

Granulocyte Colony-Stimulating Factor (G-CSF) in the Mechanism of Human Ovulation and its Clinical Usefulness

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Abstract: In 1980, Espey proposed a famous hypothesis that mammalian ovulation is comparable to an inflammatory reaction and many researches have proved the validity of his hypothesis in the last three decades. For example, interleukin (IL)-1 β , IL-6, tumor necrosis factor (TNF)- α , granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and other inflammatory cytokines presence was proven in the preovulatory follicle. Since granulocyte is the major leukocyte and it plays a very important role during inflammation, the importance of granulocyte and its related cytokine, granulocyte colony-stimulating factor (G-CSF) in the mechanism of human ovulation is easily predictable. G-CSF is one of the hemopoietic cytokines and it has strong positive effects on granulocytes. G-CSF increases the number of granulocytes and it improves the function of granulocytes. In this review, the participation of leukocytes in the ovulation mechanism is demonstrated first. Second, the participation of G-CSF is shown in comparison with the above mentioned cytokines. Finally, since G-CSF has been used for more than 20 years as a medicine without severe side effects in the field of oncology, the clinical application of G-CSF for the treatment of an ovulation disorder, luteinized unruptured follicle (LUF), will be discussed.

Keywords: Granulocyte colony-stimulating factor (G-CSF), ovulation, leukocyte, granulocyte, cytokine, luteinized unruptured follicle (LUF), clomiphene, human chorionic gonadotropin (hCG).

1. INTRODUCTION

Ovulation is a pivotal event in the female reproductive system. Its mechanism in the ovary is initiated by the luteinizing hormone (LH)-surge from the pituitary gland and terminates at 36 hours after the LH-surge in humans with the rupture of the follicle by a "physiologic injury" to the follicular wall followed by the discharge of the oocyte. During this period, the leading follicle increases its size to 20 mm in diameter and it disappears by ovulation. Due to its importance in reproduction, the clarification of the ovulation

Reich (1999) [6] and others revealed the progress of the research. Although early research was performed mainly using animal models, such as rats, rabbits and ewes, the recent prevalence of assisted reproductive technology (ART) has brought much information on human ovulation. Since we discuss human ovulation in this review, the data presented is mainly human data unless otherwise noted.

In regard to the rupture of the follicular wall in mammalian ovulation, the following theory has obtained consensus at present (Fig. (1)). The ovarian blood flow shows a rapid increase as $116.6 \pm$

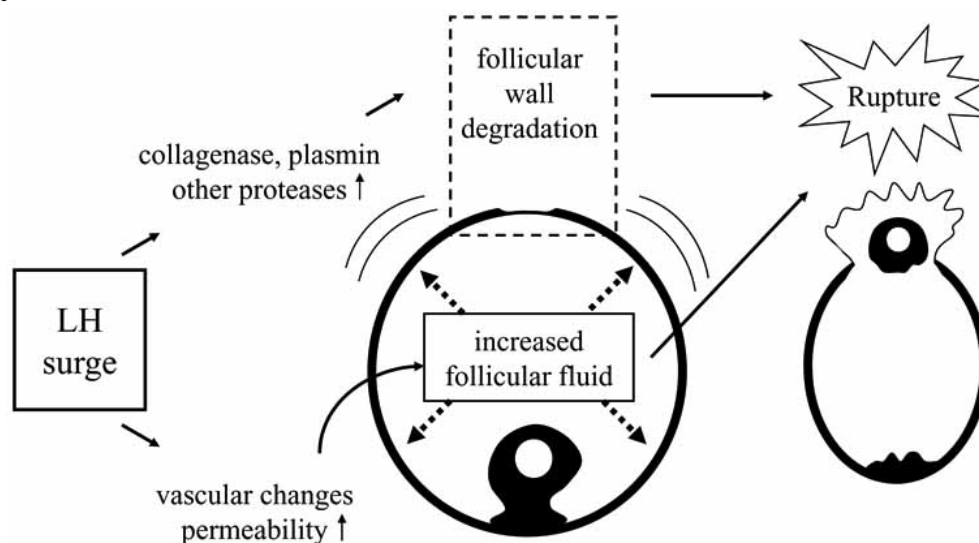


Fig. (1).

Fig. (1). Mechanism of ovulation. Details in the text.

mechanism in the ovary has been a great concern in the fields of Reproductive Physiology, Obstetrics and Gynecology. The reviews by Hartman (1932) [1], Asdell (1962) [2], Espey (1980) [3], Thibault and Levasseur (1988) [4], Le Maire (1989) [5], Tsafirri and

4.3 % (M \pm SEM) at 15 min, 122.1 ± 5.1 % at 30 min following human chorionic gonadotropin (hCG) administration and maintains its high level percentage during the subsequent 2 to 4 h, showing the peak with 156.2 ± 9.1 % (100 % = before hCG administration) in rabbits [7]. Spectrophotometric study shows that the ovary was engorged by the increased blood flow in rats [8]. Also in humans, Waseda *et al.* [9] revealed the immediate increase of ovarian blood flow after hCG injection in patients treated by clomiphene citrate

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(CC) – hCG and human menopausal gonadotropin (hMG) – hCG. Vascular endothelial growth factor (VEGF), which stimulates vascular permeability, showed increased expression by ovulatory gonadotropin stimulus in the rat ovary [10]. VEGF was present also in the human follicle [11]. VEGF and other substances increased the vascular permeability [3, 5]. By the additive effects of the increased blood flow and the increased vascular permeability, the amount of follicular fluid increases with the course of time and the follicle swells up. On the other hand, proteolytic enzymes activity in the follicular wall increased during preovulatory period [12]. These enzymes activity facilitates remodeling of the follicular wall, resulting in a decrease in the thickness of the follicular wall. Recently, Liu *et al.* [13] showed that plasminogen - deficient mice treated with the broad - spectrum matrix metalloproteinase (MMP) inhibitor galardin had successful ovulation. However even in their study, there was a mild (18 – 20%) reduction in ovulation efficiency. It means that such proteolytic enzymes play some roles in the mechanism of follicle rupture. Since the proteolytic enzymes that participated in tissue remodeling of the follicular wall were not only MMP and plasminogen activator (PA) but also cathepsin-L, pregnancy associated plasma protein A (PAPP-A), etc. [14], it is strongly speculated that other proteolytic enzymes compensate for the lack of MMP and PA in such mice. In addition, there is also the possibility that increased follicular fluid can rupture the follicular wall. In conclusion, as a balloon bursts by over inflation, increased follicular fluid ruptures the thinner follicular wall and the oocyte is expelled from the follicle as shown in Fig. (1).

In 1980, Espey [3] proposed a famous hypothesis, ovulation as an inflammatory reaction, based on hyperemia by increased blood flow, connective tissue remodeling and many other similarities. His hypothesis was widely accepted and much fruitful research was performed in the last 3 decades. For example, interleukin (IL)-1 [15], IL-6 [16], tumor necrosis factor (TNF) - α [17], granulocyte - macrophage colony-stimulating factor (GM-CSF) [18], macrophage colony-stimulating factor (M-CSF) [19, 20] and the presence of other inflammatory cytokines was proven in the preovulatory follicle. Since granulocyte is the major leukocyte and it plays a very crucial role during inflammation, the importance of granulocyte colony - stimulating factor (G-CSF) in the mechanism of human ovulation is easily predictable. However, little attention was paid to the relation between G-CSF and ovulation. In this review, we first discuss the participation of leukocyte in the ovulation mechanism and then the participation of G-CSF. Finally, the clinical applications of G-CSF in reproductive medicine will be demonstrated.

2. LEUKOCYTES IN OVULATION MECHANISM

Although the effect of the normal menstrual cycle on the leukocyte counts has frequently been investigated, the results showed no significant increases of leukocyte count around ovulation [21, 22, 23]. However, examining the data presented in the literature, a mild non-significant increase of leukocyte around ovulation was observed in many studies [21, 22]. Moreover, the leukocyte activity for alkaline phosphatase showed a significant increase at the ovulatory period even in the studies around 1970 [24, 25]. Furthermore, women undergoing ovulation induction by gonadotropin revealed leukocytosis [26, 27] and the increased polymorphonuclear leukocytes in peripheral blood at the ovulatory period were in an activated state [28]. These results in humans pointed out the importance of leukocytes in the mechanism of ovulation. More obvious reports on the participation of leukocytes in ovulation were obtained by animal studies. Hellberg *et al.* [29] reported that the LH - induced ovulation in the *in vitro* - perfused rat ovary increased more than 2 times by the supplementation of leukocytes. Using a neutrophil - depleting monoclonal antibody, the ovulation rate in rats decreased to 27% [30]. These findings conform well to the Espey's hypothesis that mammalian ovulation is compatible to an inflammatory reaction [3]. Although he himself presented the involvement of leuko-

cytes and macrophages in ovulation in 1980, Espey wrote in his later review in 1994 [31] that the central role of leukocytes in ovulation was doubtful. The basis of this change was the lack of evidence that leukocytes migrated into the connective tissue layers in a position where they could degrade the follicle wall. Moreover, Chun *et al.* [32] showed in rats that severe leukocyte depletion by vinblastine sulfate and cyclophosphamide did not affect follicular rupture. However, since this report was based on only 4 – 6 animals, it didn't have much statistical power. At least in humans, localization of leukocyte subsets in the follicle wall was clarified by Brännström *et al.* [33]. The presence of various leukocytes in follicular fluid and their production of proteolytic enzymes were also reported [34, 35]. It is common sense that cytotoxic therapy induces ovarian failure in humans [36, 37, 38]. Based on these findings, the central role of leukocytes in ovulation is evident in humans.

The accumulation of leukocytes in follicles just before ovulation in animals was pointed out by Zachariae *et al.* [39], Zackrisson *et al.* [40] and others. Early researches were mainly interested in basophils among subsets of leukocytes. Later studies revealed that not only basophils but also neutrophils [41, 42, 43] and macrophages [43, 44] were detected in great numbers in ovulating follicles. Also in humans, an increase of neutrophils and macrophages in the follicle wall was observed a few hours after LH-surge and the high population of cells was observed just before and after follicular rupture [33]. Neutrophils and macrophages are multifunctional cells which secrete classical ovulatory mediators such as eicosanoids, platelet activating factor and proteolytic enzymes. Secreting these mediators, leukocytes mainly participate in the dissociation and digestion of the follicular wall and lead to ovulation.

3. G-CSF IN OVULATION MECHANISM

In spite of the similarity of ovulation with inflammation and its dominant presence during ovulation, granulocyte and its related cytokine, G-CSF attracted little attention in ovulation research [45]. Instead of granulocyte, eosinophil and later macrophage were the focus of much study. Inflammatory cytokines, IL-1 β [46], IL-6 [16, 46], TNF- α [17], GM-CSF [18, 47] and M-CSF [20, 48, 49] were detected in human follicular fluid and their relevance to the human ovulatory process was discussed. Comparing G-CSF with these cytokines, the involvement of G-CSF in the ovulation mechanism will be revealed.

3.1. Follicular Fluid / Serum Concentration Ratio of G-CSF and Other Cytokines

ART including *in vitro* fertilization and embryo transfer (IVF-ET) (Fig. (2)) led to many discoveries in the human ovulatory process. Especially, collected follicular fluid at oocyte retrieval, which was carried out just before ovulation, was very informative to intra-follicular physiology. The presence of the above mentioned cytokines in human follicular fluid just before follicle rupture was pointed out. However, if the cytokines participate in the mechanism of follicle rupture, the concentrations in the follicular fluid must be higher than those of serum. Although there are many reports on the follicular fluid and serum concentrations of cytokines, these reports measured only one or a few cytokines and the value varies much among reports. For example, the follicular fluid / serum concentrations' ratio of M-CSF was reported as 1.3 by Nishimura *et al.* [20] and as more than 10 by Salmassi *et al.* [49]. Fujii [50] measured the follicular fluid and serum concentrations of these cytokines and G-CSF in the same samples at oocyte retrieval (Table (1)). Based on his data, only IL-6, M-CSF and G-CSF showed significantly higher concentrations in follicular fluid than in serum. IL-1 β revealed no significant differences. TNF- α and GM-CSF showed lower concentrations in follicular fluid. Moreover, G-CSF showed the highest follicular fluid / serum concentration ratio at 2.40 and the significance (p value) was the highest among IL-6, M-CSF and G-CSF.

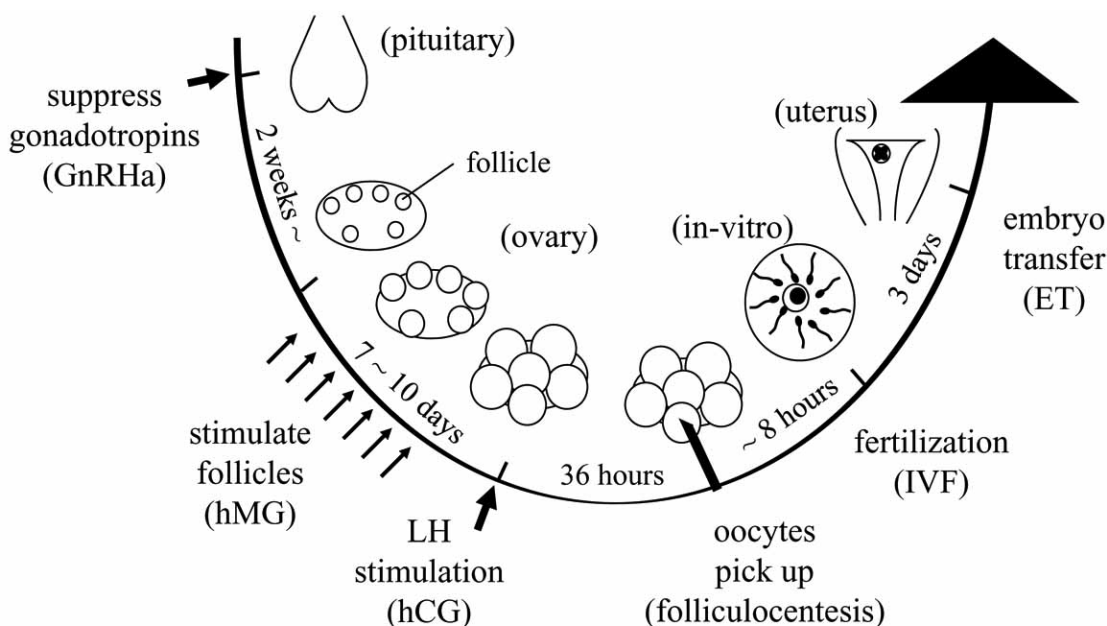
**Fig. (2).**

Fig. (2). Procedure for IVF-ET. GnRHa: gonadotropin-releasing hormone agonist; hMG: human menopausal gonadotropin; hCG: human chorionic gonadotropin.

Table 1. Concentrations of Cytokines in Follicular Fluid and Serum from IVF Patients Measured by Fujii [50]

Cytokines		Serum (S)	Follicular Fluid (FF)	FF/S	<i>p</i> value
TNF- α	[pg/ml]	6.54 \pm 0.53	5.11 \pm 0.40	0.78	<0.05
IL-1 β	[pg/ml]	2.12 \pm 0.56	1.67 \pm 0.06	0.79	<i>N.S.</i>
IL-6	[pg/ml]	2.33 \pm 0.30	3.96 \pm 0.25	1.69	<0.01
GM-CSF	[pg/ml]	2.00 \pm 0.13	1.38 \pm 0.04	0.69	<0.01
M-CSF	[U/ml]	859.8 \pm 92.1	1239.8 \pm 91.2	1.44	<0.05
G-CSF	[pg/ml]	30.71 \pm 3.55	73.75 \pm 5.51	2.40	<0.001

The data was shown as Mean \pm SEM. Statistical differences were evaluated by Mann-Whitney U test.

3.2. Changes of Serum Concentrations of G-CSF and Other Cytokines During the Normal Menstrual Cycle and Ovarian Stimulation

Makinoda *et al.* [51] reported that G-CSF showed a significant increase during the ovulatory phase compared with other phases. They later measured the changes of serum concentrations of IL-1 β , IL-6 and G-CSF during the normal menstrual cycle [22]. The results were that only G-CSF showed a significant increase during the ovulatory phase. Fujii [50] examined the changes of serum concentrations of various cytokines in patients receiving ovarian stimulation. He revealed that only G-CSF showed a significant increase on Day -5 ~ -1 (Day 0 = ovulation) compared with other periods and other cytokines (Fig. (3)). In Makinoda's report [51], ovulatory phase was estimated by basal body temperature (BBT) in women with the normal menstrual cycles. In contrast, Fujii's report [50] was carried out in patients receiving ovarian stimulation. Therefore, the days examined by Fujii were more accurate. As Hock *et al.* [26] pointed out that the G-CSF level in serum rose significantly during the stimulation cycles, G-CSF in serum increased a few days before ovulation. Based on these reports, the ovulatory phase in Makinoda's report [51] might include the late follicular phase. Yanagi *et al.* [52] indicated that G-CSF mRNA was 10 times higher in the late follicular phase than in other phases. Furthermore, she showed the localization of G-CSF mainly in granulosa cells. Salmassi *et al.*

[53] also reported the presence of G-CSF and its receptor in granulosa cells.

Considering these reports regarding G-CSF and ovulation, the following speculation is significant (Fig. (4)). With the stimulation of follicle stimulating hormone (FSH), the dominant follicle gets bigger. At the late follicular phase, especially a few days before follicle rupture, granulosa cells produce G-CSF, which induces leukocyte accumulation in the follicle and follicular wall. Leukocyte in the follicular wall markedly increases its number with the LH-surge [33] and it produces proteolytic enzymes [54]. Increased follicular fluid and decreased firmness of the follicular wall induce the rupture of the follicle, ovulation.

4. CLINICAL USEFULNESS OF G-CSF IN REPRODUCTIVE MEDICINE

The presence of G-CSF in the preovulatory follicle and its participation in the mechanism of ovulation as described above is quite clear. Salmassi *et al.* [55] proposed that G-CSF level in serum and follicular fluid was a predictor of IVF outcome. Such diagnostic use of G-CSF is very interesting and must be considered in further clinical applications. Since G-CSF has been purified, cloned and produced by the recombinant DNA technology [56] and has been safely used as a clinical medicine for more than 20 years in the field

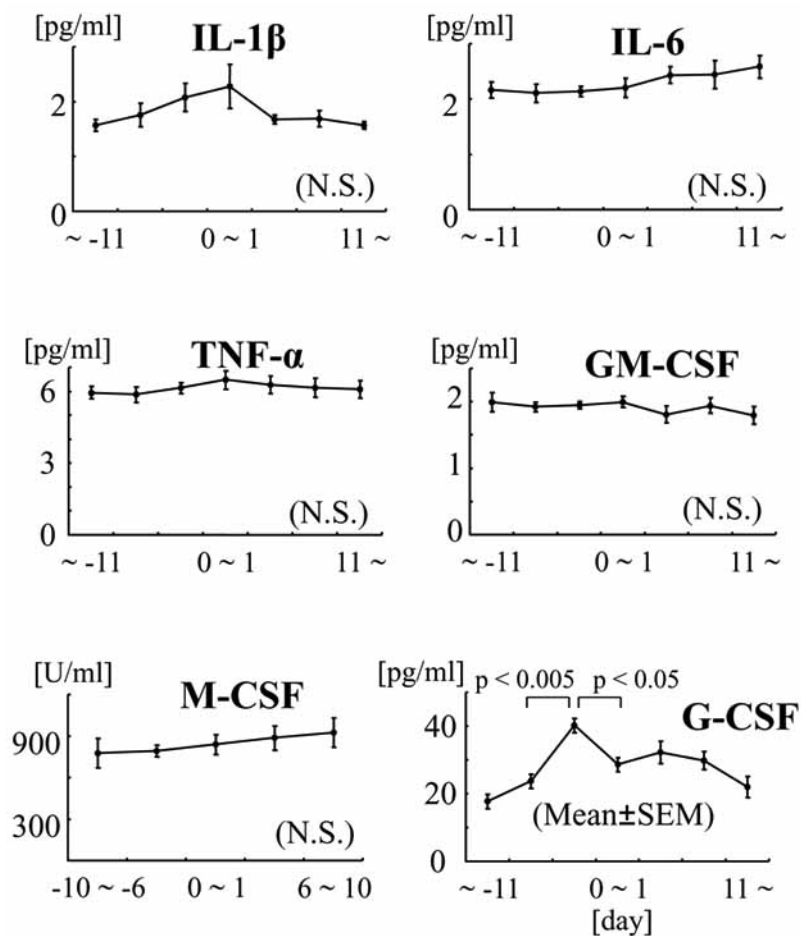


Fig. (3). Cyclic changes of cytokines measured by R. Fujii [50]. Statistical differences were evaluated by ANOVA with Scheffe test. N.S.: not significant; day 0 = ovulation.

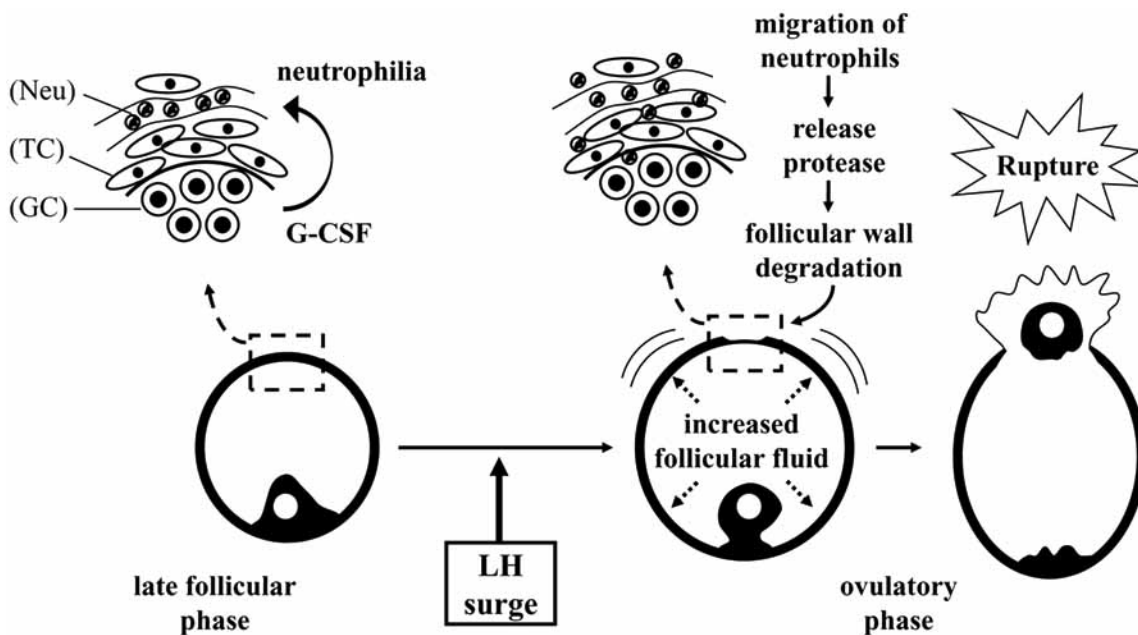


Fig. (4). G-CSF in ovulation mechanism. Neu: neutrophil; TC: theca cell; GC: granulosa cell.

of oncology [57], the use of G-CSF for ovulation disorders might be possible. In regard to this point, G-CSF is quite different from other cytokines that are not clinically used as medicine. As G-CSF plays the main role in follicle rupture, the application of G-CSF for luteinized unruptured follicle (LUF) is discussed below.

4.1. Luteinized Unruptured Follicle (LUF)

Ovulation induction using medicine started in 1960's with clomiphene citrate (CC), which is a triarylethylene compound (Fig. (5)). CC is antiestrogenic in human [58, 59]. It increases urinary gonadotropin excretion [60] and plasma levels of LH [61] and FSH

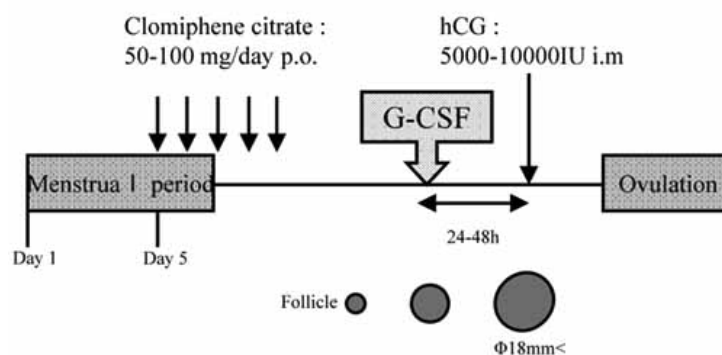
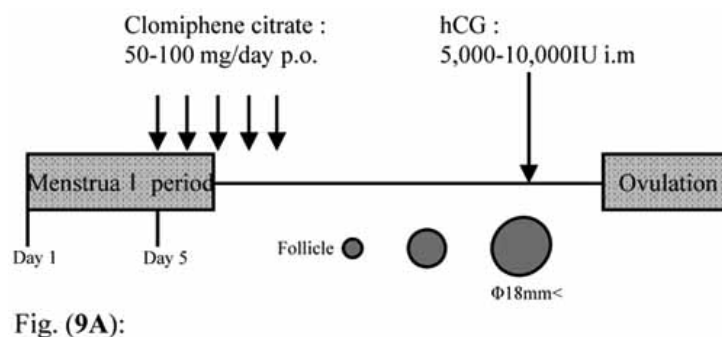


Fig. (9). A) Ovulation induction by CC-hCG. Clomiphene citrate (CC) 50 – 150 mg/day was administered consecutively for five days from Day 5 of menstrual cycle and hCG 5,000 – 10,000 units at the time when the follicular diameter reached 18 mm or more by transvaginal ultrasonography. B) G-CSF (lenograstim) 100 μg was administered subcutaneously at 24 – 48 hours before hCG administration.

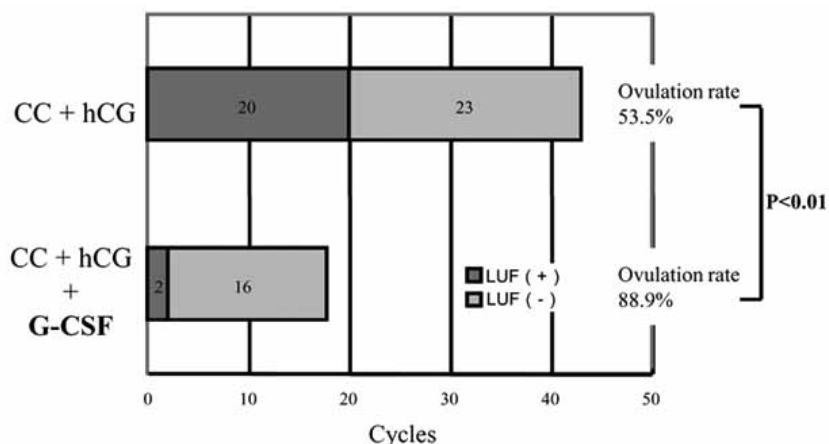


Fig. (10). Comparison of ovulation rates between CC + hCG and CC + hCG + G-CSF.

CC - hCG ovulation induction was performed with follicle monitoring. Lenograstim 100 μg was administered subcutaneously at 24 – 48 hours before hCG administration (Fig. (9B)). Ovulation was confirmed by the disappearance of the leading follicle at 2 – 5 days after hCG administration. Laparoscopic examination was performed before the clinical trial and patients with severe adhesion around the ovaries were excluded from this trial. Ovulation rate by CC - hCG only was 53.5% before the clinical trial. In contrast, the rate increased significantly to 88.9 % by the addition of lenograstim (Fig. (10)). The concentrations of G-CSF one day after the lenograstim administration were 75.9 ± 37.3 and 21.7 ± 25.8 pg/ml in LUF (-) and LUF (+) groups, respectively. Although it was $P=0.07$ and not statistically significant, the LUF group which used lenograstim

showed lower G-CSF concentrations. The changes in the number of WBC were shown in Fig. (11). Before lenograstim administration, it was $7000 \pm 797.5/\mu\text{L}$ and it increased to $19783.3 \pm 5336.8/\mu\text{L}$ one day after administration. It returned to the value before the lenograstim administration on Days 8-9. Fig. (12) shows a typical case of lenograstim induced ovulation. Since the first cycle was a LUF case, lenograstim was administered at the next cycle. In the second cycle, ovulation was successfully induced. At the 3rd cycle, lenograstim was not administered and the cycle showed to LUF again. Therefore, at the following cycles, lenograstim was administered and LUF was not observed. Of course, further trials are necessary. However, the above results indicate the possibility of G-CSF as a supportive drug to ovulation induction.

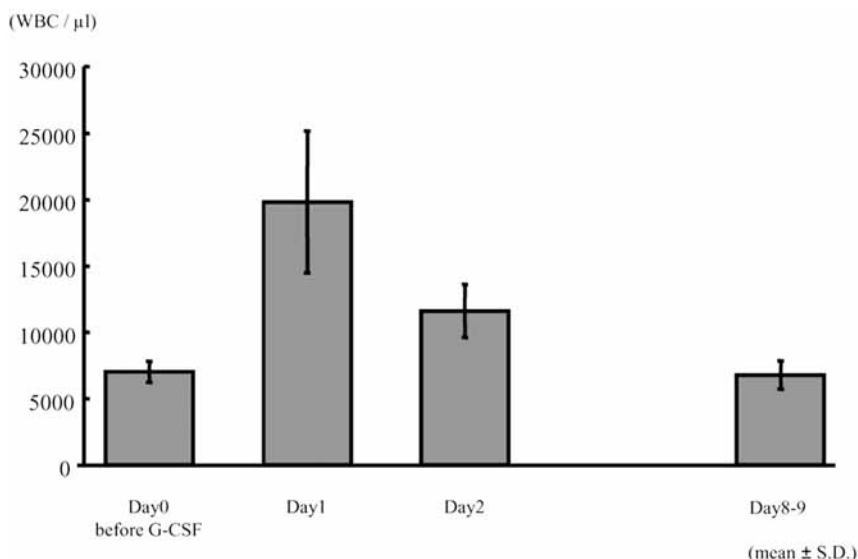


Fig. (11). Changes of numbers of white blood cells after G-CSF administration.

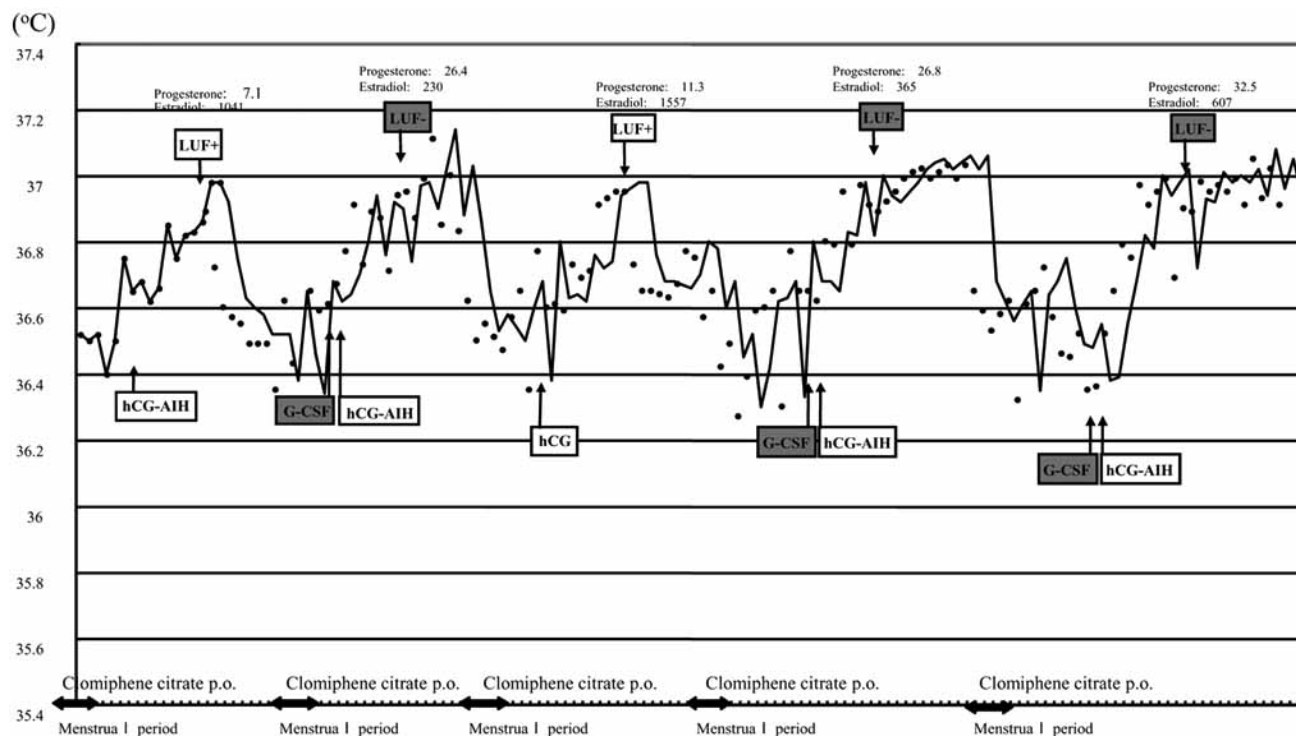


Fig. (12). A typical effective case of G-CSF for LUF

5. CONCLUSIONS

The similarity between ovulation and inflammation has been confirmed during the last 30 years. Nevertheless, little attention was paid to the granulocytes in the ovulation mechanism, which are the most mobilized leukocytes during inflammation. As shown in this review, the participation of granulocytes in the mechanism of ovulation is clear. Fortunately, the cytokine, G-CSF, which mobilizes granulocytes has been used safely for the treatment of cancer for more than 20 years. For these reasons, it is easy to assume the effectiveness of G-CSF in ovulation induction. Considering the role of granulocytes in the mechanism of ovulation, the use of G-CSF for LUF is very reasonable. Although the number of clinical trials presented in this review are few, the effectiveness of G-CSF treat-

ment in addition to the CC - hCG induction has been demonstrated. Since there are no established and definite treatments for LUF [73], the treatment with G-CSF is hopeful for LUF patients. Further clinical trials will prove the usefulness of this treatment and it is our great pleasure that many LUF patients are able to become pregnant.

ABBREVIATIONS

- ART = Assisted reproductive technology
- BBT = Basal body temperature
- CC = Clomiphene citrate
- ET = Embryo transfer

FSH	=	Follicle stimulating hormone
G-CSF	=	Granulocyte colony - stimulating factor
GM-CSF	=	Granulocyte - macrophage colony - stimulating factor
h	=	Hour(s)
hCG	=	Human chorionic gonadotropin
hMG	=	Human menopausal gonadotropin
IL	=	Interleukin
IVF	=	<i>In vitro</i> fertilization
LH	=	Luteinizing hormone
LUF	=	Luteinized unruptured follicle
M-CSF	=	Macrophage colony - stimulating factor
MMP	=	Matrix metalloproteinase
PA	=	Plasminogen activator
PAPP-A	=	Pregnancy associated plasma protein - A
PEG	=	Polyethylene glycol
SD	=	Standard deviation
SEM	=	Standard error of the mean
TNF	=	Tumor necrosis factor
VEGF	=	Vascular endothelial growth factor
WBC	=	White blood cells

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REFERENCES

- Hartman, C.G. In *Sex and Internal Secretions*, Allen E., Ed.; Williams & Wilkins: Baltimore, **1932**; pp. 647-688.
- Asdell, S.A. In *The Ovary*, Zuckerman S., Ed.; Academic Press: London, **1962**; Chap. 8, pp. 435-449.
- Espey, L.L. *Biol. Reprod.*, **1980**, *22*, 73-106.
- Thibault, C.; Levasseur, M.C. *Hum. Reprod.*, **1988**, *3*, 513-523.
- LeMaire, W.J. *Steroids*, **1989**, *54*, 455-469.
- Tsafriiri, A.; Reich, R. *Exp. Clin. Endocrinol. Diabetes*, **1999**, *107*, 1-11.
- Makinoda, S. *Hokkaido Igaku Zasshi*, **1980**, *55*, 521-526.
- Tanaka, N.; Espey, L.L.; Okamura, H. *Biol. Reprod.*, **1989**, *40*, 762-768.
- Waseda, T.; Makinoda, S.; Watanabe, Y.; Sasakura, C.; Imafuku, N.; Hiro-saki, N.; Inoue, H.; Ohshima, K.; Fujii, R.; Iura, T. *Mol. Cell. Endocrinol.*, **2003**, *202*, 71-75.
- Koos, R.D. *Biol. Reprod.*, **1995**, *52*, 1426-1435.
- Asimakopoulou, B.; Köster, F.; Felberbaum, R.; Al-Hasani, S.; Diedrich, K.; Nikolettos, N. *Hum. Reprod.*, **2006**, *21*, 3091-3095.
- Curry, T.H.; Mann, J.S.; Huang, M.H.; Keeble, S.C. *Biol. Reprod.*, **1992**, *46*, 256-264.
- Liu, K.; Wahlberg, P.; Leonardsson, G.; Häggglund, A.-C.; Ny, A.; Bodén, I.; Wibom, C.; Lund, L.R.; Ny, T. *Dev. Biol.*, **2006**, *295*, 615-622.
- Ohnishi, J.; Ohnishi, E.; Shibuya, H.; Takahashi, T. *Biochim. Biophys. Acta*, **2005**, *1751*, 95-109.
- Wang, L.J.; Norman, R.J. *Hum. Reprod.*, **1992**, *7*, 147-150.
- Buyalos, R.P.; Watson, J.M.; Martinez-Maza, O. *Fertil. Steril.*, **1992**, *57*, 1230-1234.
- Wang, L.J.; Brännström, M.; Robertson, S.A.; Norman, R.J. *Fertil. Steril.*, **1992**, *58*, 934-940.
- Jasper, M.J.; Brännström, M.; Olofsson, J.I.; Petrucco, O.M.; Mason, H.; Robertson, S.A.; Norman, R.J. *Mol. Hum. Reprod.*, **1996**, *2*, 555-562.
- Witt, B.R.; Pollard, J.W. *Fertil. Steril.*, **1997**, *68*, 259-264.
- Nishimura, K.; Tanaka, N.; Kawano, T.; Matsuura, K.; Okamura, H. *Fertil. Steril.*, **1998**, *69*, 53-57.
- Mettler, L.; Shirwani, D. *Am. J. Obstet. Gynecol.*, **1974**, *119*, 1038-1043.
- Makinoda, S.; Mikuni, M.; Sogame, M.; Kobamatsu, Y.; Furuta, I.; Yamada, H.; Yamamoto, R.; Fujimoto, S. *Int. J. Gynecol. Obstet.*, **1996**, *55*, 265-271.
- Apseloff, G.; Bao, X.; LaBoy-Goral, L.; Friedman, H.; Shah, A. *Am. J. Ther.*, **2000**, *7*, 297-302.
- Polishuk, W.Z.; Zuckerman, H.; Diamant, Y. *Fertil. Steril.*, **1968**, *19*, 901-909.
- Cochrane, P.; Weatherall, D.J. *J. Obstet. Gynaecol. Br. Commonw.*, **1972**, *79*, 1002-1008.
- Hock, D.L.; Huhn, R.D.; Kemmann, E. *Hum. Reprod.*, **1997**, *12*, 2143-2146.
- Giuliani, A.; Mitterhammer, H.; Burda, A.; Egger, G.; Glasner, A. *Fertil. Steril.*, **2004**, *82*, 1711-1713.
- Shirai, F.; Kawaguchi, M.; Yutsudo, M.; Dohi, Y. *Mol. Cell. Endocrinol.*, **2002**, *196*, 21-28.
- Hellberg, P.; Thomsen, P.; Janson, P.O.; Brännström, M. *Biol. Reprod.*, **1991**, *44*, 791-797.
- Brännström, M.; Bonello, N.; Norman, R.J.; Robertson, S.A. *J. Reprod. Immunol.*, **1995**, *29*, 265-270.
- Espey, L.L. *Biol. Reprod.*, **1994**, *50*, 233-238.
- Chun, S.-Y.; Daphna-Iken, D.; Calman, D.; Tsafriiri, A. *Biol. Reprod.*, **1993**, *48*, 905-909.
- Brännström, M.; Pascoe, V.; Norman, R.J.; McClure, N. *Fertil. Steril.*, **1994**, *61*, 488-495.
- Norman, R.J.; Brännström, M. *Pharmacol. Ther.*, **1996**, *69*, 219-236.
- Bukulmez, O.; Arici, A. *Hum. Reprod. Update*, **2000**, *6*, 1-15.
- Morgenfeld, M.C.; Goldberg, V.; Parisier, H.; Bugnard, S.C.; Bur, G.E. *Surg. Gynecol. Obstet.*, **1972**, *134*, 826-828.
- Chapman, R.M.; Sutcliffe, S.B.; Malpas, J.S. *JAMA*, **1979**, *242*, 1877-1881.
- Schilsky, R.L.; Lewis, B.J.; Sherins, R.J.; Young, R.C. *Ann. Intern. Med.*, **1980**, *93*, 109-114.
- Zachariae, F.; Asboe-Hansen, G.; Boseila, A.-W.A. *Acta Endocrinol.*, **1958**, *28*, 547-552.
- Zackrisson, U.J.; Mikuni, M.; Brännström, M. In *Ovulation, evolving scientific and clinical concepts*, Adashi, E.Y., Ed.; Springer-Verlag New York, Inc.: New York, **2000**, Vol. 16, 187-196.
- Cavender, J.L.; Murdoch, W.J. *Biol. Reprod.*, **1988**, *39*, 989-997.
- Standaert, F.E.; Zamora, C.S.; Chew, B.P. *Am. J. Reprod. Immunol.*, **1991**, *25*, 163-168.
- Brännström, M.; Mayrhofer, G.; Robertson, S.A. *Biol. Reprod.*, **1993**, *48*, 277-286.
- Russell, D.L.; Robker, R.L. *Hum. Reprod. Update*, **2007**, *13*, 289-312.
- Brännström, M.; Enskog, A. *J. Reprod. Immunol.*, **2002**, *57*, 47-60.
- Huysier, C.; Fourie, F.L.R.; Bosmans, E.; Levay, P.F. *J. Assist. Reprod. Gen.*, **1994**, *11*, 193-202.
- Zhao, Y.; Rong, H.; Chegini, N. *Biol. Reprod.*, **1995**, *53*, 923-930.
- Shinetugs, B.; Runesson, E.; Bonello, N.P.; Brännström, M.; Norman, R.J. *Hum. Reprod.*, **1999**, *14*, 1302-1306.
- Salmassi, A.; Zhang, Z.; Schmutzler, A.G.; Koch, K.; Buck, S.; Jonat, W.; Mettler, L. *Fertil. Steril.*, **2005**, *83*, 419-425.
- Fujii, R. *J. Kanazawa Med. Univ.*, **1999**, *24*, 42-49.
- Makinoda, S.; Mikuni, M.; Furuta, I.; Okuyama, K.; Sagawa, T.; Fujimoto, S. *Eur. J. Clin. Invest.*, **1995**, *25*, 877-879.
- Yanagi, K.; Makinoda, S.; Fujii, R.; Miyazaki, S.; Fujita, S.; Tomizawa, H.; Yoshida, K.; Iura, T.; Takegami, T.; Nojima, T. *Hum. Reprod.*, **2002**, *17*, 3046-3052.
- Salmassi, A.; Schmutzler, A.G.; Huang, L.; Hedderich, J.; Jonat, W.; Mettler, L. *Fertil. Steril.*, **2004**, *81* (Suppl. 1), 786-791.
- Ujioka, T.; Matsukawa, A.; Tanaka, N.; Matsuura, K.; Yoshinaga, M.; Okamura, H. *Biol. Reprod.*, **1998**, *58*, 526-530.
- Salmassi, A.; Schmutzler, A.G.; Schaefer, S.; Koch, K.; Hedderich, J.; Jonat, W.; Mettler, L. *Hum. Reprod.*, **2005**, *20*, 2434-2440.
- Morstyn, G.; Burgess, A.W. *Cancer Res.*, **1988**, *48*, 5624-5637.
- Smith, T.J.; Khatchersian, J.; Lyman, G.H.; Ozer, H.; Armitage, J.O.; Balducci, L.; Bennett, C.L.; Cantor, S.B.; Crawford, J.; Cross, S.J.; Demetri, G.; Desch, C.E.; Pizzo, P.A.; Schiffer, C.A.; Schwarzberg, L.; Somerfield, M.R.; Somlo, G.; Wade, J.C.; Wade, J.L.; Winn, R.J.; Wozniak, A.J.; Wolff, A.C. *J. Clin. Oncol.*, **2006**, *24*, 3187-3205.
- Greenblatt, R.B.; Barfield, W.E.; Jungck, E.C.; Ray, A.W. *JAMA*, **1961**, *178*, 101-104.
- Vaitukaitis, J.L.; Bermudez, J.A.; Cargille, C.M.; Lipsett, M.B.; Ross, G.T. *J. Clin. Endocrinol.*, **1971**, *32*, 503-508.
- Mishell, D.R. *Fertil. Steril.*, **1967**, *18*, 102-111.
- Jacobson, A.; Marshall J.R.; Ross, G.T. *Am. J. Obstet. Gynecol.*, **1968**, *101*, 1025-1031.
- Jacobson, A.; Marshall J.R.; Ross, G.T.; Cargille, C.M. *Am. J. Obstet. Gynecol.*, **1968**, *102*, 284-290.

- [63] Kase, N.; Mroueh, A.; Olson, L.E. *Am. J. Obstet. Gynecol.*, **1967**, *98*, 1037-1042.
- [64] Jewelewicz, R. *Am. J. Obstet. Gynecol.*, **1975**, *122*, 909-920.
- [65] Koninckx, P.R.; Heyns, W.J.; Corvelyn, P.A.; Brosens, I.A. *Fertil. Steril.*, **1978**, *29*, 266-269.
- [66] Marik, J.; Hulka, J. *Fertil. Steril.*, **1978**, *29*, 270-274.
- [67] Coulam, C.B.; Hill, L.M.; Breckle, R. *Fertil. Steril.*, **1982**, *37*, 524-529.
- [68] Evers, J.L.H. *Bailliere Clin. Obstet. Gynecol.*, **1993**, *7*, 363-387.
- [69] Hamilton, C.J.C.M.; Wetzels, L.C.G.; Evers, J.L.H.; Hoogland, H.J.; Muijtjens, A.; Haan, J.D. *Fertil. Steril.*, **1985**, *43*, 541-548.
- [70] Qublan, H.; Amarin, Z.; Nawasreh, M.; Diab, F.; Malkawi, S.; Al-Ahmad, N.; Balawneh, M. *Hum. Reprod.*, **2006**, *21*, 2110-2113.
- [71] Devroey, P.; Temmerman, M.; Naaktgeboren, N.; Van Steirteghem, A.C. *Acta Eur. Fertil.*, **1985**, *16*, 183-186.
- [72] Hirosaki, N. *J. Kanazawa Med. Univ.*, **2006**, *31*, 274-280.
- [73] Check, J.H.; Dietterich, C.; Nowroozi, K.; Wu, C.H. *Int. Fertil.*, **1992**, *37*, 33-40.

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