Effect of long-acting recombinant human follicle stimulating hormone (SAFA-FSH) on spermatogenesis

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The Master's Thesis
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

Soohyun Lee

December 2021

This certifies that the Master's Thesis of
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The Graduate School
Yonsei University

December 2021

ACKNOWLEDGEMENTS

I would like to express my very great appreciation to Professor Eun Jig Lee for invaluable support of my research. He gave me the courage to do it and the freedom to do research to my heart's content. I sincerely thank the professors who believed in me and continued to motivate and inspire me.

I would like to thank my dissertation advisor Professor Joo Yong Lee and Professor Daham Kim for their advice and constructive feedback.

I would also like to thank my laboratory colleagues Dr. Yoon Hee Cho, Dr. Yang Jong Lee, Dr. Chan Woo Kang, Mr. Joo Heon Oh, Ms. Ya Ru Bao, Ms. Eun Kyung Wang, and Ms. Juyeon Yu.

And last but not least, I wish to thank my parents and my sister for their support and encouragement throughout my study. Thank you so much for your constant love and inspire for me who belatedly said I would go back to my studies.

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ABSTRACT

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Follicle Stimulating hormone (FSH) is one of the hormones secreted by the anterior pituitary gland. In women, it promotes ovarian follicle growth and maturation, and in men, it stimulates testicular maturation to promote spermatogenesis. In the case of infertility caused by insufficient secretion of sex hormones, fertility can be restored through gonadotropin administration. Although the use of recombinant FSH has become common, it has a short duration, low dose, and difficult to maintain a constant drug concentration. It was studied whether SAFA-FSH, which is a fusion of a Fab antibody fragment that specifically binds serum albumin and a recombinant FSH protein, can act as a long-acting hormone. In this study, it was confirmed that cyclic AMP was increased by recombinant FSH or SAFA-FSH in cells expressing FSH Receptor. In addition, when recombinant FSH or SAFA-FSH were treated, the phosphorylation of CREB and ERK1/2 were also increased. A hypogonadal mice and rat model treated with a gonadotropin releasing hormone (GnRH) agonist were made, and it was confirmed that sperm production was restored even after treatment with SAFA-FSH like recombinant FSH. In conclusion, SAFA-FSH can activate cells like recombinant FSH, and because it has a longer half-life than recombinant

FSH, the drug concentration is maintained without frequent injection compared to recombinant FSH, thereby increasing the utility of long-acting hormone preparations.

Key words : FSH (Follicle stimulating hormone), FSH Receptor, SAFA Technology, Spermatogenesis

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I. INTRODUCTION

Follicle Stimulating Hormone (FSH) secreted from the anterior pituitary gland regulates sex hormones and matures reproductive cells together with Luteinizing Hormone (LH)[1]. In women, it regulates the reproductive cycle, and in men, it plays an important role in spermatogenesis[2,3]. Infertility means a low chance of getting pregnant, and there are several methods currently being used to treat infertility for men as well as women[4]. In the case of infertility, because hormone secretion does not occur properly due to an abnormality in the pituitary gland, hormones can be injected into the body to increase the chances of pregnancy[5]. Although the use of recombinant FSH has become common, it has a short duration, low dose, and difficult to maintain a constant concentration. In addition, from the patient's point of view, it is painful because it has to be administered repeatedly every day, and there is a disadvantage in that compliance with the drug may decrease. Therefore, the purpose of this study is to evaluate the development and effectiveness of long-acting FSH with increased half-life using SAFA Technology.

In the structure of the antibody, the part that specifically recognizes and binds the antigen is called Fab (antigen binding fragment), and it is divided into V_L(Variable region-Light chain), V_H(Variable region-Heavy chain), C_L(Constant region-Light chain), and C_{H1}(Constant region-Heavy chain). The antigen-binding site is formed

by combining the V_L part and the V_H part that directly binds to the antibody. Since it is a part that directly recognizes and binds an antigen, if the type of antibody changes, this part will also change.

Antibodies capable of binding specifically to serum albumin were first selected from the human antibody library, and this part is called SAFA (Serum Albumin Fragment Associated). An effective protein domain can be bound to the SAFA (Anti-serum albumin Fab) part. In this study, the target protein, FSH, was bound.

FSH is protein composed of two sub-units, an alpha sub-unit and a beta sub-unit, and the two sub-units are bound to the light chain and the heavy chain in the Fab, respectively. Literally, SAFA Technology is a fusion of a Fab antibody fragment that can specifically bind serum albumin with a recombinant FSH protein. Fab binds to serum albumin, enabling recycling in the human body and FSH protein is what enables the function of FSH itself in the human body[6]. Since there is no Fc region, side effects that may occur due to Fc can be prevented, and the possibility of side effects is also low as it is of human origin because it is selected from a human antibody library. And since it is an antibody structure, it is possible to maintain a more stable structure than other substances, and it has a longer half-life than normal FSH protein, so the duration is longer, and the blood drug concentration can be maintained stably[7].

To confirm the applicability of SAFA-FSH, we intend to investigate in vitro cell activation by FSH and SAFA-FSH in FSH receptor expressing cells, respectively[8-10]. And in vivo, we want to investigate the effect on spermatogenesis in a hypogonadal male Sprague Dawley rat model made using GnRH agonist[11].

II. MATERIALS AND METHODS

1. Cell culture

Mouse Sertoli cell lines, TM4 was purchased from Korean Cell Line Bank (KLCB). TM4 Cell were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Hyclone, USA) and 1% penicillin/streptomycin sulfate (Hyclone). HEK293FT Cell were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. The cells were cultured in a humidified atmosphere of 5% CO_2 at 37℃.

2. Plasmid construction and stable cell line generation

Human FSH Receptor gene was inserted into lentiviral vector pLECE3-GFP using HpaI-NotI restriction enzyme sites to make pLECE3-hFSHR-GFP[12]. FSH receptor gene was gift from Department of Obstetrics and Gynecology, Severance Hospital. Lentiviral particles were generated using plasmids pRSV-REV, pMD2.G and pMDLg/pRRE in HEK293FT cells co-transfected with pLECE3-hFSHR-GFP. Cells were transfected using the polyjet transfection reagent (Sinagen, USA). Two days after transfection, culture medium was harvested and sterilized 0.45um syringe filter. Purified lentiviral particles were used to infect TM4 cell. Three days after infection, Cells were separated only GFP (Green Fluorescent Protein)-positive single cells into 96well plate using BD LSRFortessa (Becton Dickinson, USA)

3. Measurement of cAMP production

TM4 and TM4-FSHR cell were cultured in 6cm plate and seeding $0.3*10^6$cells/plate. Next day, after starvation for 4hr, the cells were stimulated with Follitrope (LG Chemical) or SAFA-FSH (APRILBIO) at different concentration in 0.5 mM isobutylmethylxanthine (IBMX, sigma Aldrich) for 15min at 37℃. The reaction is stopped by aspirating the

medium and washed twice with cold Dulbecco's Phosphate buffered saline (DPBS). The cells were lysed with 80ul lysis buffer, keep cells on ice for 30min. Cell lysates are obtained clearly by centrifugation. And cAMP concentrations were measured by using cyclic AMP XP™ assay kit (cell signaling technology, USA). In each experiment, a standard curve was generated and used to calculate cAMP concentration.

4. Western blotting

Whole cell lysates were prepared and assay was carried out as standard procedures. Cells washed twice with cold DPBS and lysed in lysis buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1x protease inhibitor cocktail (Sigma Aldrich). The lysates were centrifuged at 12000rpm, 15min and obtained supernatants were used for analysis. Protein concentration was determined using Pierce BCA Protein Assay kit (Thermo Fisher Scientific). Equal quantity of protein was loaded on Bolt™ 4-12% Bis-Tris Plus Gels and separated at 200V for 35 minutes. The proteins were transferred on PVDF Membrane (Invitrogen) using Power Blotter (Invitrogen). Membranes were blocked with EveryBlot blocking buffer (BIO-RAD) for 15min at room temperature. Membranes were incubated with primary antibody for 1hr at room temperature; the primary antibodies used were rabbit anti-CREB (1:1000), rabbit anti-phospho CREB (1:1000), mouse anti-Erk1/2 (1:2000) and rabbit anti-phopho Erk1/2 (1:2000). Primary antibodies were purchased from Cell signaling Technology. Blots were washed 6 times for 5min with Tris-buffered saline containing 0.05% Tween20 (TBST) and incubated with horseradish peroxidase (HRP) conjugated anti-mouse IgG or anti-rabbit IgG secondary antibody (1:2000) for 1hr at room temperature. Immunoreactivity was detected with the Amersham™ ECL (Cytiva, USA) using iBright 1500 (Invitrogen). The intensity of protein bands was quantified using iBright analysis normalized β-actin in each sample.

5. Animal model

Twenty-six 8-week-old male C57BL/6 mice and forty Sprague-Dawley rats aged 4-5weeks (140-175g) were purchased from Orient Bio. The animals were maintained under controlled conditions (22℃, 12 hour light, 12 hour dark cycle) and received rodent chow and tap water. The animals were allowed to acclimatize for a period of 7 days before treatments. The mice were randomly divided into four groups, the control group had 5 mice and the drug injection group has 7 mice each. Mice received intramuscular injection of Diphereline (2.5 mg/kg, Ipsen). One week later, Gonal-F (65IU/kg, Follitropin alfa, MERCK) and SAFA-FSH (26ug/kg) were administered by subcutaneous injection for about 6 weeks. Gonal-F was given 3 times a week, and SAFA-FSH once every 2 weeks.

The 5-week-old male Sprague Dawley rats were randomly divided into five groups, with 8 rats in each group by body weight and housed two per cage. Body weights were measured twice a week to maintain a record of weight gain. Rats received subcutaneous injections of Diphereline (5 mg/kg, Ipsen)[11]. Two weeks later, after confirming that the testosterone concentration decreased, hCG (105IU/kg, IVF-C, LG Chem.) and Follitrope (25IU/kg) or SAFA-FSH (9.87ug/kg) were injected subcutaneously. For 6 weeks, hCG and Follitrope were injected daily, and SAFA-FSH was injected every other day in consideration of the half-life. The control group was injected with saline instead of the drugs. All experimental procedures in this study were approved by the Institutional Animal Care and USA Committee of Yonsei University Health System.

6. Testosterone measurement

Blood samples were collected by orbital sinus using capillary tubes after anesthesia at the same time every week, and only serum was isolated by centrifugation. Testosterone concentrations were measured at the Seoul Clinical Laboratories (SCL).

7. Sperm collection

Sperm were collected from epididymis cauda. Cauda was placed in 6cm plate with 10ml DPBS and minced with a scalpel, allowing sperm to be dispersed in the saline and incubated for 15min at 37℃ under 5% CO_2[13]. It was heated at 60℃ for 1min and then cooled to room temperature. Dispersed sperm solution was diluted appropriately to increase the accuracy of sperm count, sperm were counted and evaluated using a Neubauer hemocytometer (INCYTO).

8. Statical analysis

The data were presented as mean ±S.E.M. The comparison of data for statistically significant differences was done using student's 't' test and a probability level of $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ and $P \leq 0.0001$ were considered as significant.

III. RESULTS

1. Low response to cAMP when FSH treatment on TM4 cell

It is known that FSH receptor exist in TM4 cells, which are mouse Sertoli cell[14]. Since the FSH receptor is a G protein-coupled receptor it was expected that cAMP would increase when FSH was treated[15]. TM4 cells were treated with different dose of FSH, and intracellular cyclic AMP was measured by ELISA. However, the concentration of cAMP did not increase regardless of the treated FSH concentration (Figure 1). Also, although not in the figure, when the expression level of FSHR in TM4 cells was confirmed by quantitative PCR, the expression level of FSHR was very low. Based on these results, it was determined that additional FSH treatment experiments could not be performed in TM4 Cells.

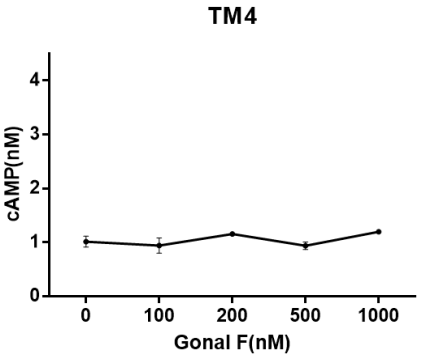


Figure 1. Low response to cAMP when recombinant FSH treatment on TM4 cells cAMP ELISA assay of TM4 cells treated with recombinant FSH (Gonal-F) at indicated dose. TM4 cells were treated with different concentrations of FSH, but no increase in cAMP was observed. The plots indicate the mean ±S.E.M.

2. Establishment of cell lines with FSHR stably overexpression

Contrary to expectations, there was no cAMP reactivity in TM4 cells. So, TM4-FSHR cell was created by overexpression FSHR gene using lentivirus in TM4 cell. After sub-cloning the human FSHR gene into pLECE3-GFP plasmid (Figure 2A), lentivirus was created by transfection with other lentiviral plasmids. The prepared lentivirus particle was infected with TM4 cells and separated into single cells by GFP (green fluorescent protein) FACS (fluorescence-activated cell sorting). It was confirmed that there are no GFP positive cells in TM4 cells, and that only TM4-FSHR cells have GFP positive cells (Figure 2B, C). Only GFP positive cells were obtained, and the obtained cells as a single cell were named TM4-FSHR cells.

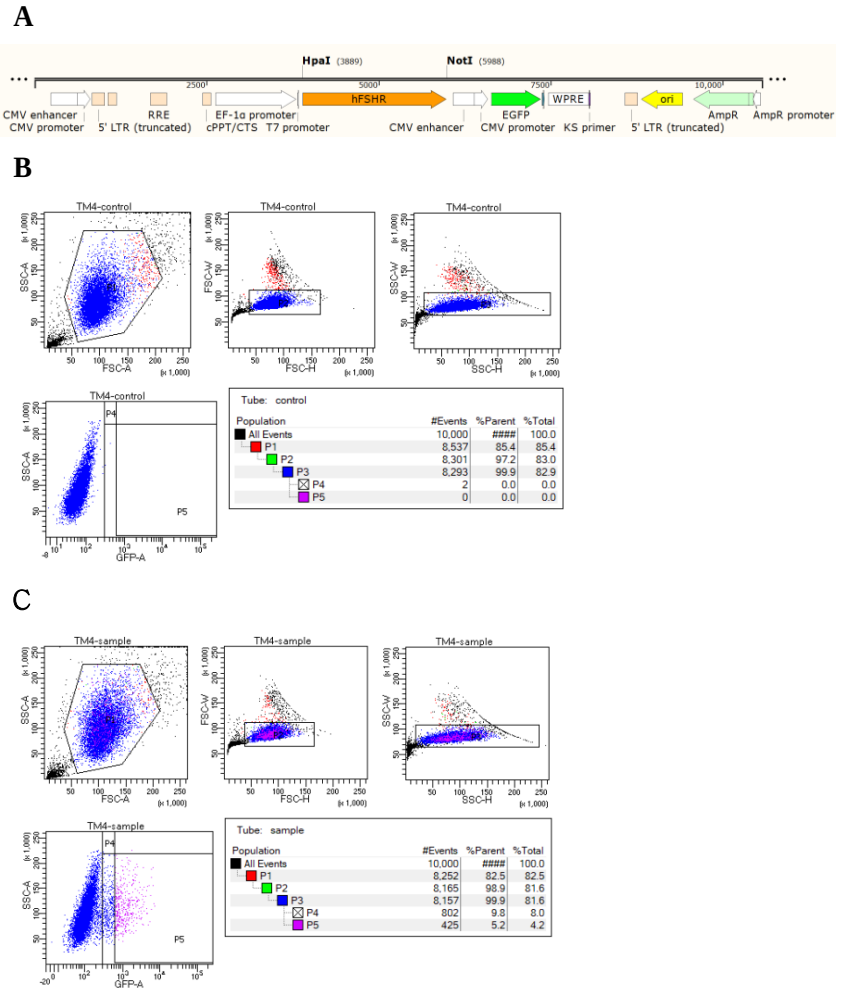


Figure 2. Establishment of cell lines with FSHR stably overexpression. (A) pLECE3-FSHR-eGFP plasmid subcloning scheme. Human FSHR gene inserted into pLECE3-GFP plasmid using restriction enzymes HpaI and NotI. (B, C) GFP-FACS result of TM4 as control (B) and TM4-FSHR (C). Each dot represents a single cell. The pink dots indicate GFP positive cells.

3. cAMP elevation in TM4-FSHR cells after FSH and SAFA-FSH treatment

FSHR is a transmembrane receptor and belongs to family of GPCR (G protein-coupled receptor). As in TM4 cell, TM4-FSHR cells were treated with recombinant FSH (Follitrope) and SAFA-FSH at different concentrations[16]. Unlike TM4 cell, as the concentration of treated FSH increased, the degree of increase in cAMP concentration increased in a dose dependent manner (Figure 3). The effect of SAFA-FSH was less than that of recombinant FSH when treated with the same moles of FSH. It was confirmed that a similar cAMP level was increased only when SAFA-FSH was treated at about 3 times that of recombinant FSH.

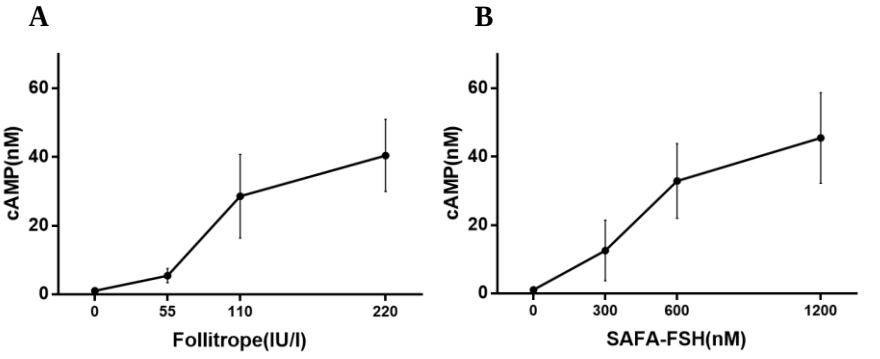


Figure 3. cAMP elevation in TM4-FSHR cells after recombinant FSH and SAFA-FSH. (A, B) The degree of cAMP increase, when Follitrope (A) and SAFA-FSH (B) treatment with different concentrations. Recombinant FSH (Follitrope) or SAFA-FSH was added for 15 min at the indicated concentrations in the presence of 0.5 mM IBMX. The plots indicate the mean ±S.E.M. of three independent experiments.

4. Recombinant FSH and SAFA-FSH treatment enhanced phosphorylation of ERK1/2 and CREB

cAMP related signaling involves ERK1/2 and CREB(cAMP response element binding protein) activation[17]. In Sertoli cells, cAMP binding to PKA and mediates phosphorylation of the extracellular signal regulated kinase 1/2 (ERK1/2)[18]. When TM4-FSHR cells were treated with recombinant FSH (Follitrope) and SAFA-FSH at different concentrations of two points, the phosphorylation level of ERK1/2 was higher than that of the negative control (Figure 4A, B). Likewise, when TM4-FSHR cells were treated with recombinant FSH and SAFA-FSH, it was confirmed that the phosphorylation of CREB was increased (Figure 4C, D). In the case of CREB, the expression level of total CREB was also unexpectedly increased by recombinant FSH and SAFA-FSH (Figure 4C). Both recombinant FSH and SAFA-FSH significantly increased the phosphorylation level compared to the control, but the recombinant FSH did not increase in a concentration-dependent manner. From these results, it can be concluded that SAFA-FSH has the same effect on the cell signaling pathway as recombinant FSH.

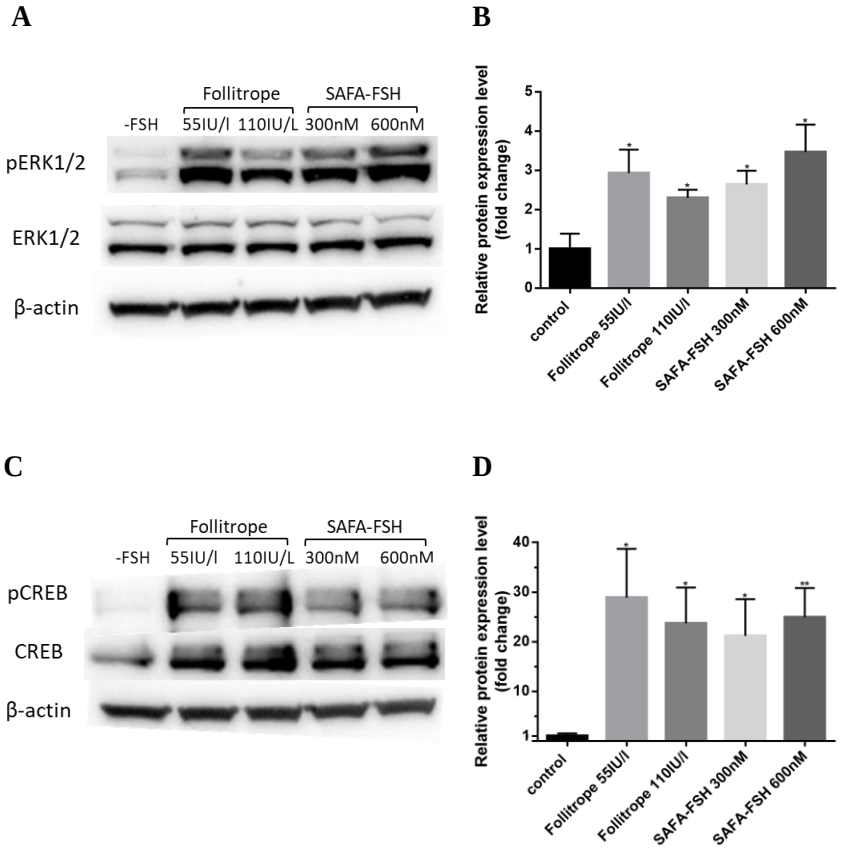
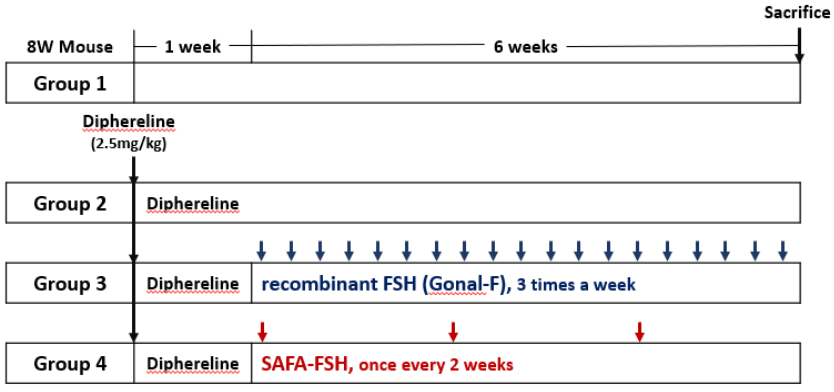


Figure 4. Phosphorylation of ERK1/2 and CREB induced by FSH or SAFA-FSH in TM4-FSHR cells at indicated dose. (A) Representative western blot analysis of protein extracts from TM4-FSHR cell showing the levels of total ERK1/2 and ERK1/2. (B) Relative ERK1/2 activity was derived as pERK1/2 normalized to total ERK. (C) Representative western blot analysis of protein extracts from TM4-FSHR cell showing the levels of total CREB and pCREB. (D) Relative CREB activity was derived as pCREB normalized to total CREB. Each sample was normalized with β-actin first. Data are representative of four independent experiments and were presented as mean ±S.E.M. *P < 0.05, **P<0.01 versus control.

5. Effects of spermatogenesis after injection of recombinant FSH or SAFA-FSH in hypogonadism mouse model

It has been confirmed that SAFA-FSH works through the same signaling pathway as recombinant FSH. Now, to confirm its function as a long-acting hormone, GnRH agonist Diphereline was injected into male mice to create an animal model of hypogonadism as a preliminary experiment. By injecting a GnRH agonist, the HPG axis (Hypothalamic-Pituitary-Gonadal axis) is shut down, resulting in a decrease in gonadotropin. First, animals were divided into four groups. The Group 1 was a control group, and the Group 2, 3, 4 were injected with Diphereline. A week later, the Group 3 was injected with recombinant FSH (Gonal-F), 3 times a week, and the Group 4 with SAFA-FSH once every 2 weeks for 6 weeks (Figure 5A, B). After the end of the experiment period, sperm was collected from the cauda of the mouse epididymis and the number of sperm was measured. Both caudas were measured per individual animal and repeated 4 times using a hemocytometer to reduce the error. Compared to the control group that was not injected with any drug, the sperm count decreased when Diphereline was injected, and the sperm count increased in the group injected with GonalF or SAFA-FSH after Diphereline injection (Figure 5C).

A



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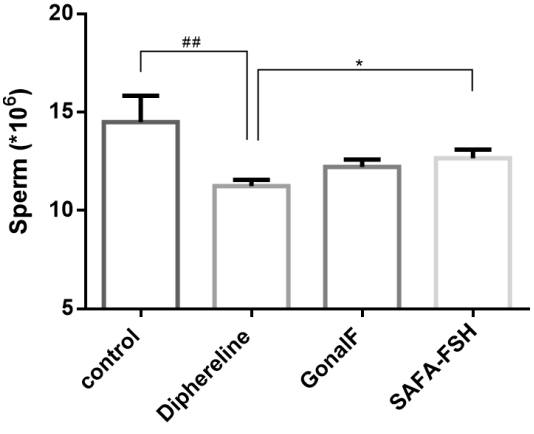

Figure 5. Increased spermatogenesis after injection of recombinant FSH or SAFA-FSH in hypogonadism mouse model (A) Time course of preliminary animal experiment per group. Arrows indicate the injection cycle of each drug. (B) Number of sperm after drug treatment. Both left and right caudas were removed from each individual and measured at two locations on a hemocytometer, respectively. Data were presented as mean ±S.E.M. ##P<0.01, Control (Group1) versus Diphereline treatment group (Group2), *P < 0.05, Diphereline treatment group (Group2) versus SAFA-FSH treatment group (Group4)

6. Recovery of spermatogenic ability after injection of recombinant FSH or SAFA-FSH in hypogonadism Rat

Based on the results of a preliminary experiment in mouse, a hypogonadism model was created in male SD rats. As in the case of mice, the GnRH agonist Diphereline was injected to suppress the production of gonadotropin. Serum testosterone concentration was measured weekly for 2 weeks after Diphereline injection, and it was confirmed that the tesotsterone concentration in the group injected with Diphereline was decreased compared to the control group (Figure 6A, B). It was found that the testosterone concentration dropped a little more at the 2nd week that at the 1st week of Diphereline injection. Two weeks after Diphereline injection, they were divided into 4 groups, one group received saline daily (Group2), one group received hCG only daily (Group3), the other group received hCG and recombinant FSH (Follitrope) daily (Group4), and the last group injected hCG daily with SAFA-FSH once every 2 days for 6 weeks (Group5) (Figure 7A, B). Unlike the mice experiment, hCG hormone, which is known to be necessary for spermatogenesis, was injected together with FSH[19]. Testosterone concentrations were measured weekly for 6 weeks of injection (Figure 7C), and subjects whose testosterone concentration did not increase despite hCG injection were excluded. The Group2 (Diphereline-only) continued to maintain low testosterone level. It suggests that the hypogonadism animal model continues to be well maintained. All three groups administered with hCG significantly increased testosterone level. After 6 weeks of hCG and FSH injection, all animals were euthanized using a CO2 chamber, testis was weighed after adipose tissue was isolated (Figure 7D). The Group2 (Diphereline-only) had significantly lower testis coefficient values than the control group, and the three groups injected with hCG had similar or greater testis coefficient values than the control group (Figure 7E). Of the epididymis part, only cauda was separated. The total number of sperm in cauda was counted by chopping in warm saline solution (Figure 7F).

Similar to testosterone concentration and testis coefficient tendency, the Group2 treated with only Diphereline remarkably reduced the number of sperm by 60% compared to the control group. Group3 injected with hCG only sperm recovered about 88%, Group4 recovered about 95%, and Group5 recovered about 93% (Figure 7G).

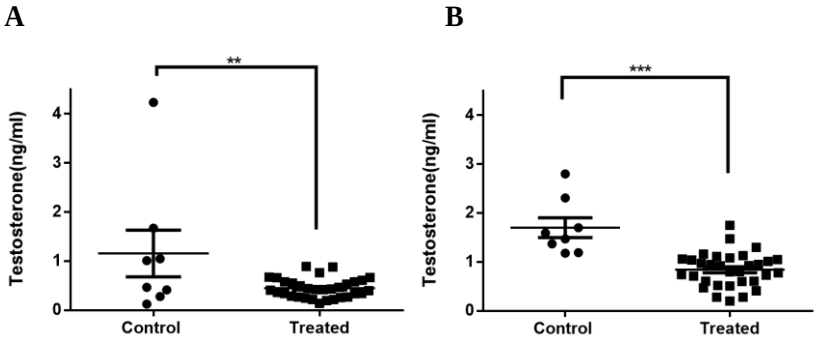

Figure 6. Establishment of hypogonadism Rat model (A) Serum testosterone concentration after 1 week of Diphereline treatment. (B) Serum testosterone concentration after 2 weeks of Diphereline treatment. Each dot represents an individual animal. Data were presented as mean ±S.E.M **P<0.01, ***P<0.0001

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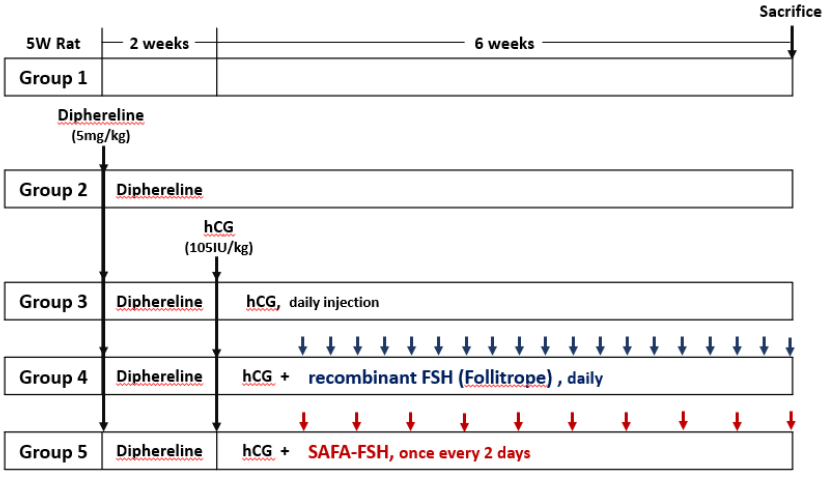

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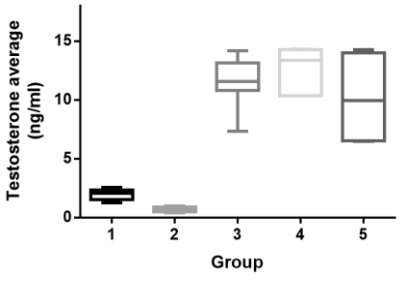

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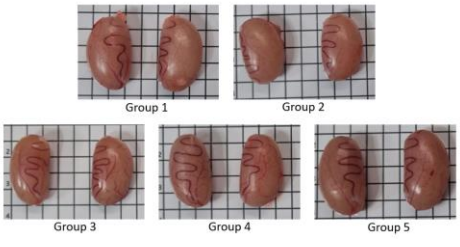

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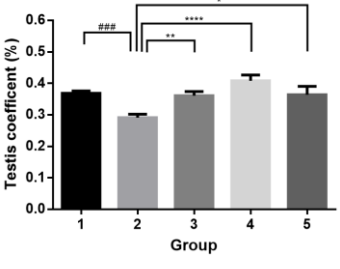

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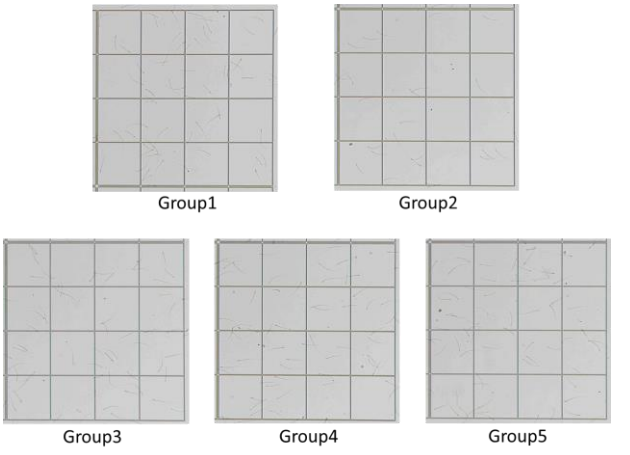

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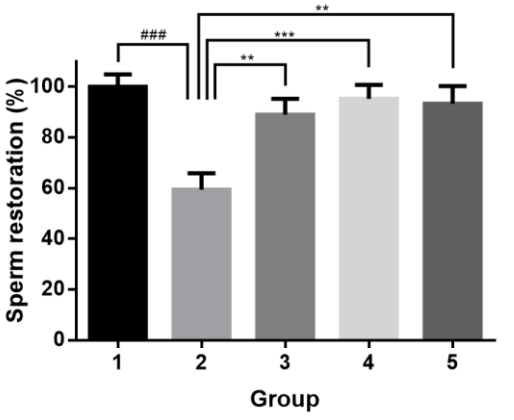

Figure 7. Recovery of spermatogenic ability after injection of recombinant FSH or SAFA-FSH in hypogonadism Rat. (A) Time course of SD Rat *in vivo* experiment per group. Arrows indicate the injection cycle of each drug. (B) Average level of testosterone concentration per group for 6 weeks of hCG, Follitrope, and SAFA-FSH injection (C) Representative testis image for each group (D) Testis coefficient (testis/body weight ratio) for each group. Testis measured the left and right, respectively, and calculated the average value. (E) Representative hemocytometer image with sperm count for each group

(F) Degree of recovery of sperm count per group. Both left and right caudas were counted per subject, and 4 zones were measured in hemocytometer per cauda. Data were presented as mean ±S.E.M. ###P<0.001, Control (Group1) versus Diphereline treatment group (Group2), **P<0.05, **P<0.01, ***P<0.001, Diphereline treatment group (Group2) versus hCG, FSH drug treatment group (Group3 or 4 or 5)

IV. DISCUSSION

Problems with the pituitary gland do not properly secrete gonadotropins, and eventually if the sex hormones are insufficient, infertility may occur. In that case, fertility can be restored through the administration of gonadotropins. Although the use of recombinant FSH is now common, the duration is short, and it is difficult to maintain a constant concentration. Therefore, it was attempted to determine whether it could act as a long-acting hormone of SAFA-FSH, which was made using the SAFA Technology that combines the Fab antibody fragment capable of binding with serum albumin and FSH protein.

In vitro, a stable cell line in with FSH Receptor was overexpressed in mouse sertoli cell, TM4-FSHR cell, was created. It was confirmed that cyclic AMP and phosphorylation of ERK1/2 and CREB were increased by treatment with recombinant FSH and SAFA-FSH, respectively. When both recombinant FSH and SAFA-FSH were treated in TM4-FSHR cells, the expression level of total ERK1/2 was almost same as that of the control, but the expression of total CREB was increased compared to the control. In view of these result, it may be seen that SAFA-FSH also acts in the same way as the pathway in which recombinant FSH acts.

In vivo, a hypogonadal model was created by injecting Diphereline into male mice, and after shutting down the HPG axis, recombinant FSH or SAFA-FSH was injected to check whether sperm formation was restored. The injection cycle was 3 times a week for recombinant FSH, once every 2 weeks for SAFA-FSH, that is, SAFA-FSH was 6 times longer than normal FSH. A hypogonadal model was also made in male rats, and after shutting down the HPG axis the sex hormones LH and SAFA-FSH were injected to determine whether sperm formation was restored. Compared to the daily injection of Follitrope, a recombinant FSH, SAFA-FSH was administered for 2 days. Both *in vivo* experiments showed similar effects to recombinant FSH, even though SAFA-FSH was injected at a longer cycle than recombinant FSH.

However, since the SAFA Technology combines the Fab antibody fragment that

binds to the human albumin protein, the half-life in mice or rats is inevitably lower than in humans. Of course, the half-life may be longer than that of the general recombinant FSH protein, but direct comparison with humans is likely to be difficult.

Testosterone concentration was still checked after hCG injection, but in some animals, testosterone concentration did not increase significantly. Because hCG also affects spermatogenesis, the efficacy of hCG was judged based testosterone level, and subjects with low testosterone level were excluded in the results. The concentration of testosterone in the three groups injected with hCG increased too much compared to the control, so it seems necessary to further reduce the amount of hCG injection later.

Additionally, since it is a long-acting hormone, experiments are needed to confirm it by increasing the injection cycle. And although hCG and FSH injections were performed for a total of 6 weeks in this experiment, it is necessary to consider a method of injecting for a longer period to extend the time for spermatogenesis to recover. Diphereline, a GnRH agonist injected to create a hypogonadism model, is also a long-acting drug, so it is necessary to wait 8 or even 12 weeks under the premise that the efficacy of Diphereline is maintained.

After the testis was removed, Histology was confirmed, but there was no significant visual difference between the experimental groups. In the future, it is considered necessary to confirm by staining with several targets that additionally check the degree of cell proliferation related factors and apoptosis-related factors.

Finally, it can be expected that it will be possible to develop infinitely in the field of antibody engineering by combining not only FSH protein but also other proteins or therapeutic agents using SAFA Technology.

V. CONCLUSION

In vitro, when TM4-FSHR cells in which FSHR is stably expressed were treated with recombinant FSH and SAFA-FSH, respectively, an increase in cAMP production and an increase in phosphorylation of ERK1/2 and CREB were confirmed.

In vivo, even when SAFA-FSH was injected at a longer cycle than recombinant FSH, sperm production was similarly restored in hypogonadism mice and rats using GnRH agonist.

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ABSTRACT (IN KOREAN)

지속형 재조합 사람 난포 자극 호르몬제(SAFA-FSH)의 정자 형성 촉진

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이 수 현

난포자극호르몬(FSH)은 뇌하수체 전엽에서 분비되는 호르몬 중 하나로, 여성에서는 난소의 난포 성장 및 성숙을 촉진하고, 남성에서는 고환의 성숙을 자극해 정자 형성을 촉진하는 역할을 한다. 성호르몬 분비가 제대로 되지 않아 생기는 불임의 경우, 성선 자극 호르몬 투여를 통해 생식 능력을 회복시킬 수 있는데, 재조합 FSH의 사용이 보편화되었지만 지속시간이 짧고, 저용량이며, 일정한 농도 유지가 어렵다. 본 연구에서는 SAFA 기술을 이용하여 혈청 알부민과 특이적으로 결합하는 F_{ab} 항체 단편과 재조합 FSH 단백질을 융합한 long-acting FSH가 지속형 호르몬으로서 작용할 수 있는지 연구하였다. FSH 수용체를 발현하는 세포에서 재조합 FSH와 SAFA-FSH로 인하여 cyclic AMP가 증가되는 것을 확인하였다. 추가로 재조합 FSH와 SAFA-FSH를 처리했을 때 CREB의 인산화와 ERK1/2의 인산화도 증가하였다. 생식샘 자극 호르몬 방출 호르몬 작용제 (GnRH Agonist)를 처리한 생식샘 저하증 (Hypogonadism) 랫드 모델을 만들었으며 재조합 FSH과 마찬가지로 SAFA-FSH를 처리하였을 때에도 정자 생성이 회복되는 것을 확인하였다. 결론적으로, SAFA-FSH는 재조합 FSH처럼 세포 활성화가 일어나고, 재조합 FSH 보다 반감기가 길어 재조합 FSH에 비해

자주 주사하지 않더라도 약물 농도가 유지되어 지속형 호르몬 제제의 효용 증대를 할 수 있다.

핵심되는 말 : 난포자극호르몬, 난포자극호르몬 수용체, SAFA기술, 정자 형성