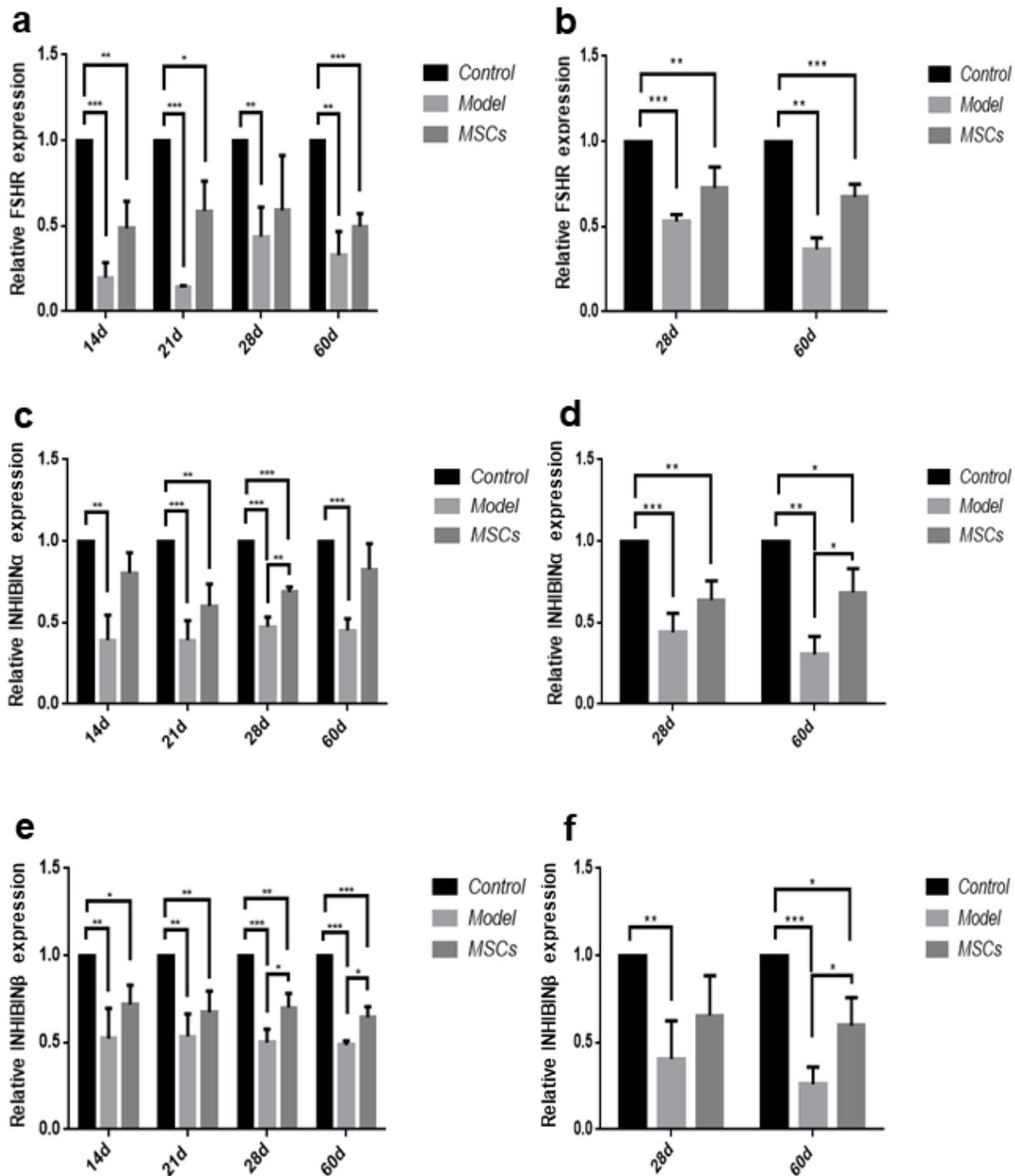


Figure 7

Effect of hUC-MSCs on the ovarian gene expression of POF mice. (a) Expression of FSHR in mouse ovary in single injection of hUC-MSCs group. (b) Expression of FSHR in mouse ovary in the hUC-MSCs group injected multiple times. (c) Expression of INHIBIN α in mouse ovary in single injection of hUC-MSCs group.

(d) Expression of INHIBIN α in mouse ovary in the hUC-MSCs group injected multiple times. (e) Expression of INHIBIN β in mouse ovary in single injection of hUC-MSCs group. (f) Expression of INHIBIN β in mouse ovary in the hUC-MSCs group injected multiple times. *P<0.05; **P<0.01; ***P<0.001.

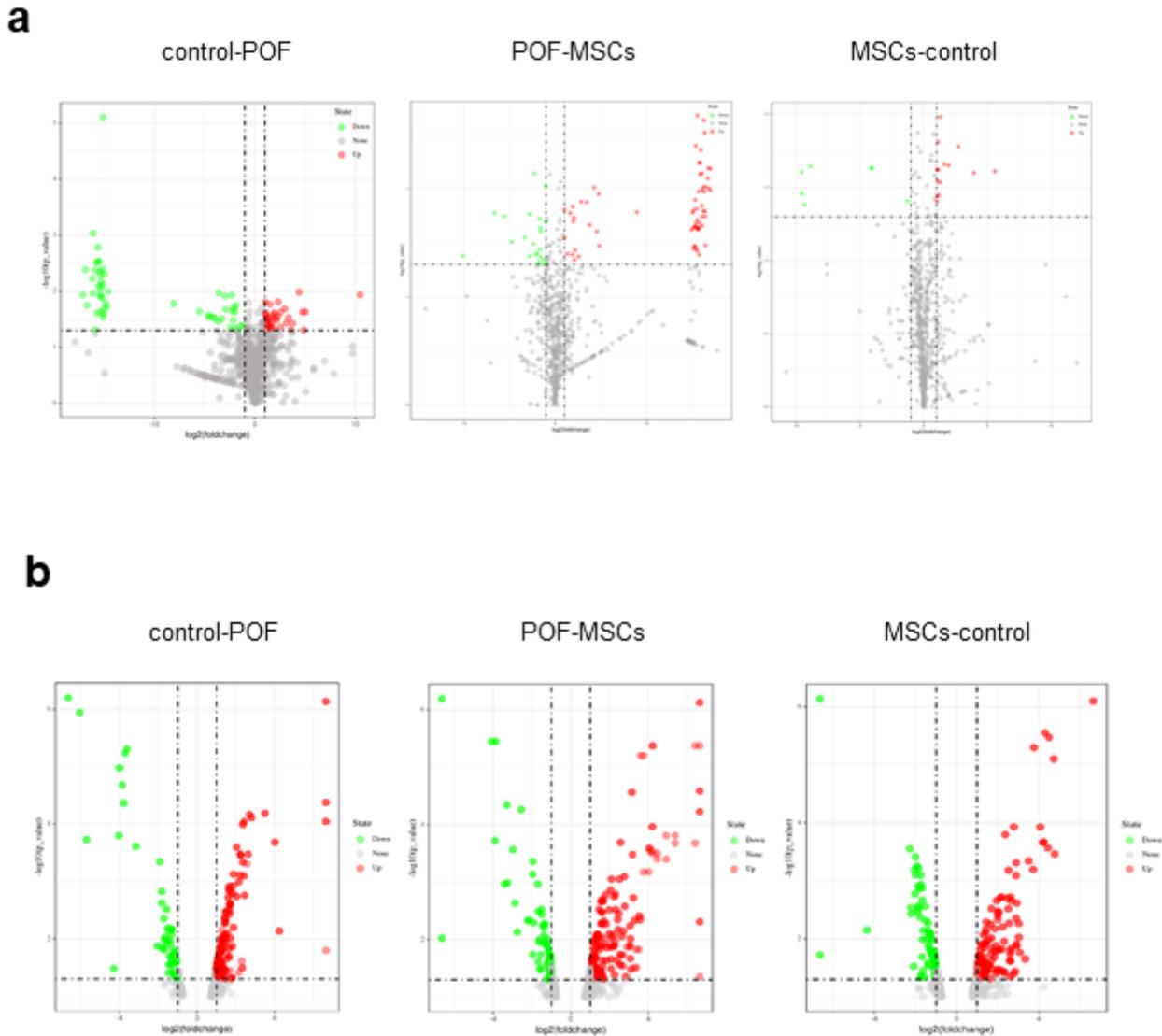


Figure 8

Grafted hUC-MSCs infiltrate the POF murine ovarian tissue. (a) Volcano map to detect differentially expressed miRNAs between different treatment groups. (b) Volcano map analysis of the mRNA chip.