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(54) **Title**: COMPOSITION FOR SPERMATOGENESIS

(57) **Abstract**: The present invention is provides compositions comprising combination of gonadotropins for promoting spermatogenesis in males. Particularly the present invention provides a composition comprising a mixture of gonadotropins selected from but not limited to group consisting of Follicle-stimulating hormone (FSH), r Luteinizing hormone (LH) and Human chorionic gonadotropin (hCG), and pharmaceutical formulations comprising the same.

Title: Composition for spermatogenesis

Field of the Invention

The present invention relates to a pharmaceutical composition for spermatogenesis. More particularly the present invention relates to a pharmaceutical composition comprising combination of Gonadotropins for promoting spermatogenesis in males.

Background of the Invention

The etiology of male infertility may be primary or secondary in nature. Spermatogenesis in humans is complex and current knowledge has its limitations. The process of sperm manufacture requires, 74 days, regardless of number of sperm produced. Potential sperms are never produced because of cell death, especially during the meiosis-1 and during meiosis-2. Many sperm released from the seminiferous epithelium are abnormal. Spermatogenesis can be deficient in quantity of sperm produced, quality of sperm produced, or both.

The prevalence of infertility in the general population is 15%–20%. Of this, the male factor is responsible for 20%–40%.1 In Indian couples seeking treatment, the male factor is the cause in approximately 23%. Due to the changing life-styles in India and other Asian countries for example consumption of carbonated beverages, alcohol, junk food and smoking may also aggravate the problems of male infertility. Though the global prevalence of male factor infertility is 40%, in

some countries like Nigeria where the prevalence seems to be higher with most of the conditions being Oligospermia, to execute conventional long term treatment protocols for spermatogenesis, compliance and cost effectiveness are most important measurables for the number of males undergoing the treatment.

In men with infertility either idiopathic infertility or on the basis of presumed pathology, various uncontrolled treatments have been applied to infertile men with often questionable patho-physiological justification or merely just on an empirical basis. What is more appalling are the results of well controlled using gonadotropins alone or in combination have not been put to the best of the therapeutic use. Testosterone, human chorionic gonadotropin (hCG) in high doses alone, Clomiphene citrate, Aromatase inhibitors, and Bromocriptine have not been shown to be effective in men with idiopathic oligo/azoospermia. Testosterone therapy can further suppress spermatogenesis.

Therefore, a need still exists for an intervention which may facilitate not only a therapeutic advantage for a quick onset and prolonged duration of action of LH activity but also the ease of administration of gonadotropins with an improved compliance and reduced cost of treatment.

Summary of the Invention

Accordingly, the present invention provides a composition comprising combination of gonadotropins for promoting spermatogenesis in males.

In one aspect the present invention provides a composition for promoting spermatogenesis in males comprising a mixture of gonadotropins selected from but not limited to group consisting of Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and Human chorionic gonadotropin (hCG).

In a specific aspect the present invention provides a composition for promoting spermatogenesis in males comprising a combination of FSH and LH at low dose and micro-dose of hCG.

Description of the Invention

The present invention is directed towards providing compositions comprising combination of gonadotropins for promoting spermatogenesis in males.

In one embodiment the present invention provides a composition comprising a mixture of gonadotropins selected from but not limited to group consisting of Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and Human chorionic gonadotropin (hCG).

The composition may be presented as a single unit. The composition comprises the combination of gonadotropins in optimum therapeutic concentrations. The composition of the present invention comprises all three gonadotropins that is FSH, LH and HCG in quantities which can offer physiological rhythm of gonadotropin release, ease of administration, compliance of the subfertile

subject with respect to the reduced number of administrations.

In a preferred embodiment the present invention provides a composition for promoting spermatogenesis in males comprising a combination of FSH and LH at low dose and micro-dose of hCG.

The LH component of the composition may range from 10 IU to 300 IU. The FSH component of the composition may range from 10 IU to 200 IU. The HCG component of the composition may range from 50 IU to 750 IU.

In one specific embodiment, the invention also provides a composition for promoting spermatogenesis in males comprising combination of 10 IU to 200 IU of highly purified FSH, 10 IU to 300 IU of highly purified LH and 50 IU to 750 IU of highly purified hCG having reduced endotoxin level of less than 0.03 EU/IU, preferably less than 0.01EU/IU per dosage.

The composition of the present invention in addition to the combination of gonadotropins may comprise of pharmaceutical acceptable excipient(s) and /or carriers.

In one embodiment, the invention also provides pharmaceutical formulations comprising composition of the present invention for promoting spermatogenesis in males comprising combination of 10 IU to 200 IU of highly purified FSH, 10 IU to 300 IU of highly purified LH and 50 IU to 750 IU of highly purified hCG.

The composition of the present invention may be formulated in dosage form suitable for oral or any other form of administration appropriate to promote spermatogenesis.

The pharmaceutical formulations of the invention may be formulated for administration by any convenient route, often in association with a pharmaceutically and/or veterinarily acceptable carrier.

In one of the preferred embodiment the present invention composition may be formulated in an injectable preparation.

The pharmaceutical formulations for parenteral administration will usually be sterile.

Pharmaceutical formulations adapted for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents are also within the scope of the invention. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to

use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets. The formulations can be administered through a prefilled syringe, an auto-injector or a multidose auto-injector.

In one of the embodiment, the present invention provides a pharmaceutical formulation comprising composition of the present invention for promoting spermatogenesis in males comprising combination of 10 IU to 200 IU of highly purified FSH, 10 IU to 300 IU of highly purified LH and 50 IU to 750 IU of highly purified hCG, 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.

In another embodiment, the present invention provides pharmaceutical formulation comprising the composition of the present invention for promoting spermatogenesis in males comprising combination of 10 IU to 200 IU of highly purified FSH, 10 IU to 300 IU of highly purified LH and 50 IU to 750 IU 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.

Oral and other pharmaceutical formulations may be presented in unit- or multi-

dose form. Oral pharmaceutical formulations may be in the form of solids, such as powders, granules, tablets, capsules (for example hard or soft gelatin capsules) or lozenges, or liquids, such as syrups or elixirs. Fillers and/or carriers may be present as appropriate, and those skilled in the art of pharmaceutical formulation will be able to provide such additional or alternative excipients as may be necessary or desirable; flavouring agents are one example. Any pharmaceutical formulation intended for oral administration may be formulated for enteric resistance, so as to assist delivery to the small intestine by avoiding or mitigating any digestion of the compound(s) as may occur in the stomach or the proximal part of the small intestine. Tablets or capsules may be enteric coated, for example by conventional procedures. Liquid pharmaceutical formulations may be effectively rendered enteric resistant by including or being co-administered with a suitable agent such as medium-chain triglycerides.

In one more embodiment, the present invention provides a kit for promoting spermatogenesis in males comprising of dose of 10 IU to 200 IU of highly purified FSH, 10 IU to 300 IU of highly purified LH and 50 IU to 750 IU of highly purified hCG for once a week or twice a week administration.

The composition comprising for promoting spermatogenesis in males comprising combination of 10 IU to 200 IU of highly purified FSH, 10 IU to 300 IU of highly purified LH and 50 IU to 750 IU of highly purified hCG, or pharmaceutical formulation comprising the same may be administered once a week or twice a week, preferably twice weekly.

The composition comprising combinations of gonadotropins thus reduces the injections to be given to the subject in the need thereof by 10-60% of the total conventional consumption for the established treatment protocols, still ensure the desired clinical outcomes, by an independent, interdependent or complementary role for spermatogenesis.

The compositions of the present invention can be given in a variety of conditions related to male sterility. The composition of the present invention may be suitable In conditions of disrupted hypothalamic axis such as IHH or in all the conditions such as Kallman's syndrome and Hypogonadotropic Hypogonadism but not just limited to these, the combination is aimed to be superior to conventionally available single gonadotropin formulations, in that the invention has a role to meet simultaneous requirement of all three gonadotropins.

While the present invention is described as above in general and with the help of various embodiments, it is apparent that various modifications may be made to the above 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 invention will now be described further in the following non-limiting examples.

Example 1

The method of Lohiya et al. using Danazol (15 mg/kg b.w./day; oral) with combination of testosterone enanthate (5 mg/kg b.w./15 days; S.C.) was administered to male rabbits for reversible suppression of spermatogenesis. The method was modified to suit our study and the dosage of Danazol was modified accordingly. Semen analyses for efficacy and reversibility were performed biweekly. The regimen used by Lohiya et al resulted in complete azoospermia or severe oligozoospermia in all the animals within 75 days of treatment accompanied with decreased motility, vitality and increased sperm abnormalities. Semen volume and pH did not alter significantly. Libido was unaffected. All seminal characteristics were within normal range after 115 days of recovery. The drug combination in given dose regimen resulted in reversible inhibition of spermatogenesis in rabbits.

Treatment Schedule

The no of animals used in the study were 18 male rabbits and each group was administered the drug as follows:

(a) Group I – Danazol[oral]+ Testosterone enanthate [Injection]

(b) Group II – Danazol [oral]+Testosterone enanthate [Injection] and Semen parameters were monitored to evaluate suppression of spermatogenesis. On confirmation of low sperm counts as assessed by manual counting, under microscopy, 37.5 IU of highly purified FSH, 37.5 IU of highly purified LH and 500 IU of highly purified hCG scaled down and adjusted as per the body-weight of rabbit was administered once weekly till sperm counts

returned to normal as assessed by microscopy.

(c) Group III - Danazol [oral]+Testosterone enanthate [Injection] and Semen parameters were monitored to evaluate suppression of spermatogenesis. On confirmation of low sperm counts as assessed by manual counting, under microscopy, 37.5 IU of highly purified FSH, 37.5 IU of highly purified LH and 500 IU of highly purified hCG scaled down and adjusted as per the body-weight of rabbit was administered twice weekly till sperm counts returned to normal as assessed by microscopy.

Sperm Collection

Male New Zealand rabbits, are easy to handle and have been used extensively for andrology use. The rabbit is well suited for infertility research because of the animal's size, relatively low cost, and accessibility of the genitalia.

Using the methods available in the literature, we constructed an inexpensive, artificial vagina using locally available materials such as a male condom and a plastic ring and a soft plastic container so as to not hurt the animal while collecting ejaculates. The device is hand-held beneath a sedated doe with the open end pointed in a caudal direction. As the buck begins to mount, the device is placed more posterior and inferior to allow penetration of the male rabbit into the artificial vagina. Ejaculation occurs rapidly and the device is removed. Animals were ejaculated approximately twice weekly. All animals were acclimatized to the artificial vagina, to avoid any undue trauma. The sperm count was assessed, by the method of Sukardi et al.

Results:

Though other models described elsewhere, have been tried, the modified method of Lohiya et.al was most suited for studying our formulation. The results are tabulated citing the following parameters:

- Maximum number of ejaculates planned
- Number of ejaculates obtained
- Milliliters of Semen /Ejaculate
- Sperm Count[Millions/ ml of semen]
- Percentage of motile sperms in ejaculate

Table 1

Group-I[Number of rabbits =6] -Danazol[Oral]+ Testosterone enanthate[Injection] for 15 Days

- Maximum Number of Ejaculates Planned / Week – One
- Total Number of Ejaculates Obtained – 9

	Milliliters of Semen /Ejaculate	Sperm Count [Millions/ ml of Semen]	Percentage of Motile Sperms in Ejaculate
Pre-Treatment	0.98 ±.0.03	71.32 ± 11	64.52 ± 0.94
Post-Treatment - In Weeks			
1	0.35 ±0.01	19.32 ± 1.12	10.12 ± 0.10
2	0.35 ± 0.01	22.35 ± 1.10	14.22 ± 0.08
3	0.38 ± 0.02	24.55 ± 1.00	14.42 ± 0.04
4	0.40 ±0.02	28.35 ± 1.35	16.32 ± 0.50
5	0.40 ± 0.03	30.35 ± 1.02	24.12 ± 0.64
6	0.50 ±0.15	38.35 ± 1.91	24.36 ± 0.83

	Milliliters of Semen /Ejaculate	Sperm Count [Millions/ ml of Semen]	Percentage of Motile Sperms in Ejaculate
7	0.65 ±0.01	47.35 ± 1.32	39.52 ± 0.20
8	0.65 ±0.01	52.35 ± 1.1 6	47.32 ± 0.14

Table 2

Group II [Number of Rabbits =6] - Danazol [Oral]+Testosterone enanthate [Injection] for 15 Days , followed by 37.5 IU of highly purified FSH, 37.5 IU of highly purified LH and 500 IU of highly purified hCG scaled down and adjusted as per the body-weight of rabbit [administered once weekly]

- Maximum Number of Ejaculates Planned / Week – One
- Total Number of Ejaculates Obtained - 9

	Milliliters of Semen /Ejaculate	Sperm Count [Millions/ ml of Semen]	Percentage of Motile Sperms in Ejaculate
Pre-Treatment	1.35 ±.0.01	77.32 ± 5. 32	69.52 ± 0.94
Danazol +TE	0.26± 0.04	20.26 ± 1..32	14.24 ± 0.39
Post-Treatment - In Weeks [Initiated form the 16th Day]			
1	0.35 ±0.01	19.32 ± 1.12	16.12 ± 0.10
2	0.38 ± 0.02	22.35 ± 1.11	16.22 ± 0.18
3	0.45 ± 0.15	26.55 ± 1.00	16.12 ± 0.04
4	0.48 ±0.12	28.35 ± 1.35	17.32 ± 0.50
5	0.53 ± 0.03	30.35 ± 1.02	22.10 ± 0.64
6	0.65 ±0.15	38.35 ± 1.91	26.36 ± 0.63
7	0.85 ±0.01	45.35 ± 1.32	39.52 ± 0.22
8	0.85 ±0.0.5	60.35 ± 1.1 6	50.42 ± 0.18

Table 3

Group III [Number of Rabbits =6] - Danazol [Oral]+Testosterone enanthate [Injec-

tion] for 15 Days followed by 37.5 IU of highly purified FSH, 37.5 IU of highly purified LH and 500 IU of highly purified hCG scaled down and adjusted as per the body-weight of rabbit [administered twice weekly]

- Maximum Number of Ejaculates Planned / Week – One
- Total Number of Ejaculates Obtained - 9

	Milliliters of Semen /Ejaculate	Sperm Count [Millions/ ml of Semen]	Percentage of Motile Sperms in Ejaculate
Pre-Treatment	1.5 ±.0.02	79.32 ± 3.22	70.52 ± 0.24
Danazol +TE	0.31 ± 0.01	20.16 ± 0.62	14.24 ± 0.27
Post-Treatment - In Weeks [Initiated form the 16th Day]			
1	0.40 ±0.01	19.32 ± 1.12	16.12 ± 0.08
2	0.41 ± 0.03	20.35 ± 1.00	18.22 ± 0.18
3	0.55 ± 0.12	26.55 ± 0.09	19.00 ± 0.14
4	0.63 ±0.08	28.35 ± 1.05	27.32 ± 0.50
5	0.78 ± 0.02	36.35 ± 1.02	42.10 ± 0.54
6	0.95 ±0.13	48.35 ± 1.21	56.36 ± 0.43
7	1.0.8 ±0.02	69.50 ± 1.32	62.52 ± 0.20
8	1.39 ±0.0.1	72.18 ± 1.1 6	63.39 ± 0.14

Daily sperm production can be estimated non-invasively as T-Sperm/hr since the previous emission / ejaculation or as daily sperm output. The difference between the 2 estimates for daily sperm production is that daily sperm output is more precise measurement of the process of spermatogenesis. Total number of sperm per ejaculate (T Sperm) is an important measure for clinicians to provide advice to patient couples. However, T Sperm per hour of abstinence (T Sperm/h) is a better measure for epidemiologist-andrologist teams or clinicians to evaluate spermatogenesis because it is a rate function.

As seen from the results above, in Table 1, 2 and 3 it is evident that composition of the present invention shows positive outcome on spermatogenesis.

Based on the above results it is contemplated that the combination of FSH and LH acts as an immediate trigger for spermatogenesis, and the micro-dose of hCG by stimulating the Leydig cells produces the male hormone Testosterone and could stimulate the process of spermatogenesis.

Claims :

1. A composition for promoting spermatogenesis in males comprising combination of 10 IU to 200 IU of highly purified FSH, 10 IU to 300 IU of highly purified LH and 50 IU to 750 IU of highly purified hCG per dosage.
2. A composition for promoting spermatogenesis in males comprising combination of 10 IU to 200 IU of highly purified FSH, 10 IU to 300 IU of highly purified LH and 50 IU to 750 IU of highly purified hCG having reduced endotoxin level of less than 0.03 EU/IU, preferably less than 0.01EU/IU per dosage.
3. The composition as claimed in claim 1 or claim 2, optionally comprising of pharmaceutically acceptable diluents, carrier or excipient.
4. A pharmaceutical formulation comprising the composition of claim 1 or 2 and pharmaceutically acceptable diluents, carrier or excipient.
5. 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.
6. 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.
7. The pharmaceutical formulations as claimed in claims 4 - 6, wherein the formulation is formulated for once a week or twice a week administration.
8. A kit for promoting spermatogenesis in males comprising of dose of 10 IU to 200 IU of highly purified FSH, 10 IU to 300 IU of highly purified LH and 50 IU

to 750 IU of highly purified hCG for once a week or twice a week administration.

9. The kit as claimed in claim 8 wherein, highly purified hCG has reduced endotoxin level of less than 0.03 EU/IU, preferably less than 0.01EU/IU per dosage.