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(54) **Title**: A COMPOSITION COMPRISING HIGHLY PURIFIED CHORIONIC GONADOTROPIN, IT'S FORMULATION AND USES OF THE SAME

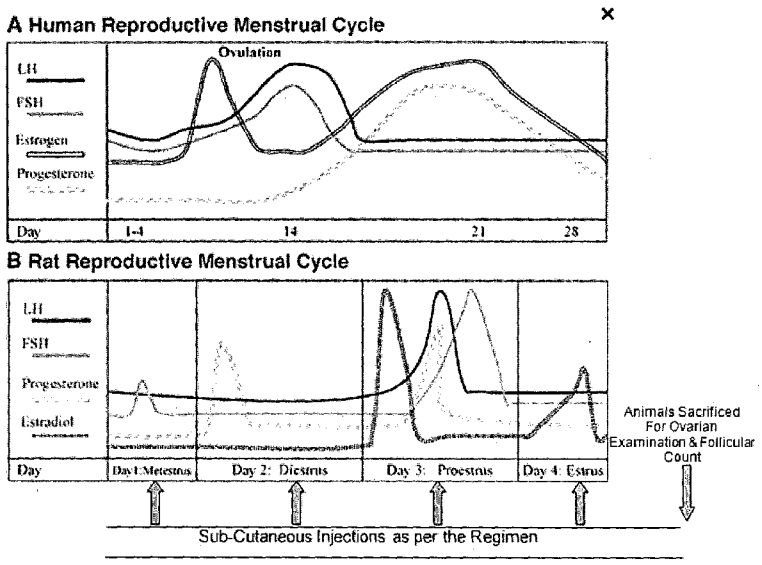


FIG 1

(57) **Abstract**: The present invention is directed to a composition comprising a low dose hCG in highly purified form intended for a novel application of promoting the estrogenic environment to support endometrial growth and receptivity and also the follicular growth with or without concomitant FSH preparation. The composition comprises highly purified hCG in the dose of 100 IU - 200 IU.

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**Title: A composition comprising highly purified chorionic gonadotropin, it's formulation and uses of the same**

**The Field of Invention**

The present invention relates to a composition comprising highly purified human chorionic gonadotropin (hCG), formulation comprising the same and uses of the composition. More particularly the present invention relates to the composition comprising highly purified hCG, pharmaceutical formulation comprising the same and uses of the same for Folliculogenesis and Endometrial Development.

**Background of the Invention**

Chorionic gonadotropin is a hormone produced by the placenta and traditionally obtained from the urine of pregnant women. The hormone is a heterodime, consisting of non-covalently bound α and β subunits and its effects are predominantly those of gonadotropin luteinizing hormone. Chorionic gonadotropin is given to women, to induce ovulation and also recently it has been included as a therapeutic for maintenance of pregnancy. The great majority of patients who are candidates for assisted reproduction technology (ART) procedures undergo controlled ovarian stimulation (COS). Pharmacological amounts of exogenous gonadotropins are used to override the process of physiological control mechanisms. The numerous ovarian follicles stimulated by COS yield the multiple oocytes that are needed to maximize the success of ART. However during the course

of folliculogenesis, the endometrial thickness is a critical factor that determines the process of nidation and continuation of the pregnancy.

Since exogenous gonadotrophins were introduced in the international pharmacopoeia almost four decades ago, physicians have tried to achieve or attempted step-down FSH regimens to recreate the follicular stimulating hormone (FSH) concentration pattern of the normal follicular phase by progressively decreasing exogenous FSH administration. Recent scientific evidence suggests that in COS it may also be possible to more closely mimic the endocrine events of the spontaneous cycle and thus limit the occurrence of unwanted stimulatory effects of exogenous gonadotrophins (Filicori and Cognigni, The Journal of Clinical Endocrinology & Metabolism Vol. 86, No. 4 1437-1441) .The key of these regimens appears to be a greater understanding of the actions of luteinizing hormone (LH) in the course of folliculogenesis, and a better and more rational use of LH activity in COS.

Ovarian response to gonadotrophins varies considerably among women undergoing COS[ Controlled Ovarian Stimualtion}. The importance of this differential response in women with previous ART (IVF/ICSI) failures has prompted researchers to investigate and determine the factors that are implicated. Though, follicular development up to the pre-antral stage is feasible in the absence of LH, an essential role for this gonadotrophin for antral formation as well as further growth and differentiation has been uniformly recognized. LH plays a key role in both oocyte and follicular cells development through modification of the steroid and protein micro- and

macroenvironment. The modification of the steroid and protein physiologic changes have a prominent role in oocyte, maturation, the process of ovulation, and subsequent fertilization and implantation.

The design of effective ovarian stimulation protocols [COS] therapy requires knowledge of basic concepts of follicular dynamics and of the respective roles of FSH and LH in regulating the development of a quality ovulatory follicle in the spontaneous ovarian cycle. Although FSH and LH are of paramount importance to the regulation of follicular development and ovulation, gonadotrophin action depends on the locally produced steroidal and non-steroidal factors that mediate and modify the actions of FSH and LH within the ovaries.

In recent years, understanding of the molecular and cellular mechanisms and gonadotrophin action on the ovaries has grown, aided by experimental work in rodent and domestic animal species. Armed with this knowledge, and the availability of genetically engineered, pharmaceutical grade, pure FSH and LH , the way is open systematically to manipulate the ovarian paracrine system to achieve single or multiple ovulation according to clinical requirements.

About 400 follicles mature sequentially and ovulate during the reproductive lifetime of an average woman. From birth to the menopause, the other approximately 99.98% of her follicles initiate development but never complete it. Instead, these follicles default to atresia due to inadequate or inappropriate stimulation by gonadotrophins.

The follicle life-cycle is a continuum, with four phases of different requirements for stimulation by FSH and LH:

1. Initiation, which occurs from birth to senescence independent of Gonadotrophic Control
2. Progression, which requires tonic stimulation by FSH [Pre-Antral, Antral & mature Follicle]
3. Preovulatory maturation, which occurs during menstrual cycles and requires appropriate stimulation by adequate amounts of both FSH and LH and
4. Ovulation, which is induced by the mid-cycle gonadotrophin surge

Inadequate FSH and lower levels of LH have been associated with the improper folliculogenesis and poor endometrial development. Unfortunately for the infertile women,not only folliculogenesis but poor endometrial receptivity contributes to pregnancy failures.

The medicaments have been used with some success in infertile women, with poor folliculogenesis and endometrial thickness [<7mm] contributing to poor success rates. Filicori et al, in their paper have "Selective Use of LH Activity in the Late Ovulation Induction Stages" have explained the rationale for use of hCG scientifically validated based on the following facts:

1. hCG has a different β-subunit amino-acid composition and greater sialic acid content compared to hLH
2. A single receptor exists for both LH and hCG

3. hCG binds to the LH/hCG receptor with higher affinity than LH, while exerting biological actions that are comparable to LH
4. hCG:hLH plasma half-life and potency 1:6 to 1:8

In addition, various studies have demonstrated, that FSH like activity is exhibited by low doses of HCG

- Stimulation of granulosa cell proliferation and growth
- Induction of granulosa cell aromatase to catalyze estrogen formation [This estrogen is thought to contribute to improved endometrial receptivity]
- Due to mid-late follicular phase interaction with granulosa cells, LH activity can:
- Increase E2 [estrogen synthesis]
- Enhanced availability of theca-derived androgen substrate
- Stimulation of aromatase
- Modulate folliculogenesis & Demonstrate synergistic activity with FSH

Studies in both human and animal experiments have shown LH acts synergistically with FSH in the process of follicular growth: FSH plays a crucial role in recruitment, selection and dominance, while LH contributes to dominance maturation and ovulation. Studies in non-humans have shown that LH may act by increasing intra-ovarian androgens, which in turn promote FSH responsive granulosa cell function.

Recently, it has been demonstrated that, in patients down-regulated by GnRH analogues (GnRHa), a short-term pretreatment with recombinant LH (rLH), prior to

recombinant FSH (rFSH) administration, increases the number of small antral follicles prior to FSH stimulation and the yield of normally fertilized (2PN) embryos. In addition,rLH pre-treatment may have a modest impact on subsequent ovarian responsiveness to FSH. LH activity,administered as a single dose of HP-hCG in combination with aromatase inhibitor in early-follicular-phase GnRH antagonist protocol has been shown to result in androgen priming and subsequent increase in the number of good quality embryos.

The ideal LH activity, administered as in hMG, rLH or hCG in ART procedures, has not been determined yet. Serum LH levels of less than 1.5 IU/L have been proven insufficient to maintain aromatase activity and E2 production. Low peri-ovulatory levels (<3 IU/L) in patients undergoing IVF are associated with impaired fertilization and increased early pregnancy loss.

Minimal or small doses of LH administered in early follicular phase during ovarian stimulation in IVF-ET cycles have a beneficial effect in the quality of oocytes, a fact of utmost importance,especially in cases where few embryos are available for transfer. Actually, in poor responders, early LH administration during COH may have a beneficial effect on the maturity and fertilizability of oocytes, as well as the number of transferable embryos.

In IUI, IVF and other ART practices, human chorionic gonadotrophin (hCG) is used as a substitute for the mid-cycle LH surge, due to the degree of homology between the two hormones. hCG has long been associated with the initiation and maintenance of

pregnancy. HCG demonstrates a bi-phasic pharmacokinetic pattern and has a slower plasma metabolic clearance, which consists of a rapid phase in the first 5-9 h following IM administration and a slower phase in the first 1-1.3 days after administration. Both LH and hCG are complex heterodimeric glycoproteins with different molecular weights (30 KD and 40 KD respectively). Difference in their carbohydrate moiety possibly explains different affinity to the LH/hCG receptor and therefore differentiated function.

In the absence or relatively low levels of endogenous FSH, low-dose hCG can support development and maturation of larger ovarian follicles (≥15 mm in diameter) that have acquired granulosa cell LH/hCG receptors and hasten the demise of smaller follicles lacking these receptors thus being dependent on FSH stimulation.

For the commercial preparations of rLH studies have not evaluated the role of combining rLH and FSH on folliculogenesis. The disadvantage with rLH, is that following a 75 IU dose, the concentration range is too small to allow proper quantification of the pharmacokinetic parameters.

In view of the above background there is a need for a cost-effective and safer ovulation induction regimens essentially to promote endometrial and follicular growth either alone or as a concomitant intervention.

**Summary of the Invention:**

Therefore, in one aspect the present invention is directed to providing a composition comprising hCG in highly purified form in low dose.

In one aspect the highly purified hCG has reduced endotoxin level of less than 0.03 EU/IU, preferably less than 0.01EU/IU.

In one more aspect the present invention is directed to providing formulations comprising the composition of hCG in highly purified form in low dose.

In another aspect the present invention is directed to providing a composition comprising a hCG in highly purified form in low dose for promoting the estrogenic environment to support endometrial growth and receptivity and also the follicular growth.

In another aspect the present invention is directed to providing a composition comprising a hCG in highly purified form in low dose with or without concomitant FSH preparation.

In another aspect the present invention is directed to providing a composition comprising a hCG in highly purified form in low dose for the treatment of infertility in patients receiving high doses of Follicle Stimulating Hormone (FSH).

**Brief Description of the Drawing:**

FIG 1. Is a graph showing the Reproductive Cycles in Humans & Female Rats. The graph typically demonstrates the differences in the cycle length. However similarities are shown in terms of the hormones present and the hormonal surge during the reproductive cycle. The studies using regimens will contribute to the ART Cycles currently being employed and bring in improved outcomes.

**Detailed Description of the Invention:**

Therefore, the present invention is directed to an hCG unconventionally in highly purified form in low dose intended for a novel application of promoting the estrogenic environment to support endometrial growth and receptivity and also the follicular growth with or without concomitant FSH preparation.

The hCG employed in the present invention is of human origin. Preferably the hCG is partially purified or purified hCG obtained from the urine of pregnant women. The process of the present invention comprises subjecting hCG to steps of purification by chromatographic technique and lyophilizing the purified hCG to obtain highly purified hCG. The entire process of obtaining the highly purified hCG has been described in the co-pending Indian Patent application No. 2067/MUM/2010 by the Applicant, the contents of said application has been incorporated herein by reference.

The highly purified hCG as obtained by said process has reduced endotoxin level of less than 0.03 EU/IU, preferably less than 0.01EU/IU. Such highly purified hCG is

contemplated to be without structural damage or loss of potency.

In one embodiment, the present invention provides a composition comprising highly purified hCG and a pharmaceutically acceptable diluent, carrier or excipient. The highly purified hCG has reduced endotoxin level of less than 0.03 EU/IU, preferably less than 0.01EU/IU.

The pharmaceutically acceptable diluents, carriers or excipients suitable to formulate a pharmaceutical composition are those which can be included without affecting the activity and stability of the highly purified hCG. Such diluents, carriers or excipients will be known to a person skilled in the art. Preferably the compositions comprise a daily dose of hCG of 100-200 IU.

In one embodiment, the present invention provides use of the composition comprising highly purified hCG at the dose of 100-200 IU for preparing the medicament for promoting the estrogenic environment to support endometrial growth and receptivity and also the follicular growth.

In another embodiment, the present invention provides pharmaceutical formulation comprising the composition of highly purified hCG, formulated as a unit dosage in the form of a solid ready for dissolution to form a sterile injectable solution for intramuscular or for subcutaneous use. The solid usually results from lyophilisation.

Typical excipients and carriers include sucrose, lactose, sodium chloride, buffering agents like sodium phosphate monobasic and sodium phosphate dibasic. The solution may be prepared by diluting with water for injection immediately prior to use.

In another embodiment the comprising the composition of highly purified hCG, may also be formulated as a solution for injection, comprising any of the excipients and buffers listed above, and others known to one skilled in the art.

In one of the embodiment, the present invention provides a pharmaceutical formulation comprising composition of high purified low dose hCG 100-200 IU, and pharmaceutically acceptable suitable excipients such as Lactose, Sucrose etc. which may aid in the stabilization of the lyophilized product. Such formulation is filled into glass ampoules.

The pharmaceutical formulation of the highly purified hCG is suitable for subcutaneous administration in IVF /ICSCI procedures.

The daily dose of the composition comprising the highly purified hCG or formulation comprising the same may be administered once a day from day 1 to about day 4 stimulatory cycle.

The composition of the present invention optionally may comprise of other active drugs. Examples of other drugs that can be included in the composition may be selected from but not limiting to gonadotropin releasing hormone, gonadotropin releasing hormone agonists, gonadotropin releasing hormone antagonists, preparations with luetinizing hormone activity, progesterone preparations, or aromatase inhibitors. Dose ranges for these drugs are at the dose range that provides bioactivity desired for the composition of the present invention.The composition comprising the highly purified low dose hCG ranging from 100 IU to 200 IU may be used for various applications including but not limited to those including for promoting endometrial and follicular growth either as a sole agent or as a concomitant intervention as a part of protocol.

In one embodiment, the composition comprising a hCG in highly purified form in 100 IU to 200 IU dose or formulation comprising the same is used for promoting the estrogenic environment to support endometrial growth and receptivity and also the follicular growth.

In one embodiment, the composition comprising a hCG in highly purified form in 100 IU to 200 IU dose or formulation comprising the same is used with or without concomitant FSH preparation.

In another embodiment, the composition comprising a hCG in highly purified form in 100 IU to 200 IU dose or formulation comprising the same is used for the treatment of infertility in patients receiving high doses of Follicle Stimulating

Hormone (FSH).

In another embodiment, the composition comprising a hCG in highly purified form in 100 IU to 200 IU dose or formulation comprising the same may optionally be used in women undergoing pituitary suppression, or in patients of hypogonadotrophic hypogonadism or in patients presented with any other hypoestrogenic states (typically presented with low basal LH & estradiol levels ) at base line, not being limited to any of these categories of patients only; to promote the estradiol levels during the early to mid follicular phase for an appropriate endometrial pattern and thickness; use concomitantly with FSH or as a sole agent for its ability to enhance the follicular growth, in mid to late follicular phase or throughout the follicular phase of the cycle; for enhancing the FSH efficacy to improve the ovulation induction outcome such as hastening the development of larger antral follicle, to reduce the chances of ovarian hyperstimulation by reducing the number of small preovulatory follicles (<10mm); for use as cited in the published research and owing to its additional applications such as shortening COH duration, lowering HP FSH requirements for stimulation, reduced COH cost for providing the concomitant activity of LH along with HP-FSH to optimise COH for an improved fertilization rates and embryo quality; as a sole intervention of the treatment protocol in the mid to late follicular phase to support the stimulation of larger follicle ( > 14 mm ) even in the absence of FSH; for augmenting uterine receptivity by enhancing endometrial quality with respect to pattern and thickness, not limited to any one of these parameters or both these parameters; as a part of stimulation protocols employing aromatase inhibitor for a more favourable estradiol / androgen ratios so as to

ensure oocytes with appropriate competence with respect to fertilizability and eventual development of quality embryos.

The low dose highly purified hCG may confer the following advantages:

Low-dose hCG spiking in late COS:

1. Increases estrogens and estrogen/ androgen ratios in serum and follicular fluid

2. Does not augment preovulatory and follicular fluid progesterone levels when up to hCG 200 IU/day are used

3. Modulates folliculogenesis: large follicles are stimulated while small follicles are restrained

4. Results in oocyte quality, embryo yield, and pregnancy rates at least comparable to traditional FSH regimens [as demonstrated in human subjects}

While the present invention is described by various embodiments, it is apparent that various modifications may be made or embodiments can be altered to provide other embodiments which utilize the processes of this invention. Such embodiments are construed to be within the scope of the present invention.

The present invention is illustrated by the following non-limiting example.

Example 1:

The Study - COH Using 100 IU hCG:

Inbred female 3-4 month old Wistar rats, weighing 130 – 150 gms grams were chosen for the study. COS [Controlled Ovulation Stimulation] protocols as described in literature were used for the study using either a n agonist or antagonist were carried to evaluate the efficacy of the HP-HCG formulation.

Female rats, with regular estrus patterns were chosen after microscopic examination of vaginal smears. The phases studied were Diestrus, Proestrus and Estrus.The animals were allowed to rest and acclimatise to the day-night cycles of the laboratory.

The Endometrial & Follicular Study

1.Six groups of 36 rats [n=36] were chosen for the study.

2. Rats were fed normally with no dietary restrictions and any form of pre-treatment

3.Blood was collected from the tail vein for analysis of the hormones FSH,LH and Prolactin.

4.Treatment was initiated with either an agonist or antagonist and the animals were carefully marked for easy identification

5.The injections were administered 48 hrs prior the next metaestrus cycle and continued till the estrous phase

6.The dosages of GnRH agonsit/ GnrH agonist, 200 IU hCG, 75 IU FSH were scaled down and adjusted as per the rats body-weight prior to administration

7.The Treatment Groups:

- Control Group [n=6]

- HP -HCG 200 IU + HP-FSH [n=6]
- HP-HCG 200 IU + GnRH Agonist
- HP -HCG 200 IU + GnRH Agonist + HP-FSH
- HP-HCG 200 IU + GnRH Antagonist
- HP -HCG 200 IU + GnRH Antagonist+ HP-FSH

The control group had no treatment at all and were purely for evaluation of the follicular patterns. The injections were administered with 1 ml disposable syringes causing minimal trauma so as to not stress the animal.

8.Hormonal Estimations

Blood was collected from the tail vein, FSH,LH were carried out at the beginning of the study and immediately after sacrifice using the cervical dislocation using ELISA [Table I,I & III]

9.Post sacrifice, ovaries were removed surgically and were damped with tissue paper and weighed. The ovaries, were then introduced into glass vials containing formaldehyde diluted with normal saline.

10. Endometrial Thickness, was carried using the Hematoxylin and Eosin Staining. After fixed with 10% formalin for 24 hours and embedded in paraffin, the uteri were cut into a thick 5 μm transverse sections and then mounted on slides. Parts of these tissue sections were stained with hematoxylin (Harris) and eosin according to the standard procedure. Morphological changes were observed under light microscope, and morphometric parameters were evaluated. The stromal area and glandular area

were also measured [Table I,II and III]

**Hormonal Levels & Endometrial Thickness**

**Table I**

| Groups | | FSH [ng/mL] | LH [ng/mL] | E2 levels [pg/mL] | Endometrial Thickness [μm] |
|---|---|---|---|---|---|
| Control | Pre- | 600.21 ± 75.9 | 350. 19 ± 42.75 | 830.37 ± 238.52 | 540.69 ± 13.80 |
| | Post- | 632.37 ± 62.01 | 369. 32 ± 22.75 | 1045.00 ± 345.68 | 580.97 ± 23.96 |
| HCG 200 IU + HP-FSH | Pre- | 615.16 ± 52.75 | 384. 65 ± 63.75 | 8955.68 ± 432.76 | 565.21 ± 16.32 |
| | Post - | 900.42 ± 98.16 | 695. 54 ± 72.75 | 1100.63 ± 298.73 | 685.34 ± 38.75 |

**Table II [Agonist]**

| Groups | | FSH [ng/mL] | LH [ng/mL] | E2 Levels | Endometrial Thickness |
|---|---|---|---|---|---|
| HCG 200 IU + GnRH Agonist | Pre - | 638.21 ± 55.9 | 346.09 ± 22.75 | 837.84 ± 278.52 | 547.39 ± 33.80 |
| | Post - | 800.37 ± 73.20 | 565. 32 ± 32.60 | 1145.00 ± 345.68 | 623.97 ± 13.96 |
| HCG 200 IU + GnRH Agonist + HP-FSH | Pre - | 650.16 ± 42.75 | 334.05 ± 23.75 | 995.27 ± 331.06 | 565.21 ± 16.32 |
| | Post - | 1100.18 ± 87.16 | 795.54 ± 42.75 | 1380.63 ± 432.92 | 685.34 ± 38.75 |

**Table III [Antagonist]**

| Groups | | FSH [ng/mL] | LH [ng/mL] | E2 Levels | Endometrial Thickness |
|---|---|---|---|---|---|
| hCG 200 IU + GnRH Antagonist | Pre - | 578.42 ± 67.58 | 399.83 ± 49.75 | 965.84 ± 278.52 | 537.89 ± 53.80 |
| | Post - | 964.18 ± 53.20 | 865. 42 ± 75.60 | 1345.00 ± 465.68 | 729.07 ± 23.96 |
| hCG 200 IU + GnRH Antagonist+ HP-FSH | Pre - | 650.16 ± 42.75 | 564.05 ± 42.15 | 920.87 ± 631.06 | 589.21 ± 16.32 |
| | Post - | 890.18 ± 49.32 | 665.14 ± 52.73 | 1400.63 ± 432.92 | 795.34 ± 38.75 |

Based on the above studies it can be concluded that HCG not only plays a crucial role in the ovulation process, but is also capable of exerting all the physiologic actions of FSH on granulosa cells. Using serum E2 concentration as a marker of follicular stimulation efficacy improvement of endometrial thickness seems an opportunity to reduce the incidence of OHSS in humans and also reduce the dosage of FSH in the late follicular phases.

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**Claims :**

1. A composition comprising hCG in highly purified form in a dose hCG ranging from 100 IU to 200 IU.
2. The composition as claimed in claim 1, wherein the highly purified hCG has reduced endotoxin level of less than 0.03 EU/IU, preferably less than 0.01EU/IU.
3. The composition as claimed in claim 1, optionally further comprising of pharmaceutically acceptable diluent, carrier or excipient.
4. Use of the composition as claimed in claim 1 for preparing the medicament for promoting the estrogenic environment to support endometrial growth and receptivity and also the follicular growth.
5. A pharmaceutical formulation comprising the composition of claim 1 or 2 and pharmaceutically acceptable diluent, carrier or excipient.
6. The pharmaceutical formulations as claimed in claim 5, wherein the formulation is formulated as a unit dosage in the form of a solid ready for dissolution to form a sterile injectable solution for intramuscular or for subcutaneous use.
7. The pharmaceutical formulations as claimed in claim 5, wherein the formulation is formulated as a solution for injection, comprising any of the excipients and buffers
8. Use of the composition as claimed in claim 1 or formulation as claimed in claim 5 for promoting the estrogenic environment to support endometrial growth and receptivity and also the follicular growth.
9. Use of the composition as claimed in claim 1 or formulation as claimed in claim 5 with or without concomitant FSH preparation.
10. Use of the composition as claimed in claim 1 or formulation as claimed in claim

5 for the treatment of infertility in patients receiving high doses of Follicle Stimulating Hormone (FSH).

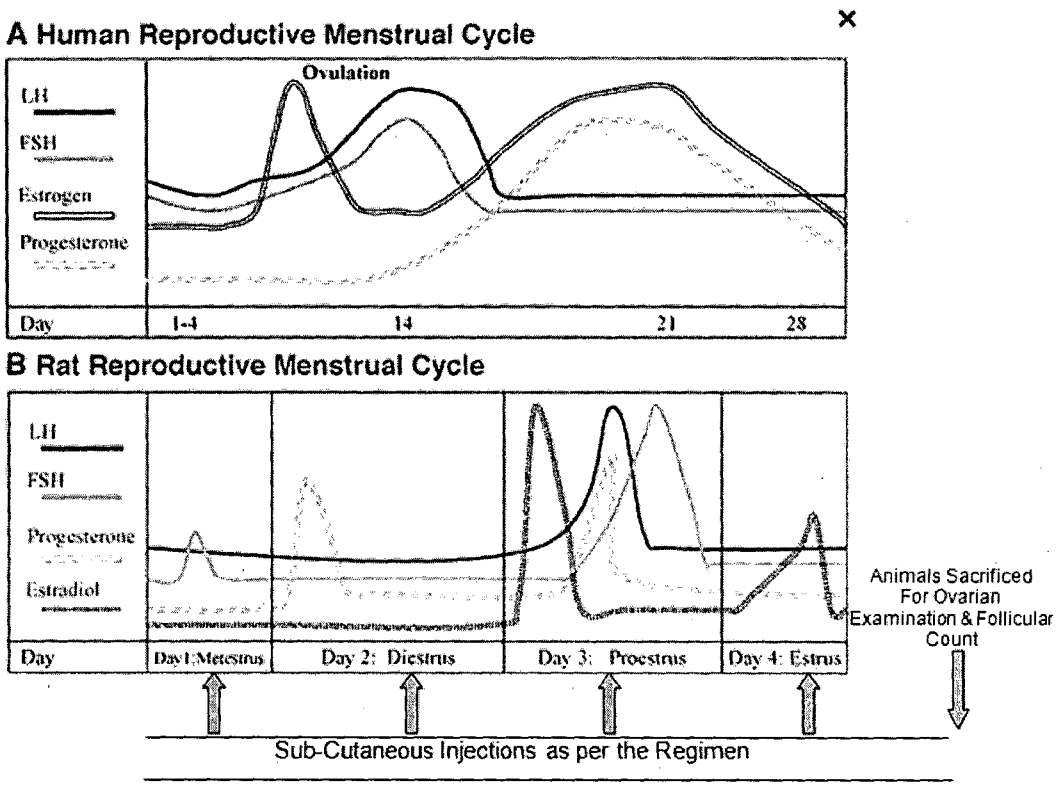
A Human Reproductive Menstrual Cycle
LH
FSH
Estrogen
Progesterone
Ovulation
Day
1-4
14
21
28
B Rat Reproductive Menstrual Cycle
LH
FSH
Progesterone
Estradiol
Day
Day1:Metestrus
Day 2: Diestrus
Day 3: Proestrus
Day 4: Estrus
Animals Sacrificed For Ovarian Examination & Follicular Count
Sub-Cutaneous Injections as per the Regimen

FIG 1