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Effect of lactate and pH on mouse pluripotent stem cells: Importance of media analysis

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ABSTRACT

Pluripotent stem cells have generated a great deal of excitement concerning their use for clinical therapy and regenerative medicine. Similar to other mammalian cells, the growth of these cells is dependent on various culture conditions. The presence of lactic acid as a metabolic by-product has been reported to be detrimental for mammalian cells in most cases. However, the use of lactate as a source of energy has also been reported for certain cell types. The current study was carried out to examine the effect of media lactate concentration and extracellular pH change, two important parameters that occur due to lactic acid production, on mouse pluripotent stem cells, both embryonic and induced pluripotent stem cells. We examined both feeder dependent and feeder independent embryonic stem cell lines for a comprehensive observation. It was noted that increase or decrease in pH affected cell proliferation, viability and pluripotency of all three cell lines. The effect of lactate was less obvious. Supplementation with lactate decreased cell proliferation and cell number. However, from the data obtained, we hypothesize that the surviving cells were able to adapt to the change in environment and utilize lactate as an energy source. There was no significant effect of lactate on the pluripotency of the cells.

1. Introduction

Pluripotent stem cells have the unique properties of extensive replication in an undifferentiated state while retaining the ability to differentiate into derivatives of all three germ layers on receiving appropriate stimuli. By virtue of these properties, pluripotent stem cells have been deemed to be of immense importance in the fields of tissue engineering, regenerative medicine, drug discovery, disease modelling, etc. The field of stem cell biology has undergone enormous advancements over the past few years with extensive studies being carried out for the various intrinsic and extrinsic parameters involved. Biocompatible materials have been used to replace the feeder cells as attachment matrices [1–5]. Various growth factors, mouse embryonic fibroblast (MEF) conditioned media and specialised media have been used for the expansion and pluripotency maintenance of the cells [6–11]. Efforts have also been made to scale up pluripotent stem cell (PSC) cultures using different types of bioreactors with or without the use of 3 dimensional constructs [12–18]. The effects of various culture parameters like metabolites, pH, dissolved oxygen, etc., have also been studied [19–21].

Lactic acid, a by-product of glucose metabolism, has been reported to be one of the potential growth inhibitors for mammalian cells as early as 1958 [22]. It is known to have significant effect on growth, proliferation, metabolism, antibody production and even differentiation of various mammalian cell lines. The detrimental effect of lactic acid accumulation can be due to the presence of lactate ions or the accompanying change in pH and osmotic pressure. To date, various studies have shown the effect of all three on different cells. It is well established that the influence of lactic acid on different cell lines is diverse and is dependent on their tolerance level as well as their response to the resultant change in culture conditions. Addition of sodium lactate externally to the

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culture system with various cell lines showed that baby hamster kidney (BHK) cells have a higher lactate tolerance when compared to Hybridoma cell lines [23]. Studies have also demonstrated that apart from inhibiting cell proliferation, addition of sodium lactate or lactic acid also inhibited glucose metabolism (mainly due to increased accumulation of NADH), decreased lactate production and also in certain cases it increased the production of byproducts like antibodies Erythropoietin and recombinant proteins in different cell lines [24–27].

In recent years, stem cells have also been subjected to similar studies. It was observed that proliferation of hematopoietic stem cells ceased at a lactic acid concentration higher than 20 mM [28] while mesenchymal stem cells could tolerate a lactate concentration of no more than 10 mM [29]. Patel et al., also showed that hematopoietic stem cells are more sensitive to changes in pH rather than a change in the accumulated lactate concentration. However, Chen et al., showed that mesenchymal stem cell expansion is affected by both pH change and lactate accumulation. They also showed that increasing pH or lactate concentration significantly depressed osteogenic differentiation but promoted adipogenic differentiation of mesenchymal stem cells. Similar to this, it was reported that addition of exogenous lactic acid induced chondrogenic differentiation of dermal fibroblasts [30].

Studies have also been carried out to investigate the effect of lactate on mouse embryonic stem cells (mESC), although there are contradictions in the available data. As a part of their study of embryonic stem cell expansion in 3D matrix, Ouyang et al. [31], showed that mESC are extremely sensitive to the presence of lactic acid in media. They inferred that the growth of mESC was inhibited above a lactate concentration of 16 mM and that high lactic acid concentration affected the pluripotency of the cells. The data published by Chaudhry, Bowen and Piret [19], however, contradict this. This group showed that neither growth rate nor embryoid body formation potential of mESC were affected by increasing the initial media lactate concentration up to a value of 40 mM [19]. In another brief communication, Martinez-Outschoorn et al., [32] showed that 10 mM sodium salt of L-Lactate boosted feeder dependent ESC growth by increasing the cell colony size as well as colony numbers. Recently it has also been reported that growth and metabolism of human embryonic stem cells (hESC) are also affected by external addition of lactate to the medium. Chen et al. showed that sodium lactate concentration above 1 g/L resulted in lower cell density of hESC, along with a loss in expression level of the pluripotency marker *Tra 1-60* [33]. They also reported that lactate supplementation beyond 2 g/L decreased lactate production by the cells themselves.

By contrast, studies regarding the effect of pH on mammalian cells highlight two things. In most cases, a change in pH was detrimental to cell growth. It was reported that pH change was detrimental to cell growth and embryoid body formation capability of mouse ESC [19]. Increasing or decreasing the culture pH modulated differentiation of various stem cells [34,35]. However, detailed studies of its effects on pluripotent stem cells have not been carried until now, especially on induced pluripotent stem cells.

Here, we attempt to study the effect of the addition of exogenous sodium lactate, not only on cell growth and on pluripotency of mPSC, but also recorded its effect on cell proliferation and metabolic activity. Additionally, we studied the effect of pH change on proliferation, cellular metabolic activity and pluripotency of mouse pluripotent stem cells. We have tried to generalise the effects by including not only feeder free ESC but also studying the effect of sodium lactate and pH individually on feeder dependent mESC and mouse induced Pluripotent Stem Cells (iPSC).

2. Materials and methods

2.1. Mouse pluripotent stem cell culture

Feeder independent mESC (Oct4B2) were generated in the lab on a D3 embryonic stem cell (ESC) background and were transfected with an Oct4GFP constructs. They were maintained on 0.1% gelatin coated tissue culture dishes in ES media containing DMEM, 15% fetal bovine serum, 1X Glutamax, 1X non-essential amino acid, 1X Pen/Strep Solution, 0.1 mM 2- mercaptoethanol and 1000 U/ml LIF (Leukemia Inhibiting Factor) solution (Merck-Millipore, Billerica, MA). The feeder dependent mouse ESC and iPSC were also generated previously in the lab [36]. They were maintained on mitomycin C inactivated feeder cells. Cells were incubated in a humidified incubator at 37 °C with 5% CO_2. Unless otherwise mentioned, all materials were purchased from Invitrogen (Life Technologies, Carlsbad, CA). All three cell lines have enhanced GFP (Green Fluorescent Protein) transgene under the control of the regulatory elements of the promoter of the pluripotency gene *Oct4*.

2.2. Effect of sodium lactate on mouse pluripotent stem cells

Cells were seeded in 96 well plates at a seeding density of 1000 cells/well. Media containing various concentrations of sodium L-lactate (SIGMA) in the range of 0–4 mg/ml was added to the cells. After 3 days, assays were carried out to measure cell proliferation, cell metabolism and pluripotency. A sodium lactate concentration of 0 mg/ml was considered as the control.

2.3. Effect of pH on mouse pluripotent stem cells

DMEM without sodium bicarbonate was used for the maintenance medium. The desired media pH of the media was obtained by adjusting the concentration of sodium bicarbonate [19,37]. This was based on the equilibrium bicarbonate concentration ([HCO^-_3], mM) at 37 °C, which depends on the medium pH and the partial pressure of CO_2 (pCO_2, mmHg) in the gas phase according to

$$\log\left[HCO_3^-\right] = pH + log\,[pCO_2] - 7.543. \quad (1)$$

Cells were seeded in 48 well plate at a seeding density of 2500 cells/well. Media adjusted to different pH values within the range of 6.0–8.5 were added to the cells, cultured for 3 days followed by assays to measure cell proliferation, metabolic activity and pluripotency. Cells cultured in basic ESC media were used as control.

2.4. Cell proliferation assay

18–24 h prior to termination of the experiment, an appropriate volume of BrdU (Bromodeoxyuridine) stock solution was added to the cells to achieve a final BrdU concentration of 10 μM. At the end of the experiment, cells were trypsinized and replated onto clear bottom 96 well Optilux plates. The cells were allowed to attach for 6–8 h followed by fixation using 4% paraformaldehyde and stained with anti BrdU- FITC (Fluorescein isothiocyanate) antibody (Santa Cruz Biotechnology, Santa Cruz, CA) as per optimized protocol. Briefly, the fixed cells were treated with 0.1% Triton X for permeabilization. They were then incubated with 1M HCL for 30–45 min at 37 °C for DNA denaturation and then neutralized by washing 3 times with borate buffer (pH = 8.5) followed by 30 min blocking using 2% BSA solution. The cells were then incubated overnight with anti BrdU – FITC secondary antibody at 4 °C followed by Hoechst staining. Fluorescence intensity was quantified using Array Scan High Content Screening instrument (Thermo Scientific, Waltham, MA).

A manual cell count using trypan blue solution was carried out for the feeder independent ESC.

2.5. Cellular metabolic assay

Metabolic activity of the cells was measured using Cell Titer Glo luminescent assay (Promega, Madison, WI). The luminescent signal generated by this assay was directly proportional to the ATP content, giving a measurement of the mitochondrial activity. The assay was conducted according to the manufacturer's instructions. Briefly, the cells were equilibrated to room temperature for 10 min and a volume of Cell Titer Glo equal to the volume of cell culture medium was added. The contents were mixed for 2–5 min to induce cell lysis and the signal was allowed to stabilise at room temperature. The solution was then transferred to an Optilux plate and luminescent signal was measured on FLUOstar Optima (BMG Labtech, Australia).

2.6. Cell pluripotency measurement

Immunostaining for Stage Specific Embryonic Antigen 1 (*SSEA 1*) was carried out as a measurement for cell pluripotency. At the end of day 3, the cells were trypsinized and replated on to Optilux plates. They were allowed to attach for 6–8 h followed by fixation using 4% paraformaldehyde. This was followed by 30 min blocking using 2% BSA (Bovine Serum Albumin). Overnight incubation was done with mouse *IgM anti SSEA 1* antibody (Merck – Millipore) at 4 °C. After washing, the cells were incubated with anti mouse – IgM rhodamine (Chemicon International, Merck – Millipore) for 1 h followed by 30 min incubation with 1 μg/ml Hoechst solution. The fluorescence intensity was measured using Array Scan High Content Screening instrument.

The GFP expression of the *Oct4 – GFP* transgene was also measured to get an estimation of *Oct4* specific pluripotency.

2.7. Lactate concentration measurement using HPLC

Lactate concentration in spent media was measured using HPLC (High Performance Liquid Chromatography). Spent media from the culture were collected, centrifuged to remove debris and analysed using HPLC 1100 (Agilent Technologies). A C18 XBD column was used. The buffers used were 0.1%TFA (Trifluoroacetic acid) and 80% Acetonitrile with 0.08%TFA at a flow rate of 0.15 ml/min at 25 °C. Detection was done using a UV detector at 210 nm.

2.8. Statistical analysis

All quantitative results were expressed as mean ± SEM. Two Way Anova followed by Dunnet's multiple comparison test was performed using Graph Pad Prism, version 6.00 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com) in order to find statistical significance of the data. Statistical significance analysis was carried out by comparing the experimental set ups with control set-ups. In the case of the lactate effect, a sodium lactate concentration of 0 mg/ml was considered to be the control set up, while for the effect of change in pH, the normal ES cell maintenance medium was considered to be the control. Differences among data were considered to be statistically significant if $p < 0.05$.

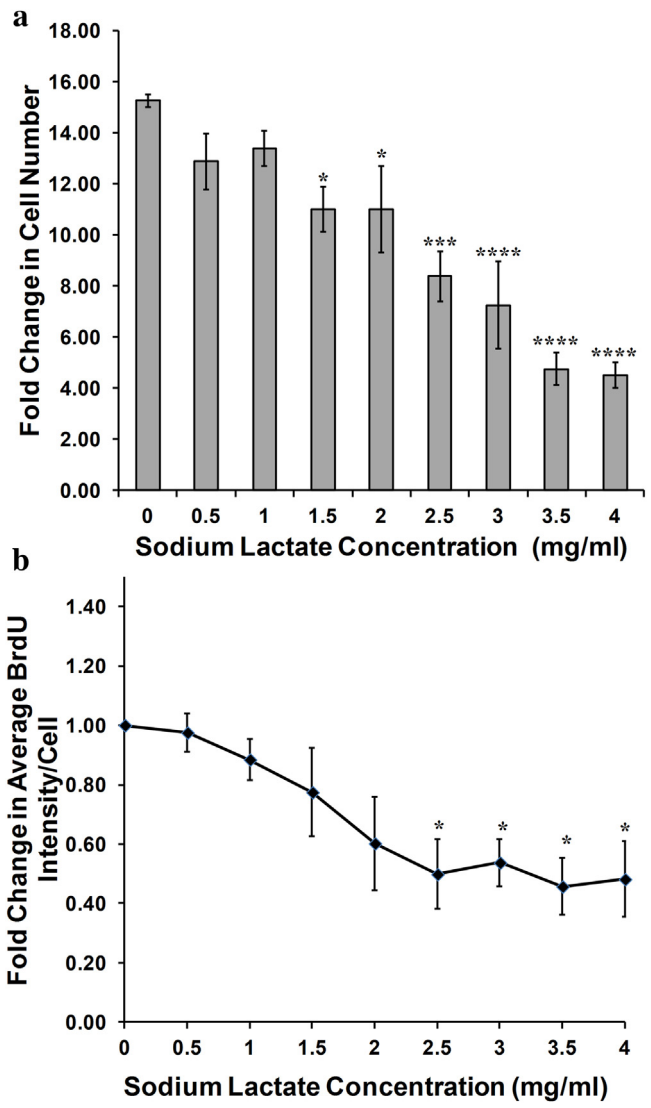

Fig. 1. Influence of sodium lactate concentration on cell count and proliferation of feeder free ESC. (a) Fold change in cell number (mean ± SEM for n = 4 independent experiments), (b) Fold change in average BrdU fluorescence intensity/cell as a measurement of cell proliferation (mean ± SEM for n = 3 independent experiments).

3. Results

3.1. Effect of exogenous sodium lactate addition on pluripotent stem cells

3.1.1. Cell count and cell proliferation

The main aim of this experiment was to determine if increasing lactate concentration in the media had any effect on the cell growth and proliferation of mouse pluripotent stem cells in the absence of any change in media pH. For this purpose, sodium lactate was added to the culture media at various concentrations and viable cell number was counted using trypan blue (for feeder free ESC only) while cell proliferation was measured using BrdU incorporation assay. The result for trypan blue cell count was represented as fold change with respect to day 0 for different lactate concentration, while for the BrdU assay they were presented as fold change with respect to the control concentration (0 mg/ml). As seen in Fig. 1a, total viable cell number for feeder free ESC decreased with increase in sodium lactate concentration. The decrease was not significant up to a sodium lactate concentration of 1 mg/ml but, thereafter, the

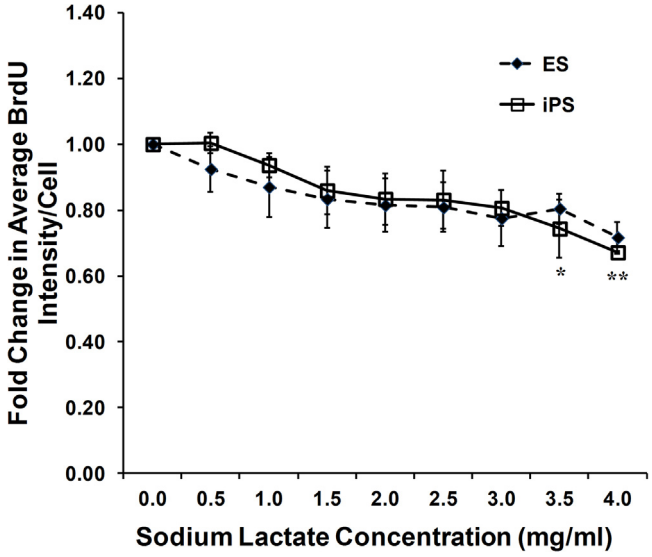

Fig. 2. Effect of sodium lactate on cell proliferation of feeder dependent ES and iPSC measured by fold change in BrdU fluorescence intensity/cell. Data represent mean ± SEM for n = 3 independent experiments.

viable cell count continued to decrease significantly with increasing sodium lactate concentration.

BrdU incorporation assay also supported the above data. Fig. 1b shows the fold change in average BrdU-FITC fluorescence intensity/cell. Although not significant at the lower concentrations, cell proliferation showed a decreasing trend with increasing sodium lactate concentration. The decrease became significant once the concentration reached a value of 2.5 mg/ml.

Fig. 2 shows that cell proliferation for feeder dependent ES and iPSC followed a trend similar to that of the feeder independent ESC. The decrease in cell proliferation for the ESC was not statistically significant across the entire range of sodium lactate. For iPSC, the decrease in cell proliferation became significant from a sodium lactate concentration of 3.5 mg/ml. Comparison of Figs. 1b and 2 shows a significant decrease in cell proliferation for feeder independent mESC at a lower concentration of sodium lactate compared with feeder dependent mESC and miPC. It is possible that the presence of feeder cells acts as a protection for the cells.

3.1.2. Cellular metabolic activity

Metabolic activity of the cells was estimated by measuring the luminescent signal of Cell Titer Glo assay. As mentioned previously, the results were denoted as fold change with respect to the control concentration (0 mg/ml). Usually the cellular metabolic activity is a direct measurement of cell viability and proliferation and shows a similar trend. Interestingly, in this case it was observed that the metabolic activity assay showed almost uniform values across the entire range of tested sodium lactate concentration for all three cell lines (Fig. 3).

This scenario was possible if the metabolic activity of the viable cells increased corresponding to an increase in sodium lactate concentration in the medium. Therefore, we hypothesized that the cells were able to alter their metabolic pathway to use the externally added lactate as a source of energy along with glucose, thus increasing their metabolic activity and resulting in these contrasting data.

3.1.3. Lactate concentration estimation by HPLC

Based on the cell proliferation and cellular metabolic activity data, we hypothesized that the cells may be utilizing exogenously added lactate as a source of energy along with, or in place of, glucose. In order to test our hypothesis to some extent, HPLC of the

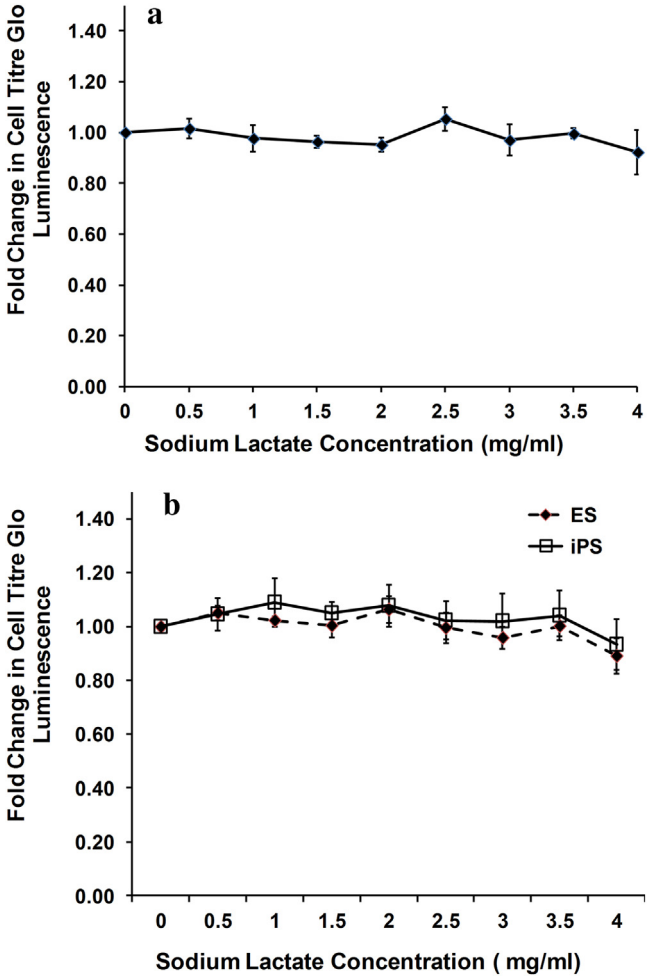

Fig. 3. Fold change in cellular metabolic activity of (a) feeder free ESC, (b) feeder dependent ES and iPSC measured by Cell Titer Glo assay. Data represent mean ± SEM of n = 3 independent experiments.

spent media was carried out to estimate the lactate concentration. It was expected that if the hypothesis were to be supported, HPLC analysis would show a decrease in concentration of lactate in the spent media with externally added lactate over time.

Fig. 4 shows the result for HPLC analysis of spent media to assess the change in lactate concentration. The fold change was measured with respect to the initial lactate concentrations in each case. As expected, when there was no external addition of sodium lactate, lactate concentration of the spent media increased with time confirming the fact that lactate is indeed produced by the cells. However, we observed that when external sodium lactate was added to the culture, the lactate concentration proceeded to decrease with time. The decrease was significant at and above concentrations of 2 mg/ml and 1 mg/ml for feeder independent and feeder dependent cell lines, respectively. These data support to some extent our hypothesis of lactate consumption by the pluripotent cells although further analysis is indeed required.

3.1.4. Cell pluripotency

Apart from immunostaining of the surface marker *SSEA 1*, pluripotency was also estimated by measuring the fluorescence intensity of the GFP reporter gene controlled by *Oct4* promoter. Average fluorescence intensity/cell was measured and the results were shown as fold change with respect to the control concentration (0 mg/ml). For feeder independent ESC, although there is a slight decrease in the SSEA 1 intensity with increasing lactate

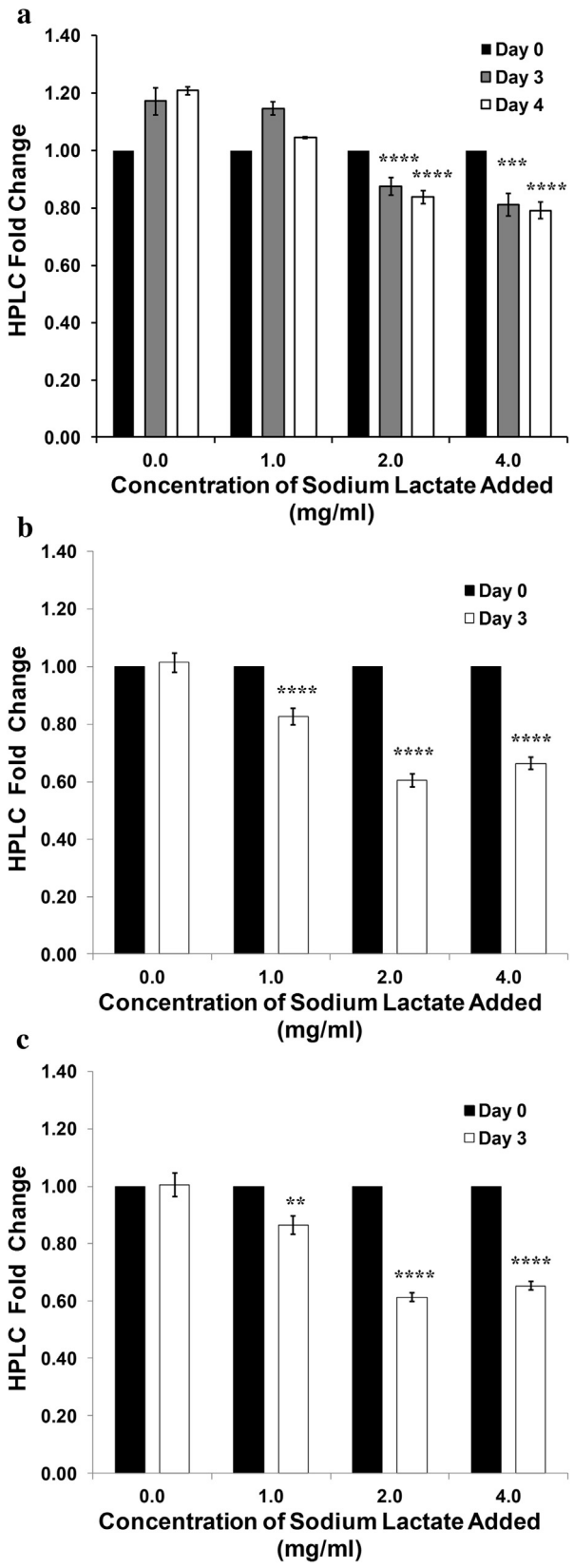

Fig. 4. Fold change in measurement of lactate concentration in spent media from (a) feeder free ESC, (b) feeder dependent ESC and (c) feeder dependent iPSC by HPLC. Data represent mean ± SEM of n = 3 independent experiments.

(Fig. 5a), there is no significant change in pluripotency with increasing sodium lactate concentration. There was also no measurable change in the GFP intensity (Fig. 5b).

Similarly, *Oct4-GFP* fluorescence intensity and *SSEA 1* immunostaining fluorescence intensity/cell were also measured for feeder dependent cell lines. As observed in Fig. 5(c,d), although there was variation in the fluorescence intensity fold change for both *Oct4-GFP* and *SSEA 1* immunostaining, there was no specific trend or significant change in cell pluripotency with increasing sodium lactate concentration.

3.2. Effect of pH on mouse pluripotent stem cells

Change in extracellular culture media pH was found to have significant effect on cell proliferation, cellular metabolism and cell pluripotency of mouse pluripotent stem cells. All data are represented as fold change compared with regular ESC media.

3.2.1. Cell proliferation

Both acidic and alkaline media pH affected the proliferation of mouse pluripotent stem cells irrespective of the absence or presence of feeder layer. As seen in Fig. 6, cell proliferation decreased significantly if the pH decreased or increased beyond 7-7.5. The feeder independent cells seem to require a more precise control of pH, since it showed a significant decrease in proliferation even at a pH of 7.0.

3.2.2. Cellular metabolic activity

The cellular metabolic activity (Fig. 7) for the pluripotent stem cells was reduced with both decrease or increase in pH and followed a trend similar to cell proliferation. The decline was significant for all three cell lines.

3.2.3. Cell pluripotency

Unlike the effect of lactate concentration, change in media pH significantly affected the cell pluripotency (Fig. 8). Decrease in pluripotency for the feeder free ESC was significant due to media acidity in comparison to alkalinity of media. However, pluripotency of the feeder dependent cells was equally affected by increase or decrease in media pH; in particular, the iPSC looked to be especially sensitive.

4. Discussion

Lactic acid has been reported to be one of the major waste products in mammalian cell culture, produced predominantly during glycolysis. The influence of lactic acid on cells can be explained mainly by change in pH or effect of lactate ions. To date, a number of papers have reported the detrimental effects of both pH change and lactate ion accumulation on mammalian cells. However, very few studies are available that elucidate the effect of lactate on pluripotent stem cells. In addition, the current literature has various discrepancies within. In this paper, we have reported the effect of exogenously added lactate on cell proliferation, pluripotency and cellular metabolic activity on three different mouse pluripotent stem cell lines to have a more general trend.

No significant change in pluripotency of mouse pluripotent cells was observed on addition of exogenous sodium lactate. These data support previously published literature on mouse ESC, which demonstrates that the presence of lactate does not affect pluripotency of mouse ESC [32]. On the other hand, similar to Ouyang et al.'s observation, total cell number for feeder free mouse ESC decreased with increase in lactate concentration [31]. Chen et al. have reported similar findings for human ESC too, wherein they observed a decrease in cell number as well as pluripotency of human ESC on supplementation of medium with sodium lactate

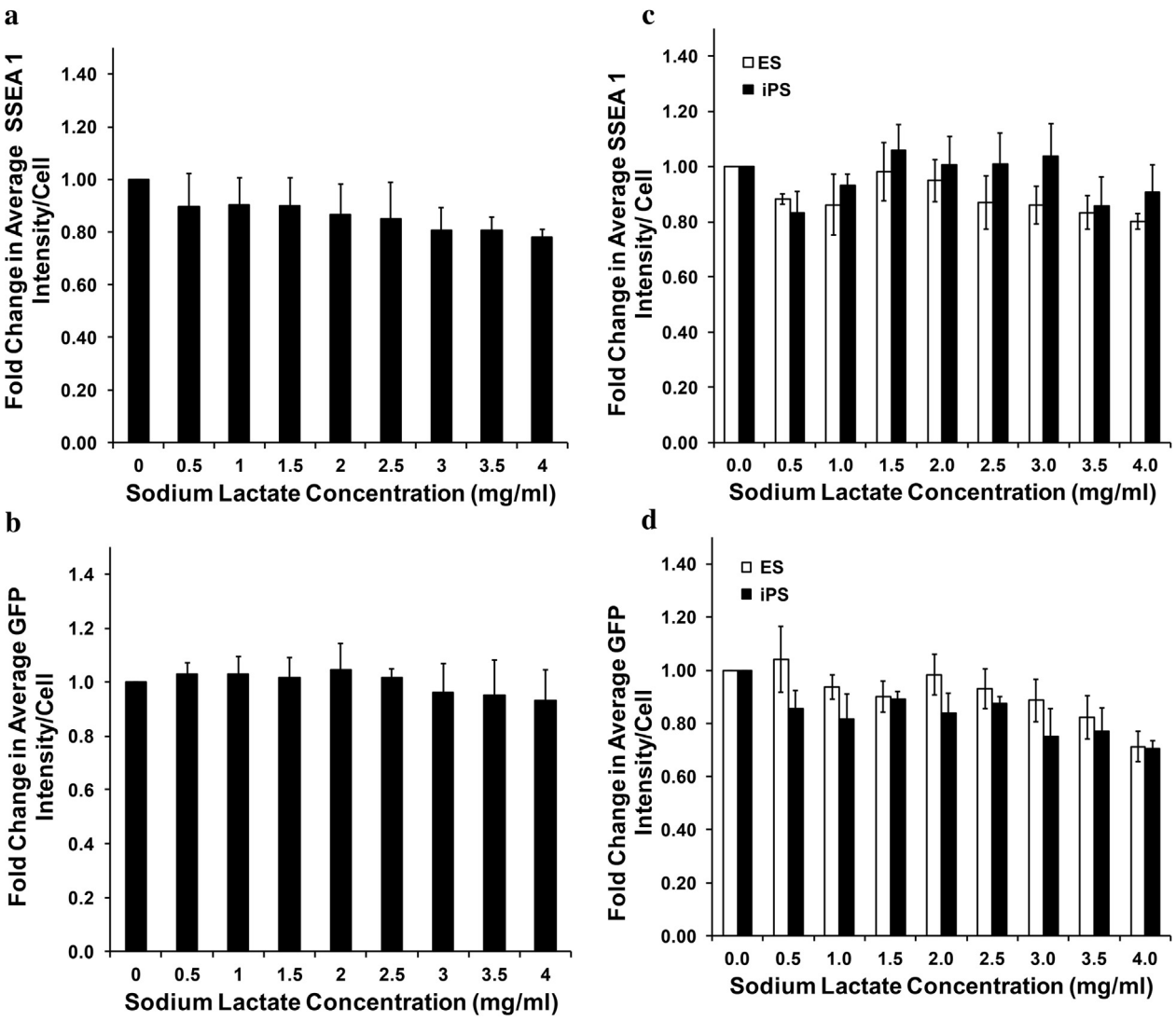

Fig. 5. Effect of sodium lactate concentration on pluripotency. (a,c) Fold change in SSEA 1 fluorescence intensity/cell (b,d) Fold change in Oct4 promoter driven GFP intensity/cell. (a,b) feeder free cells, (c,d) feeder dependent cells. Data represent mean ± SEM for n = 3 independent experiments.

[33]. The difference regarding the effect of sodium lactate on pluripotency between our observation and Chen et al.'s data can be attributed to the fact that human cells are known to be more sensitive than mouse cells to a change in growth environment. The decrease in cell number became significant from a sodium lactate concentration of 1.5 mg/ml and the significance of the change in cell number increased with increasing lactate concentration. Extending the scope of our work beyond this, we observed that the cell proliferation rate for feeder free mouse ESC also decreased with increasing sodium lactate concentration and became significant once the sodium lactate concentration reached 2.5 mg/ml. Experimentation with feeder dependent mouse ES and iPSC to determine the effect of lactate on their proliferation rate showed a similar decreasing trend in cell proliferation rate. The decrease in cell proliferation for iPSC showed statistical significance only when the sodium lactate concentration reached a value of 3.5 mg/ml. It is entirely possible that the feeder cells were in some way responsible for protecting the cells from the detrimental effect of lactate.

In most cases, the cellular metabolic activity is proportional to cell proliferation and cell number. However, we observed that there was no measurable change in the cellular metabolic activity for any of the cell lines. These data were in contrast to the cell proliferation results. Based on the cell proliferation and cellular metabolic activity data, we hypothesized that either addition of sodium lactate to the medium decreased lactate production by the cells themselves or the cells may have been able to utilize exogenously added lactate as a source of energy along with glucose.

In order to support our hypothesis to some extent, we carried out HPLC analysis of the spent media from cell culture with sodium lactate concentration between 1 and 4 mg/ml. We found that, when no additional sodium lactate was added to the media, the lactate concentration increased over time, suggesting that lactate was being produced by the cells as a metabolic by product. However, when sodium lactate was added to the culture, we observed a decrease in the fold change in measured lactate concentration over time. Similar observations were made by Chen et al. on hESC wherein they reported a decrease in lactate production by the cells upon supplementation of the media with sodium lactate [33]. Further studies are, however, required to elucidate fully this interesting phenomenon and to understand if the externally added sodium lactate is merely quenching the production of lactate by the cells or if they are using it as an energy source.

Although relatively less studied, alteration of cell metabolism due to externally added lactate and utilization of lactate as a preferred energy source have been reported earlier for a number of cell lines. Recombinant CHO cells have been shown to have increased

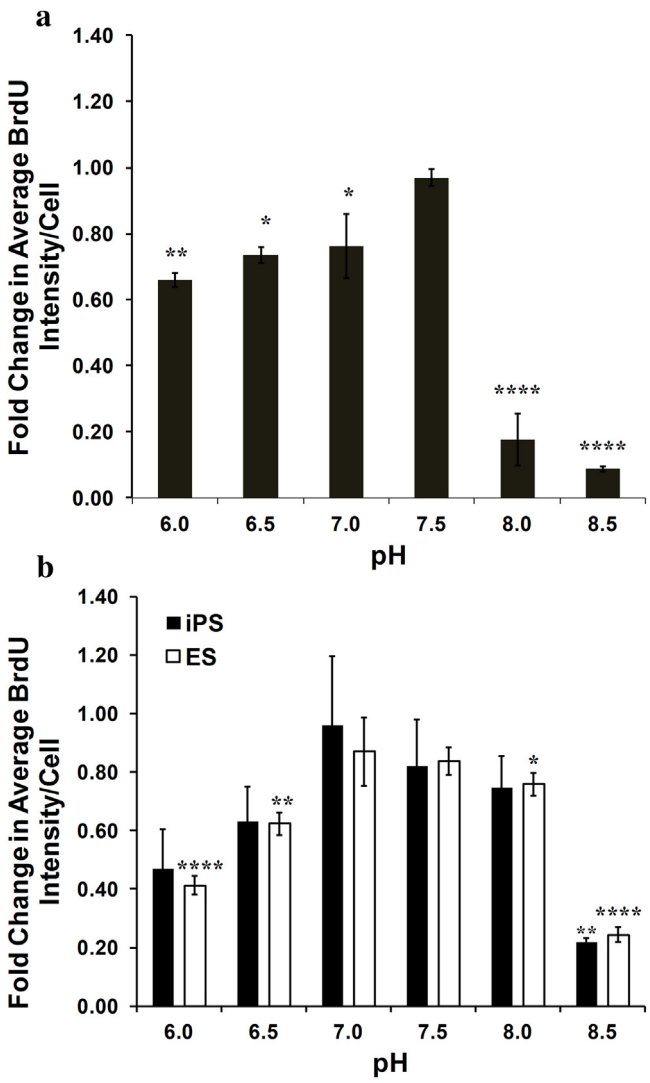

Fig. 6. Effect of pH on cell proliferation of mouse pluripotent stem cells (a) Feeder free ESC, (b) feeder dependent ES and iPSC. Data represent mean ± SEM of n = 3 independent experiments.

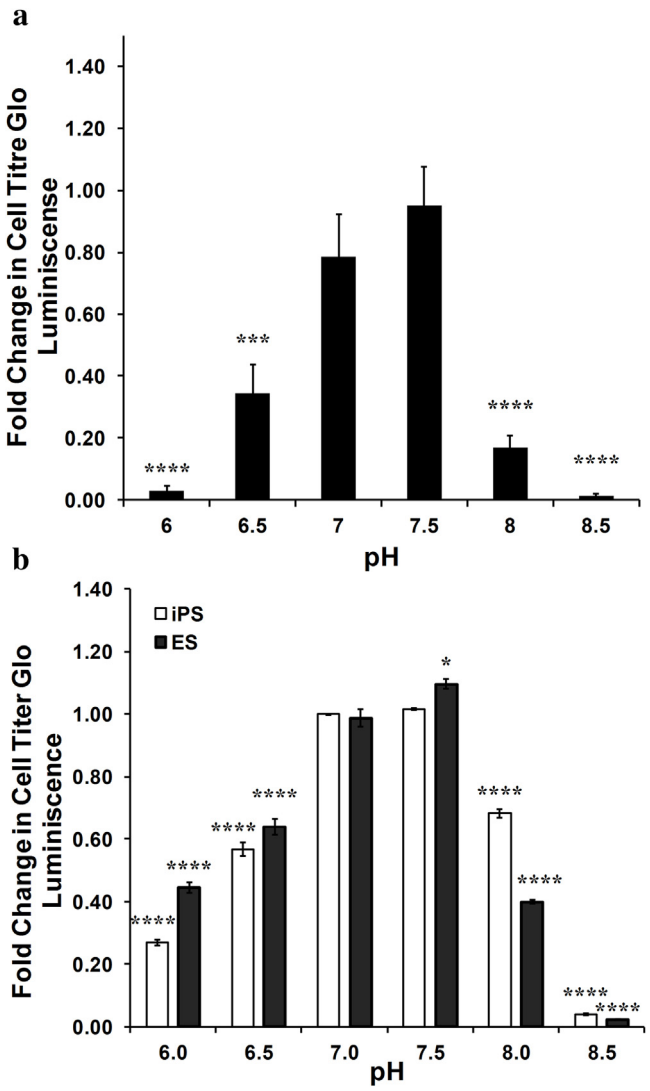

Fig. 7. Effect of pH on cellular metabolic activity of mouse pluripotent stem cells (a) Feeder free ESC, (b) feeder dependent ES and iPSC. Data represent mean ± SEM of n = 3 independent experiments.

erythropoietin production on addition of exogenous sodium lactate, although there was a decrease in specific cell growth and glucose consumption [26]. The group suggested that the high concentration of sodium lactate led to oxidation of lactate to pyruvate at a high rate by the LDH (lactate dehydrogenase) enzyme. This resulted in an accumulation of NADH and high concentration of pyruvate. The energy thus generated, however, was used for amino acid and protein synthesis. Zagari et al., [38] have also carried out studies on lactate metabolism by CHO cells. They reported that consumption of lactate by the CHO cells was associated with an increased mitochondrial activity and oxygen consumption. The same group also identified the malate – aspartate shuttle as a key factor in lactate consumption by CHO cells [39]. The use of lactate as a preferred source of energy has also been reported for germ cells along with the ability of lactate to inhibit germ cell apoptosis [40–42].

Interestingly, a positive effect of lactate on mouse ESC has also been reported. It was shown that media enriched with lactate increase colony number and size for feeder dependent ESC [32]. They suggested that lactate was converted to Acetyl CoA, which can be used either in mitochondrial metabolism or in increasing gene expression via histone acetylation. Our HPLC data that show decrease in lactate concentration are consistent with these data.

Findings on the effect of change in extracellular pH on mouse pluripotent stem cells were congruent to the available literature. Cell proliferation, cellular metabolic activity as well as pluripotency of the mouse pluripotent stem cells were significantly decreased with any change in the media pH towards acidity or alkalinity.

To conclude, we have shown here the effect of exogenously added lactate and change in media pH on mouse pluripotent stem cell culture. Pluripotent stem cells are extremely sensitive to any change in media pH, which affects the cells' growth, viability, proliferation and pluripotency. We have also shown that although pluripotency is not affected, cell proliferation is negatively affected by increase in lactate concentration. Further studies are also needed to fully understand the effect of these interesting parameters. Eg, it is possible that addition sodium lactate to the media and/or change in media pH, changes the intracellular pH of the cells, which in turn can have interesting effects on cell behaviour. Effect of osmolality is also an important factor and needs to be studied. Long term studies for these parameters would also add interesting information in this area. In conclusion, these studies suggest that careful consideration of media pH and media composition is of utmost importance for proper growth and maintenance of pluripotent stem cells.

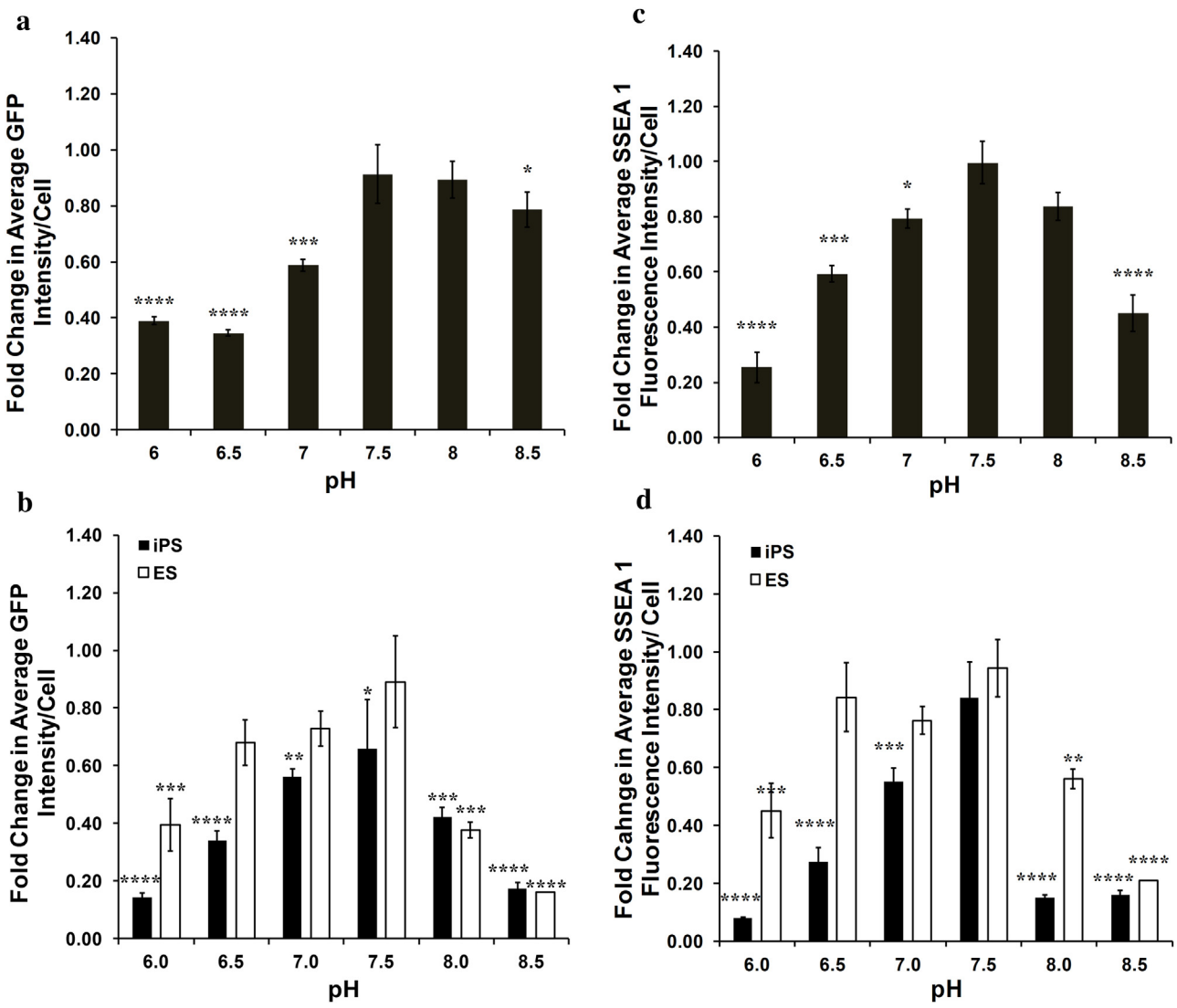

Fig. 8. Effect of pH on pluripotency of mouse pluripotent stem cells (a, c) Feeder free ESC, (b, d) feeder dependent ES and iPSC. Data represent mean ± SEM of n = 3 independent experiments.

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