

COMMERCIAL PREPARATION OF GnRH: Are differences of biopotency the causes of variability in patient response?

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=Abstract=

Ovulation induction in hypothalamic amenorrhea using gonadotropin-releasing hormone(GnRH) pulse therapy is complicated by widely variant patient responses ranging from anovulation to multiple pregnancy. Route of administration(intravenous vs subcutaneous), pulse therapy, GnRH dose, infusion interval, or hormone preparation may contribute. We evaluated the bioactivity of 4 GnRH preparations(Relisorm, Serono; Lutrelaf, Ferring; Factrel, Ayerst; GnRH, Sigma) in a rat anterior cell bioassay. Dispersed rat anterior pituitary cells were placed for 48 hrs at 5×10^5 cells/well, washed and incubated with GnRH. The GnRH was diluted according to the manufacturer's culture medium (10^{-12} to 10^{-5} M). GnRH stimulated immunoreactive luteinizing hormone(LH) production was assayed in culture medium after 4 hrs by radioimmunoassay(RIA). A linear dose-response relationship was exhibited by all preparations from 10^{-10} to 10^{-7} M. Maximal LH production was 249 ± 24 ng/ml/4hrs(mean \pm SEM) and was not different among the preparations tested(ANOVA, $p > 0.05$). The minimal effective dose of GnRH was 10^{-10} M for all preparations(basal = 27 ± 4 ng/ml/4hrs: mean \pm SEM). No significant differences were noted for MED, or dose-response slope ($p < 0.05$, ANOVA and slope test for parallelism, respectively). In addition, bioactive LH and immuno and bioactive follicular stimulating hormone(FSH) dose responses were confirmed. We concluded that the principal variability of patient response seen with GnRH pulse therapy cannot be attributed to the bioactivity of these commercial GnRH preparations. But rather, most of the variability is due to the inherent individualism in patient response or other factors of the treatment protocol.

Key Words : GnRH, Biopotency

책임저자 : 문영진

I. Introduction

During recent about half century, the very fast development has been performed on reproductive endocrinology for ovulation induction. After Hohling and Junkmann postulated the existence of a hypothalamic center which controls the anterior pituitary gland in 1932¹, Harris hypothesized the release of a hypothalamic factors into the portal system, which in turn stimulated the release of tropic hormones from the pituitary gland². After 4 decades from postulation of hypothalamic-pituitary relationship,

Matsuo et al purified and identified the structure of luteinizing hormone releasing factor(LHRF, Figure 1) in 1971³. A few months later, LHRF, a decapeptide was synthesized by Geiger et al⁴ and Matsuo et al⁵. After that GnRH has been used in many fields of gynecological endocrinologic abnormalities, including, anovulation⁶⁻¹⁰, delayed puberty¹¹⁻¹³, myotonic dystrophy¹⁴, luteal phase deficiency¹⁵⁻¹⁷ and deficient cervical mucous¹⁸. Among them, hypothalamic

Figure 1. Structural formula of GnRH.

PyroGLU-HIS-TRP-SER-TYR-GLY-LEU-ARG-PRO-GLY-NH₂

amenorrhea using GnRH pulse therapy is complicated by widely variant responses ranging from anovulation¹⁹ to multiple pregnancy²⁰. For induction of ovulation without these complications in a mature follicle by GnRH, several conditions must be considered. We figured out the possible conditions which contribute to make these complications. These are the route of administration, pulse frequency, dosage of GnRH, infusion interval, infusion volume and hormone preparation. Among them, to supply the best GnRH preparation, many pharmacy company invested their unique GnRH preparations. So, every time when we apply GnRH preparation to patients for ovulation induction, we have been into a delimita to select a best one for the patients. Therefore, we evaluated the bioactivity of 4 GnRH preparations(Relisorin, Lutrelif, Factrel, GnRH) with rat anterior pituitary cell bioassay. Our findings confirmed that in the pituitary cells, the synthetic GnRH is a potent stimulator of LH and, to a less extent, of FSH. And every preparation has similar effect on the production of FSH and LH in the pituitary cells.

II. Material and Method

This study was designed to compare the bioactivity for the production of FSH and LH with anterior pituitary cell bioassay according to the concentration of each commercial GnRH preparation. GnRH preparations were obtained from manufacturer or

purchased from hospital pharmacy. They are diluted to the manufacturer's recommendation and then diluted over a wide concentration(10^{-12} to 10^{-5} M) with culture medium M199. The generic names of 4 GnRH preparations are shown in the table 1.

Cell isolation and culture:

Dispersed pituitary cells were prepared according to the method of Vale et al.²¹ with minor modification²². Anterior pituitary glands are removed from adult female Sprague-Dawley rats after rapid decapitation, Quartered and rinsed several times with HEPES (n-2 hydroxyethyl piperazine ethanesulfonic acid, Gibco Laboratories, Grand Island, NY) buffer(NaCl, 137mM; KCl, 5mM; Na_2HPO_4 , 0.7mM; HEPES pH7.2, 25mM; Glucose, 10mM; CaCl_2 , 360 μ M) at room temperature. The pituitary quarters were dispersed for 1hr at 37°C using Earle's Balanced salts solution(pH 7.4) containing 0.4% collagenase(Gibco Laboratories, Grand Island, NY), 0.1% hyaluronidase(Sigma Chemical Co., St. Louis, MO) 0.1% DNAase(Sigma Chemical Co., St. Louis, MO) to prevent the clumping of cells²³, 3.0% bovine serum albumin(BSA: Miles Laboratories, Naperville, IL), 2.5mM HEPES, 10mM dextrose (Abbott Laboratories, North Chicago, IL), and 100 units/ml penicillin/ 100 μ g /ml Streptomycin (Hazleton Laboratories, Denver, PA) in a humidified 95% air-5% CO₂ incubator. After dispersion, cells were washed twice with culture medium to remove the remnants of lysed cells disrupted during mincing of the tissues²⁴, viable cells were counted(using Trypan Blue

Table 1. Generic names of the GnRH preparations.

Commercial name	Company name	Generic name
Relisorin	Serono Laboratories, Inc. Randolph, MA.	Gonadorelin Hydrochloride
Lutrelif	Ferring Laboratories, Inc., Ridgewood, NJ.	Gonadorelin Acetate
Factrel	Ayerst Laboratories. New York, NY.	Gonadorelin Hydrochloride
GnRH	Sigma Chemical Co., St. Louis, MO.	LHRH, Acetate salt

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exclusion) and plated for 48hrs at a concentration of a 5×10^5 cells/well. These were incubated in Medium 199(M.A. Bioproducts, Walkersville,MD) tissue culture medium supplemented with Penicillin/Streptomycin, 10% horse serum, 2.5% fetal bovine serum and each of the GnRH preparations being tested. To compare the activity of residual GnRH in rat cells in culture medium standard samples were made with GnRH dilution reagents instead of GnRH preparations. Every samples were quadruplicated in each concentration with all preparations tested.

Bioassayable FSH:

Bioactive FSH(b-FSH) in the pituitary cell culture medium was determined using an in vitro rat granulosa cell estradiol production assay[granulosa cell aromatase bioassay(GAB)]. GAB was prepared according to the method of Dahl et al²⁵. Intact female Sprague-Dawley rats(21-22 days old) were implanted with subcutaneous silastic capsules(10mm) containing approximately 10mg diethylestilbesterol(DES, Sigma Chemical Co.,St.Louis,MO), to stimulate granulosa cell proliferation. 4 days after incubation, animals were dissected for granulosa cell collection. The ovaries were decapsulated, follicles were punctured with 27-gauge hypodermic needles, and granulosa cells were carefully expressed into MaCoy's 5a medium(modified, without serum, Gibco, Santa Clara,CA). The cells were centrifuged on low speed for 5min tabletop Whiserfuge (Damon/IEC Co., Needham Heights, MA). The supernants were discarded, the cells were washed with fresh medium and centrifuged. An aliquot was diluted with Trypan Blue stain and cultured in 16-mm 24-well culture plates (Corning Glass Works, Corning,NY) for 3 days at 37°C in a humidified, 95% air-5% CO₂ incubator. Each well contained 5×10^4 viable cells in 0.5ml MaCoy's 5a medium supplemented with 2mM L-glutamin (Gibco, Santa Clara,CA), 100units/ml Penicillin/100µg/ml Streptomycin, 10^{-6} M androstenedion (Andronase substrate, Sigma Chemical Co, St.Louis,MO), 10^{-7} M DES, 1 µg/ml insulin(Lilly Research Laboratories, Indianapolis,IN), 30ng/ml hCG

(human chorionic gonadotropin, National Hormonal and Pituitary Distribution Program, NIKKAD), and 0.125mM MIX (1-methyl-3 isobutylxanthine, Sigma Chemical Co., St.Louis.MO). Androstenedion and DES were added to enhance FSH-stimulated aromatase activity. MIX (phosphodiesterase inhibitor) were added to minimize cAMP breakdown. Insulin and hCG were added to increase the granulosa cell response to FSH. For the measurement of FSH bioactivity in serum samples, 20 µl of each serum sample was added to the each well. All assay samples were performed in triplicated. The tubes were placed in ice and 3.48ml 0.01M phosphate buffered saline (pH 7.0) were added. The tubes were centrifuged (1500xg for 15 min) at 8°C; the supernants were collected and stored at -20°C until assayed for estrogen. Estrogen content was measured by radioimmunoassay. These results were converted to ng/ml b-FSH using a cynomolgus FSH reference preparation (NICHD cyn-FSH-RP1).

Bioassayable LH:

Bioactive LH(b-LH) in the culture medium was determined using a mouse interstitial Leydig cell testosterone production assay. The assay was based on testosterone by mouse Leydig cell preparation. This assay was a modification of the procedures described by Van Damme et al²⁶ and Dafin et al²⁷. Two 10 weeks old Swiss-Webster mice were sacrificed by cervical dislocation. The testes were removed and placed in a petri dish containing 0.3ml aerated preincubation medium[medium 199 with Hanks' salts, L-glutamine, and 25mM HEPES buffer: penicillin; streptomycin; 0.1% BSA, and 10% NaHCO₃ (0.1ml/100ml); pH adjusted to 7.4 with NaOH]. The testes were cut with scissors into small pieces and 20ml(5ml/1 testis) of the same medium was added. The cells were dispersed for 10-15min with magnetic stirrer surrounded by an ice bath; then, the medium was repeatedly drawn into and gently squeezed from a Pasteur pipette over several minutes until a homogeneous was obtained. The medium was filtered through a fine nylon and then preincubated for 1hr at

34°C. The cell suspension was cooled in ice water and centrifuged at 250xg for 15min at 8°C. Sedimented cells were resuspended in 10ml incubation medium (preincubation medium plus 0.125 mM MIX plus 100IU/ml sodium heparin). Addition of sodium heparin during mouse interstitial cell testosterone assays performed in the presence of serum is important to reduce electrostatic interactions and cell clumping, and to prevent secondary clotting by tissue thromboplastins during cell incubations²⁸. The interstitial cells numbered approximately 3.0×10^5 /0.1ml medium. Incubations were performed in polyethylene tubes (13x100mm) at 34°C for 3hrs; the tubes were shaken at 90 cycles/min in a Dubnoff metabolic incubator under continuous aeration of 95% O₂ and 5% CO₂. The Tubes ran for the standard curve each contained 0.1ml dispersed cell medium, 0.02ml 5% BSA in preincubation medium and 0.1ml incubation medium. The tubes for the assay of serum samples each contained 0.1ml cell medium, 0.1ml incubation medium and constant 20 µl of serum sample. At the end of incubation, the tubes were placed in ice and 3.78ml 0.01M phosphate-buffered saline(pH 7.0) were added. The tubes were centrifuged(1500xg for 15min) at 8°C; the supernants were collected and stored at -20°C until assayed for testosterone. Testosterone production was assested using an established radioimmunoassay and the results were converted to ng/ml b-LH using pituitary LH reference preparation (NICHD rh-LH-RP1).

Radioimmunoassayable FSH and LH:

GnRH stimulated immunoassayable FSH(ir-FSH) and immunoassayable LH(ir-LH) production were assested in culture medium after 4 hrs with each concentration of GnRH preparations. Additionally, GnRH free culture mediums were also assested for basal condition. Immunoreactive FSH and LH were measured by a double antibody method²⁹ according to the procedure recommended by the National Pituitary and Hormone Distribution program, NIDDK, employing I¹²⁵ iodo-LH-I-6 as tracer, NIADDK

anti-rFSH-S-11 and NIADDK anti-rLH-S-9 as the antisera (the first antibody) and NIADDK rFSH-RP-2 and NIADDK rLH-RP-2 as the reference preparation (standard).

III. Result

We evaluated the bioactivity of 4 GnRH preparations(Relisorin, Serono; Lutrelf, Ferring; Factrel, Ayerst; GnRH, Sigma) in a rat anterior pituitary cell bioassay. In ir-LH assay, a linear dose-response relationship was exhibited by all preparations from 10^{-6} to 10^{-13} M of GnRH(Table 2). Maximal LH production was 249 ± 24 ng/ml/4hrs in 10^{-6} M. The minimal effective dose(MED) of GnRH for LH was 10^{-10} M. No significant differences were noted for MED, or dose-response slope between all preparations($p < 0.05$, ANOVA and slope test for parallelism).

In bio-LH assay, a linear dose-response relationship was exhibited from 10^{-6} to 10^{-10} M by all preparations(Table 3). Minimal LH preparation was 71 ± 4.5 ng/ml/4hrs in 10^{-7} M, and was not different from the other tested preparations. The MED of GnRH for LH in bio LH assay was 10^{-9} M. No significant differences were noted for MED or dose-response slope between all preparations($p < 0.05$, ANOVA and slope test for parallelism).

In ir-FSH assay, a linear dose-response relationship was exhibited by all preparations from 10^{-5} to 10^{-12} M(Table 4). The maximal FSH production was 87.2 ± 1.7 ng/ml/4hrs in 10^{-7} M, and was not different among the preparations tested($p < 0.05$, ANOVA and slope test for parallelism).

In bio-FSH assay, a linear dose-response relationship was exhibited by all preparations from 10^{-5} to 10^{-12} M(Table 5). The maximal FSH production was 150 ± 12 ng/ml/4hrs in 10^{-7} M, and was not different among the preparations tested($p < 0.05$, ANOVA and slope test for parallelism). The down-regulation effect on pituitary cells was exhibited in 10^{-5} and 10^{-6} M more definitely than in ir-FSH assay. The MED of GnRH

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Table 2. The results of the LH release in each GnRH preparation by ir-LH assay.

Dose(M)	LH release(ng/ml/4hrs)				
	Rel. I	Rel. II	Lutre.	Factr.	GnRH
10 ⁻⁶	194±11	249±24	224±7	134±14	245±9
10 ⁻⁷	153±12	202±12	209±7	128±20	243±17
10 ⁻⁸	149±12	188±12	199±11	140±24	176±2
10 ⁻⁹	91±7	155±5	109±4	121±9	107±3
10 ⁻¹⁰	55±2	81±2	117±12	76±4	68±4
10 ⁻¹¹	62±2	76±4	57±4	64±3	60±1
10 ⁻¹²	60±3	67±2	63±4	62±2	63±6
10 ⁻¹³	58±3	60±2	63±2	62±2	59±2
Basal	27.0±4				
Rel.I.:Relisorm I, Lutre.:Lutrefef,	Rel.II.:Relisorm II, Factr.:Factrel.				

Table 3. The results of the LH release in each GnRH preparations by bio-LH assay .

Dose(M)	LH release(ng/ml/4hrs)		
	Relisorm	Luterlef	Factrel
10 ⁻⁷	52±4	71±4.5	53±2.5
10 ⁻⁸	36±4	52±4	50±2.5
10 ⁻⁹	32±4	19±3	36±4
10 ⁻¹⁰	2.3±4	1.8±2	2.9±2
Basal	16±1.5		

Table 4. The results of FSH release in each GnRH preparations by ir-FSH assay.

Dose(M)	FSH release(ng/ml/4hrs)			
	Relisorm	Lutrefef	Factrel	GnRH
10 ⁻⁵	78.9±1.7	82.3±1.7	79.6±3.4	84.4±2.4
10 ⁻⁶	84.4±3.9	84.4±4.5	85.8±3.9	69.9±4.5
10 ⁻⁷	87.2±1.7	84.6±4.1	86.2±3.9	71.7±3.9
10 ⁻⁸	79.3±2.9	69.2±3.9	74.2±1.2	73.9±3.4
10 ⁻⁹	58.9±2.6	54.4±1.7	55.0±2.1	50.7±2.1
10 ⁻¹⁰	27.3±0.69	27.9±1.1	28.5±1.2	
10 ⁻¹²	20.2±0.26	19.7±1.0	20.2±1.3	19.8±0.43
Basal	21.5±2.0			

preparations for FSH was 10⁻¹⁰M.

IV. Discussion

No significant differences were noted for MED or dose-response slope (P<0.05, ANOVA and slope test for parallelism, respectively). All the GnRH

preparations tested produced similar log linear dose-responses, MED, and maximal secretion of both immuno and bioactive FSH and LH. The principal variability of patient response seen with GnRH pulse therapy cannot, therefore, be attributed to differences in bioactivity of these commercial GnRH preparations. On the contrary, most of the variability must be due to inter-individual differences or other factors of the

Table 5. The results of FSH release in each GnRH preparation by bio-FSH assay.

Dose(M)	FSH release(ng/ml/4hrs)		
	Relisorm	Lutrelaf	Factrel
10 ⁻⁵	27.5±3	15.0±2	10.0±2
10 ⁻⁶	57.5±6	45.0±4.5	35.0±4.3
10 ⁻⁷	150.0±12	57.5±8	62.5±7.4
10 ⁻⁸	137.5±15	95.0±8.4	67.5±7
10 ⁻⁹	52.5±6.5	80.0±6	35.0±5.5
10 ⁻¹⁰	47.5±6.8	20.0±1.8	50.0±10
10 ⁻¹²	12.5±5.5	20.0±1.6	
Basal		14.0±2	

treatment protocol. In spite of many failures including anovulation¹⁹ and multiple pregnancy²⁰ with GnRH treatment, a lot of studies have been tried to develop the clinical application for ovulation induction. After Kastin et al's report in 1971⁶ about induction of ovulation by an infusion of porcine LRH in an amenorrheic woman, many factors; patient selection, route of administration, pulse frequency, GnRH dose per pulse, infusion interval, infusion volume, binding to and metabolism at the GnRH receptor, has been proved to increase the success rate for ovulation induction.

GnRH actions on gonadotropin and synthesis are mediated by two parallel pathways; extracellular calcium-dependent mechanism and independent mechanism. In one, the processing of GnRH signals involves the sequential interaction of three membrane-bound proteins; receptors for extracellular binding and interaction with G-protein; that permits exchange of a tightly bound guanosine diphosphate (GDP, an inactive ligand) for guanosin triphosphate (GTP, an active ligand). The GTP-bound G protein, in turn, activates the enzyme phospholipase-C (PLC), thereby initiating hydrolysis of the precursor phosphoinositol(4,5)-biphosphate(PIP₂)³¹ to generate two active second messengers: IP₃(inositol triphosphate) and DAG(diacyl glycerol). These two messengers then increase a bifurcating signaling system responsible for regulating cellular processes. IP₃ functions to induce an increase in the cellular Ca⁺² pool from the

endoplasmic reticulum as well as opening the gated Ca⁺² channels with influx of extracellular Ca⁺²^{32,33}; the rise in intracellular Ca⁺² triggers the release of preformed gonadotropin by exocytosis. Like the rise in the intracellular Ca⁺² gonadotropin secretion is associated with redistribution of calmodulin(the cellular calcium cation receptor). Calmodulin has been shown to move from the cytosol to the plasma membranes of pituitary cells after GnRH administration with an associated rise in LH^{34,35}. On the other hand, DAG activates protein kinase-C(PK-C) and transports from membrane bound to intracellular targets, thereby activating gene transcription for gonadotropin synthesis. Repeated pulses of GnRH probably either stimulate an increase in the number of receptors or modulate postreceptor calcium dependent mechanism on the pituitary gonadotrope. This thought to account for the mechanism of GnRH self-priming, or so called up-regulation. The constant infusions of GnRH down-regulate by depleting receptors(or desensitize postreceptor response). With the concept on the relationship between transmembrane Ca⁺² flux and GnRH activity, Ca⁺² channel blocking agents(verapamil) inhibit the stimulated LH release^{36,37}, and LH release is activated by Ca⁺² channel-activating agents(Maitotoxin)³⁸.

In the choice of administration route, with the review of other reports, Taymor³⁹ summated the success rates by the pulsatile GnRH administration

within the physiological frequencies between 60 and 120 min are 90%^{40,41} by subcutaneous route and 78%⁴² by intravenous route, respectively. But, some authors claim that the intravenous route appears to be more predictable to ovulate. In our review with others' data using GnRH with subcutaneous or intravenous administration in hypothalamic insufficiency^{15,40,43-48,50,52-58} and polycystic ovarian disease(PCOD)^{40,51,56,59-62} regardless to interval or dosage of GnRH administration, the intravenous administration(89.4% and 69.4%, respectively) is superior than the subcutaneous route(69.0% and 45.4%, respectively) in ovulation induction.

Among these findings, the ovulation rate(80.4%) in hypothalamic amenorrhea by intravenous administration is very similar with the recently published result by Dessole et al(83%)⁶⁴. In spite of these results, the intravenous route treatment is less tolerated and can make uncommon complications^{45,47,51,63}, such as chemical phlebitis, back bleeding, leakage of GnRH solution and infection, like bacteremia. In the other hand, the intravenous administration gives the economical benefits of a lower daily dose of GnRH. Therefore, with these different advantage between intravenous and subcutaneous administration, we made the conclusion that the subcutaneous infusion can be used initially in almost patients to anovulatory women who failed to ovulation induction with clomiphene citrate and who are adjustable to GnRH treatment after pretreatment intravenous-GnRH test⁶⁵. Because of anticipated lesser success rate, in some cases with severe obesity, they are not recommended to use the subcutaneous administration⁴⁵. These findings that peak levels in obese subjects are significantly more attenuated and that the time to peak is extended 15-20 min in comparison with intravenous and subcutaneous route. Subcutaneous administration in obese subjects consistently produced peak levels two to three times lower than those in the subcutaneous route in the subjects, and the sharp pulse pattern was lost⁶⁶. These findings are helpful to explain the suggestion of intravenous route in the obese patients

Within the well established monthly pattern, gonadotropins are secreted in a pulsatile fashion with a frequency and magnitude that varies with phase of cycles. Pulsatile increments in gonadotropin release occur every 60 to 90 minutes throughout most of cycles, but decrease in frequency to 3 to 4 hrs during the mid and late luteal phase⁶⁷. In details, mean LH puls frequency is declining from 15.2 pulses/24hrs in the early to 8.4 pulses/24hrs in the late luteal phase⁶⁸. These findings can be applied to treatment of anovulation in hypothalamic amenorrhea and PCOD. In addition, the invention of automatic portable pulsatile infusion pump has been helpful to treatment of anovulation⁶⁹.

After detection of pulsatile rhythmic pattern of gonadotropin secretion on monkey⁷⁰ and human⁷¹, several investigations were tried to determine to adjustable frequencies of GnRH administration. For the proper secretion of FSH and LH, Hauser et al compare the effect of 4 different frequencies(1 pulse/hr, 1 pulse/3 hrs, 5 pulse/hr, 2 pulse/hr) in GnRH administration. Every frequency except 1 pulse/hr was the sequency of reduced pituitary response to each GnRH stimulus⁷². And then, by means of automatic portable pump designed to deliver a pulse of GnRH every 60 to 120 minutes, a large body of data has been collected that has shown this mode of ovulation induction to be effective and safe, particularly in patients with endogenous GnRH deficiency^{53,67,71,72}, such as Kallmann's syndrome and functional hypothalamic amenorrhea. In the pulsatile pattern, subcutaneous dose of 10-20 µg/pulse seems to be adequate and in intravenous administration, smaller dose (1-20µg/pulse, usually lesser than 5 µg/pulse) are used^{69,74-76}.

Within these data, the best way with intravenous administration may be to start on a dosage of 3 µg every 90 minutes and increase it as needed up to a maximum of 15 µg per dose. And we must consider the reports about multiple pregnancy with dose of 5 µg and greater^{41,43,77,78}.

V. Conclusion

We reviewed many possible conditions which can improve the ovulation success rates in the clomiphene-resistant anovulatory women with hypothalamic insufficiency and PCOD. We can get the higher success rate in ovulation induction by choosing the proper conditions; pulse interval, route of administration, selection of patients, the using of portable automatic pump machine, etc. Although, within our data about differences in bioactivity to produce FSH and LH in pituitary cells, there is no definite

Differences between 4 GnRH preparations. Finally, we get the conclusion that we don't need any more to be into a delimma to choose the best GnRH preparation to improve the ovulation rate in these anovulation patients. The best way is to choose the any kinds of synthetic GnRH which you can buy in lowest price.

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