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Investigation of the Effect of Granulocyte Colony-Stimulating Factor on Endometrial Thickness in the Wistar Albino Rat

Wistar Albino Sıçanında Granülosit Koloni Uyarıcı Faktörün Endometrial Kalınlık Üzerindeki Etkisinin Araştırılması

Sadun SUCU[1], Ülkü ÖZMEN[2]

[1]Department of Perinatology, Ankara Etlik City Hospital, Ankara, Türkiye
[2]Department of Gynecology and Obstetrics, Marmara University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Aim: A thin endometrium is a difficult situation for infertile patients and the doctors who treat them. In our study, we wanted to investigate the efficacy of G-CSF, which can be used for the treatment of infertile couples with thin endometrium, by examining the effect of GCSF on the endometrium in rats.

Material and Methods: In our study, which we used as an animal model, we divided 50 rats into five groups and investigated the effect of G-CSF on thin, rat endometriums obtained using alcohol. Endometrial and uterine thickness were performed semi-quantitatively by an impartial pathologist. At the end of the study, the rats' hemoglobin levels and weight were measured.

Results: When the pathological samples were examined, it was found that G-CSF increased the thickness of the endometrial surface epithelium at the dose and duration administered, but had no effect on the thickness of the uterus and endometrium. Weight and hemoglobin levels were lower in the groups of rats exposed to the surgical procedure than in the control group.

Conclusions: The effect of G-CSF on the endometrium is evaluated differently in various studies in the literature. The main reason for this is that studies on the thin endometrium are not physiopathologically standardized. In our study, we found that G-CSF increased the thickness of the endometrial surface epithelium. We believe that this promotes embryo implantation.

Keywords: Infertility, G-CSF, endometrium, rat

ÖZ

Amaç: İnce endometrium, infertil hastalar ve onları tedavi eden doktorlar için zorlu bir durumdur. Bu çalışmada, infertil çiftlerin tedavisinde kullanılabilecek olan G-CSF'nin etkinliğini araştırmak ve G-CSF'nin endometrium üzerindeki etkisini incelemek amacıyla bir hayvan modeli kullanarak çalışmamızı gerçekleştirdik.

Gereç ve Yöntemler: Hayvan modeli olarak kullandığımız bu çalışmada, 50 sıçan beş gruba ayrıldı ve alkol kullanılarak elde edilen ince endometriumlu sıçanlarda G-CSF'nin etkisi araştırıldı. Endometrial ve uterin kalınlık, tarafsız bir patolog tarafından yarı kantitatif olarak değerlendirildi. Çalışmanın sonunda sıçanların hemoglobin düzeyleri ve kiloları ölçüldü.

Bulgular: Patolojik örnekler incelendiğinde, uygulanan doz ve sürede G-CSF'nin endometrial yüzey epiteli kalınlığını artırdığı, ancak uterus ve endometrium kalınlığı üzerinde etkisinin olmadığı görüldü. Cerrahi işleme maruz kalan sıçan gruplarında kilo ve hemoglobin düzeylerinin kontrol grubuna göre daha düşük olduğu tespit edildi.

Sonuç: G-CSF'nin endometrium üzerindeki etkisi, literatürdeki farklı çalışmalarda farklı şekilde değerlendirilmiştir. Bunun başlıca nedeni, ince endometrium üzerine yapılan çalışmaların fizyopatolojik olarak standardize edilmemiş olmasıdır. Bizim çalışmamızda, G-CSF'nin endometrial yüzey epiteli kalınlığını artırdığı sonucuna ulaştık. Bunun embriyo implantasyonunu desteklediğine inanıyoruz.

Anahtar Kelimeler: İnfertilite, G-CSF, endometrium, sıçan

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Sorumlu Yazar/Corresponding Author: Sadun SUCU, Ankara Etlik City Hospital, Department of Perinatology, Ankara, Türkiye
E-mail: medical.academic.sucu@gmail.com

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INTRODUCTION

Worldwide, 15-20% of couples suffer from infertility. With advances in assisted reproduction and therapy, pregnancy rates have risen. In vitro fertilization (IVF) cycles require a healthy embryo, a successful treatment procedure and an endometrium appropriate for implantation.(1) Since a thin endometrium typically causes implantation failures and cycle cancellation, long-term estrogen therapy, vasoactive drugs and endometrial scraping have been used to thicken it. None of these techniques have consistently helped patients. Increased endometrial thickness and responsiveness was studied with immunotherapy. (2,3)

Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein produced by bone marrow, stromal, mononuclear, fibroblast, natural killer and endometrial cells. G-CSF primarily stimulates the growth and differentiation of neutrophils in the bone marrow and controls their release into the bloodstream. Studies have examined its use in the treatment of couples with recurrent IVF failure.(4,5) G-CSF levels in follicular fluid are thought to influence ovulation and pregnancy.(6) G-CSF influences trophoblast development and placental metabolism, and trophoblast cells express the receptor. These data suggest that G-CSF affects the trophoblast, placenta and embryo implantation.(7) In this study, the effects of G-CSF on the thin endometrium and uterus were investigated in an animal model.

MATERIAL AND METHODS

The local ethics committee for animal experiments at Bülent Ecevit University approved our study (protocol no.: 2015-16-01/07). Fifty female Wistar albino rats weighing between 250 g and 280 g were obtained from the Bülent Ecevit College animal laboratory for our project. The experimental animals were divided into five groups and monitored daily to assess their estrous cycles, while cellular changes in the cervix uteri were analyzed microscopically by cervical smears. Using the Papanicolaou staining method, the predominant cell type (intermediate, basal, superficial, cornified epithelial cells, etc.), the relative abundance of leukocytes, the ratio of superficial cells to other epithelial cells, eosinophilic cytoplasm and nuclear shrinkage were examined to determine the phase of the estrous cycle.

- Experimental group 1: (alcohol+G-CSF) with endometrium thinned by intrauterine alcohol administration and s.c. G-CSF treated group.
- Experimental group 2: (alcohol + PSS) with endometrium thinned by intrauterine administration of alcohol and s.c. physiological saline solution (PSS) was injected.
- Experimental group 3: (G-CSF)The group in which only s.c. G-CSF was injected.
- Experimental group 4: (PSS) The group in which only s.c. physiological saline solution was injected.
- Experimental group 5: (Control) The group that did not undergo any surgical or medical intervention.

The alcohol+G-CSF group was formed to demonstrate the effect of G-CSF on thin endometrium; the alcohol+PSS group was formed to confirm the effect of G-CSF on thin endometrium; the G-CSF group was formed to demonstrate the effect of G-CSF on normal endometrium; the PSS group was formed to study the effect of stress induced by daily G-CSF injection on endometrium; the control group was formed to confirm the results of the other groups.

Iatrogenic Protocol for the Creation of a Thin Endometrium

The abdomen of the rat was inserted through a 2 cm incision in the lower ventral segment, with the timing chosen according to the determination of the phases of the estrous cycle. During the estrus phase, intraperitoneal anesthesia was administered with ketamine at a dose of 80-100 mg/kg and the area was then shaved and cleaned with povidone-iodine. The uterine horns were pulled out of the abdomen and ligated as close as possible to the ovaries with a silk 2-0 suture. At the level of the cervix, the uterus was clamped with a non-crushing clamp and 0.1 ml of 95% ethyl alcohol was injected into both horns while the filling of the horns was monitored. After a waiting period of 3 minutes, the injection of 0.1 ml was repeated in both horns and after a further waiting period of 2 minutes the cervical clamp was opened. The uterine horns were placed in the abdominal cavity, the muscle layer and the anterior abdominal wall were sutured with 2-0 polyglactin 910 and the skin of the rats was sutured with 2-0 silk. After the surgical procedure, the rats were monitored for 4-6 hours to wake up from anesthesia. All animals participating in the experiment were given commercial pellets (12-16 mm diameter) and water ad libitum in a 14-hour light/10-hour dark cycle in the Bülent Ecevit College animal laboratory.

Procedure for G-CSF Administration

The cycle phase was determined in the group in which a thin endometrium was applied after waiting for two–estrous cycles and in the other groups by observation in the following cycle. A subcutaneous G-CSF injection of 40 µg/kg/day was administered for 5 days from the first day of the estrous cycle. The G-CSF injection was continued for a total of 4 cycles and administered during the first 5 days of the estrous cycle.

A similar procedure was used in the PSS group as in the groups with G-CSF injections.

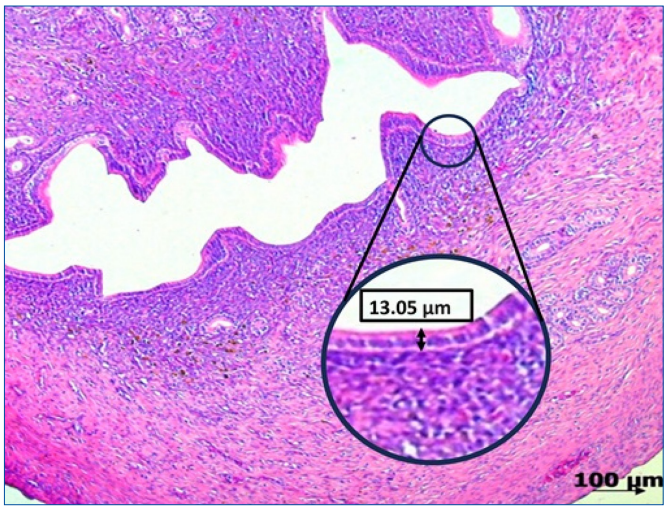
Measurement of the Hemoglobin Level and Body Weight of Rats

The weights of the rats, which had a homogeneous weight distribution before the experiment, were measured under anesthesia shortly before they were euthanized by hysterectomy and exsanguination. Blood samples obtained by exsanguination were evaluated in the microbiology laboratory of Bülent Ecevit College Health Application and Research Center using the CA USA brand Beckman Coulter Unicel DXH 800 Slidemarker Stainer for a complete blood count. Coulter DxH Cell Lysey, Coulter DxH Diff Pack, Coulter DxH Retic Pack and Coulter DxH Cleaner solutions were used in this evaluation.

Histopathological Examination Procedure

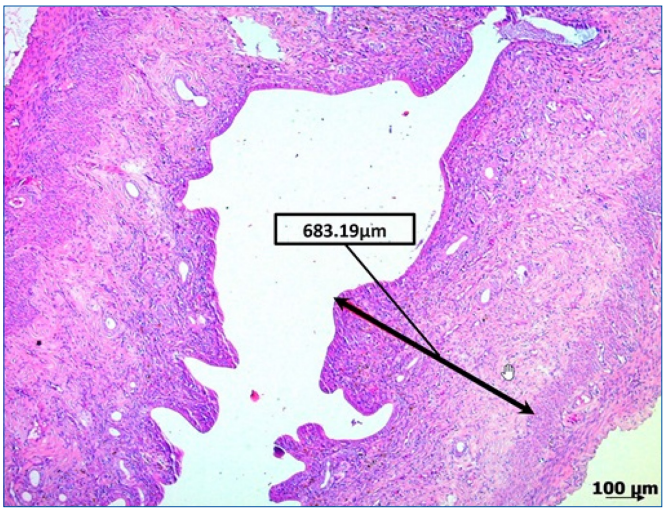
Hysterectomy was performed in rats in the estrus phase of the last cycle treated with G-CSF. The removed specimen was preserved in 10% formaldehyde solution and collected for histologic and pathologic examination. For histopathologic examination, the tissues were embedded in paraffin and hematoxylin-eosin (H&E) sections were prepared from these paraffin blocks. The H&E sections prepared for the uterus were analyzed by light microscopy (Leica DM2500 Optical Microscope Systems, Germany) by an impartial pathologist who did not know the groups. Microscopic images (Imaging System, Zeiss Microscope Axio Imager. A2m, U.S.) were obtained for each study group. The thickness of the endometrial surface epithelium, the thickness of the endometrium and the total thickness of the uterus were measured digitally semi-quantitatively. Considering the endometrial and total uterine thickness of all groups and the areas with the least epithelial indentations, 6 areas of each sample were determined and the average values were calculated by measuring these areas. (Figure 1-5) The pathological specimens were blinded for the pathologists.

Figure 1. Examples for measuring the thickness of the endometrial epithelium.



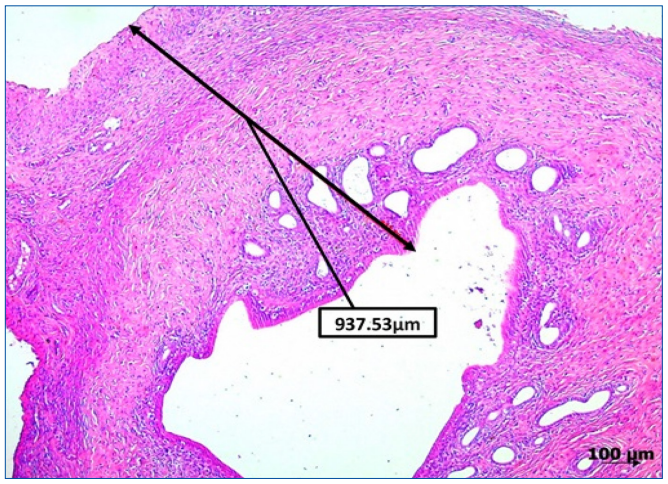
Experimental group 1: (alcohol+G-CSF) " " It shows the measurement of the epithelium thickness in the zoomed part within the circle.

Figure 2. Examples for measuring the thickness of the endometrium.



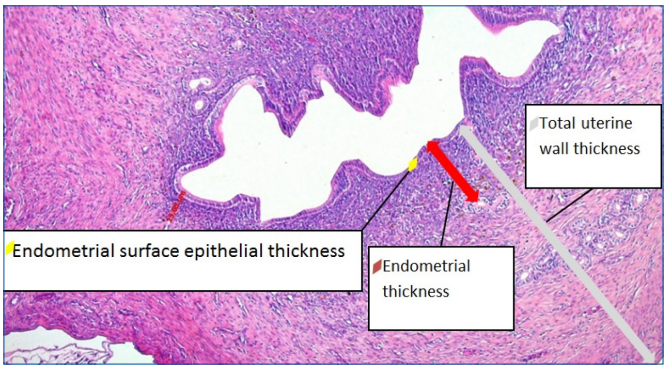
Experimental group 5: (Control) The black arrow shows the total thickness of the endometrium.

Figure 3. Examples for measuring the thickness of the uterus.



Experimental group 4: (PSS) The black arrow shows the total thickness of the uterus.

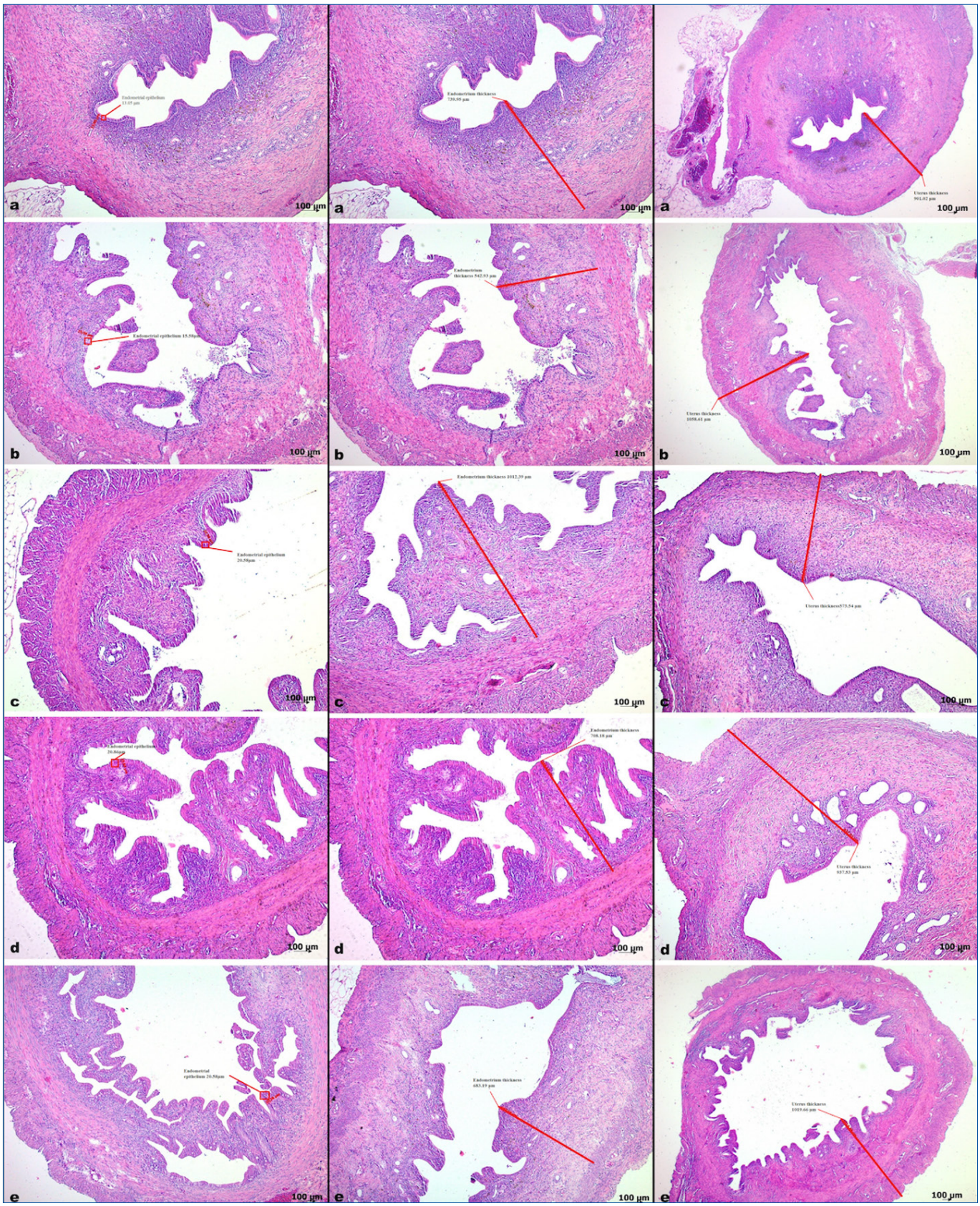
Figure 4. Examples for measuring the thicknesses.



Experimental group 1: (alcohol+G-CSF)

The yellow arrow shows the thickness of the surface epithelium of the endometrium; the red arrow shows the thickness of the endometrium; the gray arrow shows the total thickness of the uterine wall.

Figure 5. Endometria of the rats in the different groups



a: alcohol+G-CSF; b:alcohol+PSS; c:G-CSF; d:PSS; e: Control

This figure shows the measurement methods on similar or identical histological sections.

Table 1. Mean, standard deviation, median, minimum and maximum values for the thickness of the endometrial surface epithelium, the thickness of the endometrium and the total thickness of the uterine wall for each group.

Groups	Endometrial surface epithelial thickness (µm)	Endometrial thickness (µm)	Total uterine wall thickness (µm)
Alcohol + G-CSF (n=10) mean±SD median (min-max)	23.4±7.56 24.6 (11.6-32.6)	570±287.2 592 (238-855)	987±200.6 978 (773-1228)
Alcohol + PSS (n=9) mean±SD median (min-max)	10.6±3.24 9.1 (7.6-15.6)	711±157.9 765 (490-878)	1087±270.4 1067 (782-1412)
G-CSF (n=10) mean±SD median (min-max)	18.4±3.05 17.4 (15.3-23.4)	731±245.4 601 (526-1118)	1068±300.7 1088 (740-1472)
PSS (n=10) mean±SD median (min-max)	19.7±2.95 19.2 (15.6-24.9)	690±130.8 692 (540-869)	1105±168.9 1165 (851-1316)
Control (n=10) mean±SD median (min-max)	20.3±3.62 20.1 (15.6-26.1)	667±73.2 670 (542-746)	1021±135.9 1046 (797-1197)
Total (n=49) mean±SD median (min-max)	18.6±6.0 18.7 (7.6-32.6)	673±195.9 691 (238-1118)	1053±217.9 1061 (740-1472)
p- value	**<0.001**	0.803	0.751

G-CSF, granulocyte colony-stimulating factor ; PSS, physiological saline solution.

Table 2. p-values comparing the groups in terms of the thickness of the surface epithelium of the endometrium.

Groups	Alcohol + G-CSF	Alcohol + PSS	G-CSF	PSS	Control
Alcohol + G-CSF		**0.001**	0.750	0.105	0.393
Alcohol + PSS	**0.001**		**0.001**	**<0.001**	**<0.001**
G-CSF	0.750	**<0.001**		0.315	0.190
PSS	0.105	**<0.001**	0.315		0.684
Control	0.393	**<0.001**	<0.190	0.684	

G-CSF, granulocyte colony-stimulating factor ; PSS, physiological saline solution. Statistically significant p-values are in bold.

Monitoring postoperative well-being: Postoperative welfare monitoring and follow-up of the rats participating in the study were conducted according to the recommendations of the Institutional Animal Care and Use Committee (IACUC). For rats undergoing midline incision surgery, daily postoperative monitoring was performed for the first 7-10 days (including weekends and holidays). This monitoring was performed simultaneously by the study investigators and the veterinarian of the Bülent Ecevit College Animal Experimentation Laboratory . The areas of surgical incisions, diet, body temperature and water intake of the rats were monitored. The rats were not given analgesic or prophylactic antibiotic treatment so as not to cause a reaction with G-CSF, which was the subject of the animal experiment, or to affect the elimination process of the drug. Since the suturing of the midline incision was performed with absorbable suture material, it was not necessary to remove the sutures later. No lethargy, loss of appetite, fever, wound dehiscence, signs of infection (redness, excessive swelling or discharge), missing suture material or wound dehiscence were observed at the controls.

Table 3. Mean, standard deviation, median, minimum and maximum values for weight, hemoglobin for each group.

Groups	Weight (gr)	Hb (g/dl)
Alcohol + G-CSF (n=10) mean±SD median (min-max)	247±24.9 249 (210-280)	12.2±0.5 12.0 (11.5-13.0)
Alcohol + PSS (n=9) mean±SD median (min-max)	249±11.4 248 (231-269)	12.5±1.4 12.3 (10.9-14.4)
G-CSF (n=10) mean±SD median (min-max)	266±16.4 260 (249-295)	14.2±1.1 14.5 (12.5-15.2)
PSS (n=10) mean±SD median (min-max)	249±14.6 258 (249-274)	13.7±0.9 13.6 12.2-14.9
Control (n=9) mean±SD median (min-max)	274±9.1 274 (263-286)	13.8±0.9 13.4 (12.8-14.9)
Total (n=48) mean±SD median (min-max)	257±19.0 258 (210-295)	13.3±1.3 12.9 (10.9-15.9)
p-value	**<0.001**	**<0.001**

Hb, hemoglobin; WBC, white blood count; G-CSF, granulocyte colony-stimulating factor ; PSS, physiological saline solution.

Table 4. p-values comparing the groups in terms of weight, hemoglobin, white blood count, neutrophils.

	Alcohol + G-CSF	Alcohol + PSS	G-CSF	PSS	Control
WEIGHT					
Alcohol + G-CSF		0.971	0.165	1.000	**0.006**
Alcohol + PSS	0.971		**0.009**	0.971	**0.001**
G-CSF	0.165	0.009		**0.019**	0.095
PSS	1.000	0.971	**0.019**		**0.001**
Control	**0.006**	**0.001**	0.095	**0.001**	
HEMOGLOBIN					
Alcohol + G-CSF		0.661	**0.001**	**0.002**	**<0.001**
Alcohol + PSS	0.661		**0.002**	0.095	**0.024**
G-CSF	**0.001**	**0.002**		0.353	0.604
PSS	**0.002**	0.095	0.353		0.604
Control	**<0.001**	**0.024**	0.604	0.604	

G-CSF, granulocyte colony-stimulating factor ; PSS, physiological saline solution. Statistically significant p-values are in bold.

Statistical Analysis

The statistical analysis was performed with the program SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were expressed as arithmetic mean±standard deviation and median (minimum-maximum). Kruskal-Wallis analysis of variance was used to compare the groups. In the Kruskal-Wallis analysis of variance, the pairwise comparison of the subgroups was carried out using the Dunn test. For all evaluations, the p-value < 0.05 was considered significant. The groups were blinded for the statisticians.

RESULTS

Of the 50 subjects enrolled in the study, which were randomly divided into five groups according to age and body weight, one rat in the alcohol + PSS group was considered unsuitable for histopathologic examination and blood count evaluation due to an intra-abdominal infection. One rat in the control group was excluded from the blood count analysis because the blood sample had clotted prior to analysis.

There was no statistically significant difference between the groups with regard to the total thickness of the uterine wall and the endometrium. When evaluating the thickness of the endometrial surface epithelium, the alcohol + PSS group had the lowest value compared to the other groups. ($p < 0.005$) (Table 1) There was no statistically significant difference between the other groups in terms of endometrial surface epithelium. ($p > 0.05$) (Table 2)

At the end of the study, the group with the highest weight in the weight measurements taken shortly before the rats were euthanized by exsanguination was the control group. The alcohol+PSS group had the lowest median weight. Compared to the control group, the median and mean values were lower in the alcohol+G-CSF, alcohol+PSS and PSS groups. ($p < 0.001$) Hemoglobin levels were statistically significantly lower in the groups exposed to the surgical intervention (alcohol + G-CSF and alcohol + PSS) than in the control group. ($p < 0.001$; $p=0.024$) The lowest hemoglobin level was found in the alcohol + G-CSF group. The G-CSF group had the highest hemoglobin level. (Table 3) The p-values for the pairwise comparisons of the groups in terms of weight and hemoglobin are shown in Table 4.

DISCUSSION

There are numerous studies in the literature on the effects of a thin endometrium on infertility and its treatment. Some of these relate to drug treatment and others to the receptivity of the endometrium. In any case, a thin endometrium and its treatment is a challenge. Multiple studies consistently demonstrate a correlation between a thin endometrium and unfavorable outcomes in the process of implantation. (8-10) Thin endometrium is difficult to treat and attempts are made to increase the pregnancy and implantation rate. Various treatment methods (low-dose acetylsalicylic acid, estrogen replacement, vitamin E, etc.) have been tried in the literature. Various etiopathogeneses have been investigated and treated in studies. In the past, vitamin E, sildenafil and acetylsalicylic acid were the most prominent among the studies in which the effects of various medications and dietary supplements on the thin endometrium were investigated. (11) It has been shown that the use of pentoxifylline and tocopherol in thin endometrium leads to positive pregnancy results and improves the pregnancy rate. (12) There is evidence that vaginal sildenafil improves uterine artery blood flow and endometrial development in IVF patients with previously poor endometrial response. (13) In addition, Ikoma T and

colleagues have shown that bone marrow cells transplanted from male donors were detected by immunofluorescence labeling of the Y chromosome in endometrial curettage pathologies from female patients. This suggests that bone marrow-derived stem cells may be a source of endometrial stem cells and that G-CSF may have an effect on the endometrium via this mechanism. (14) Although these similar studies have inspired many new studies, they suggest that the agents used act on the thin endometrium via hypothetical pathways, as they do not define a clear pathophysiological pathway for the cause of the thin endometrium and patients are not standardized in this way. To avoid this situation, we believe that a study investigating the effect of a single agent on the thin endometrium, standardized in the same way, will provide more accurate results. In our study planned for these reasons, we found that G-CSF increased the thickness of the endometrial epithelium. There was no significant difference in endometrial epithelial thickness between the alcohol+G-CSF group and the control, PSS and G-CSF groups, but higher values of epithelial thickness in the alcohol+G-CSF group than in the alcohol+PPS group. This shows that G-CSF increases the epithelial thickness of the endometrium. The observation of the lowest endometrial epithelial thickness in the alcohol + PSS group among the study groups showed that the method was successful in producing a thin endometrium epithelium. The fact that only the G-CSF-treated group showed no significant difference from the control group and the PSS group in terms of endometrial epithelial thickness indicates that G-CSF has no significant effect on rats whose endomerium is not thin. Since we assume that the effect of G-CSF in the studies mentioned is directed at the surface epithelium of the endometrium, we think that the treatments with medical agents (VEGF, sildenafil, etc.) have indirect effects. In this context, it appears that only the right indication for the use of G-CSF will lead to successful treatment. In the PSS group, which was exposed to a daily injection, it was shown that the daily injection stress had no influence on the thickness of the endometrial epithelium, which did not differ from the control group. The fact that there was no difference between the groups in terms of total uterine thickness and total endometrial thickness suggests that the effect of G-CSF on these parameters is not significant. Fatemeh Sarvi and colleagues found that using GCFS in women with thin endometrium on HCG injection, oocyte retrieval, and embryo transfer increased endometrial thickness and implantation rate. (15) Maryam Eftekhar et al. observed that G-CSF may thicken endometrium. It has not been found to increase chemical and clinical pregnancy rates in infertile women with thin endometrium in frozen embryo transfer. This may be because G-CSF (Leukemia Inhibitory Factor) promotes LIF and reduces CD16, CD56, which are crucial to implantation. (16) In the study by Won et al. on the best time of intrauterine GCSF injection and its effects on the endometrium, adhesion molecules increased and natural killer cell activity decreased. (17) All these results are indeed related to the surface epithelium of the endometrium, the importance of which was emphasized in our study.

G-CSF can be synthesized by multiple cells at the maternal-fetal interface and contributes to the regulation of trophoblast development, endometrial decidualization, placental metabolism and angiogenesis. It is an important means of intercellular communication. The study by Jinli Ding and colleagues showed that G-CSF from M2 macrophages can promote trophoblast invasion and migration by activating the PI3K/AKT/Erk1/2 signaling pathway and thus may be involved in the normal course of pregnancy. (18) Combining the results of these studies with the results of our study, we believe that the success of implantation is achieved by increasing the thickness of the endometrial epithelium where the embryo's first point of contact with the mother, and that this is related to adhesion molecules and inflammatory processes. We believe that the discrepancy between chemical pregnancy and clinical pregnancy rates between studies is due to the lack of standardization of the physiopathology of the thin endometrium. In these studies, when investigating the effects of G-CSF on the thin endometrium, the part of the endometrium on which it mainly has a morphological effect should be considered.

The meta-analysis of 14804 individuals from 20 research between 2014 and 2022 found that in vitro fertilization in patients with endometrial receptivity problems is not only dependent on endometrium state. The comprehensive analysis found that women with endometrial receptive dysfunction need a tailored strategy with high-quality diagnostic and successful therapy to attain appropriate thickness and enhanced endometrial receptivity. Due to methodological flaws in the included studies, more study is needed to determine endometrial thickness, structure, treatment procedures, and other parameters' independent value. (19) G-CSF improves endometrial receptivity and conception rates, according to 10 research from 2011 to 2017 that included 475 individuals. The research is conflicting and hard to compare due to the small number of studies on this issue and the diverse study styles. More controlled, randomized studies with more people are needed to identify the right prescription, dosage, and duration. (20) The common point of these meta-analyses is that a thin endometrium has different outcomes in implantation, biochemical pregnancy, clinical pregnancy and live birth rate and that further studies are needed. Furthermore, as shown in studies examining the relationship between infertility and the endometrium, many molecular factors and demographic changes are influential. (21) We believe that the reason for this is that the physiopathology of thin endometrium is not clearly understood. In our study, we have shown that G-CSF increases epithelial surface epithelium thickness

in thin endometrium obtained by the same method (cytotoxic alcohol). However, we think that it may also be useful in women with similar causes such as Asherman's syndrome or previous endometritis. We can also imagine that G-CSF contributes to the success of implantation and IVF by increasing the receptivity of the endometrium due to the enlarged surface epithelium.

Looking at weight and postoperative hemoglobin levels, which are the secondary outcomes of the study, the highest body weight is seen in the control group which was not exposed to any intervention. The fact that the G-CSF group has a higher body weight on average than the PSS group and there is no statistically significant difference between it and the control group shows that the G-CSF group has no effect on weight loss. The fact that there is no statistically significant difference in postoperative hemoglobin in the G-CSF group compared to the control and PSS groups shows that G-CSF is not a risk factor for anemia. The hemoglobin levels in the alcohol+G-CSF and alcohol+PSS groups exposed to the surgical procedure are statistically significantly lower than in the control group. The postoperative hemoglobin level in the G-CSF group does not differ from that of the PSS group and the control group. For this reason, we believe that G-CSF has no negative effects on anemia.

Our animal model study, which was designed to shed light on treatment with G-CSF to increase endometrial thickness, differs from other studies on the effect of G-CSF in the relationship between drug dose, application method and time. Many studies on the same topic and more generally studies on the relationship between the endometrium and implantation, pregnancy rates and live birth rates show that there are many different factors in this cascade. Pathology occurring at any point in this cascade has an impact, from obtaining a suitable endometrium to embryo implantation and even live birth rates. With our study, in which we investigated the endometrial epithelium thickness aspect of this cascade, we aimed to contribute to the literature and demonstrate the histologic effects of G-CSF application on the endometrium. As this was an animal study, the physiopathology of the thin endometrium was standardized in our study and the effect of G-CSF was more clearly visible thanks to this standardization. We believe that we differed positively from other studies in this respect. Unfortunately, due to the nature of our study, the uterus of the rats was removed, so we could not determine the implantation and live birth rates after G-CSF application in rats.

CONCLUSION

Consequently, infertility due to a thin endometrium is a problem whose physiopathology and treatment is difficult. In this study, which can serve as a benchmark for the animal model we created and subsequent studies, we recommend clinicians and researchers to determine the cause of thin endometrium or plan treatment for the possible cause. We believe that not every infertility patient with thin endometrium can be treated with G-CSF and that randomized controlled trials are needed to develop the right treatment protocols for the right patient. Future studies on the treatment of infertility with a thin endometrium or G-CSF therapy should focus on the endometrial surface epithelium and aim to determine whether the increase in epithelial endometrial thickness induced by G-CSF treatment in thin endometrium is due to increased migration of bone marrow precursors with subsequent differentiation and maturation or due to G-CSF treatment-induced proliferation of epithelial cells. It should be noted that the effect of G-CSF is morphologically based on the surface epithelium of the endometrium. The effect of G-CSF on endometrial surface epithelium thickness has been demonstrated at the histopathological level, but its direct clinical implication for infertility treatments has not yet been clearly established.

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