Clinical Study Protocol

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A multicenter and randomised clinical study on pubertal replacement therapy in boys - Treatment of boys with absent or delayed puberty with rhFSH and two different formulations of testosterone in low dose: Testoviron Depot® and Nebido®

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The following Amendment(s) have been made to this protocol since the date of preparation:

Amendment No.	**Date:**

PROTOCOL SYNOPSIS

A multicenter and randomised clinical study on pubertal replacement therapy in boys - Treatment of boys with absent or delayed puberty with rhFSH and two different formulations of testosterone in low dose: Testoviron Depot® and Nebido®

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Principal Investigator

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Study period

Estimated date of first subject enrolled: March 2013

Estimated date of last subject completed: December 2015 (group II) and December 2022 (group I)

Objectives

The primary objective of this study is to evaluate the effect of Testoviron Depot® and Nebido® on pubertal development in young males with delayed puberty and the effect of Testoviron Depot® and Nebido® in combination with Puregon in young males with absent of puberty.

Secondary objectives are serum testosterone levels during treatment with Testoviron Depot® and Nebido®, signs of masculinization and health-related quality of life (KIDSCREEN, JTJÄ).

Endpoints and variables

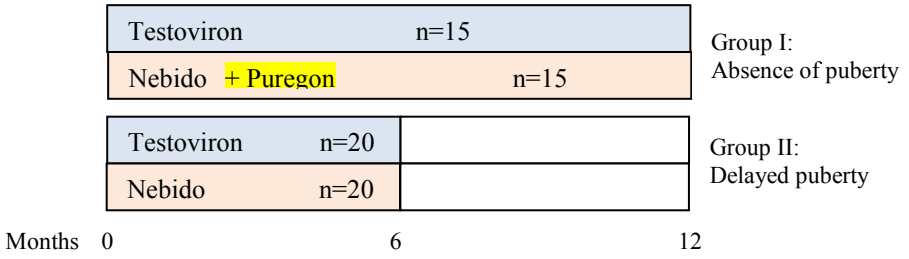
The primary endpoint in group I (boys with abscent puberty) will be increase in blood LH after GnRH test. Testis size ≥8 mL will be the primary endpoint for group II (boys with delayed puberty).

Secondary endpoints are increase in testis size from baseline to 1 year (group I), serum testosterone levels during treatment with Testoviron Depot® and Nebido®, signs of masculinization from baseline to 3, 6 and 12 months, body growth, hair growth measured as tanner stage and inspection of axillaries hair, deepening of voice, gender identity and health-related quality of life.

LH, FSH, Estradiol, DHEAS, Inhibin B, SHBG, AMH and mass spectrometric analysis of steroids will be analyzed as explorative parameters.

Study design

This is a prospective, randomized, controlled, open clinical study with parallel-group design. A total of 70 young males, 14-16 years old, with delayed or absent puberty will be randomized to receive hormone replacement during 6 or 12 months. The study includes 9 or 10 visits at the clinic.



Study subjects

The subjects will be divided to two groups depending on whether they have absences of puberty or (N=30) or delayed/slow progress of puberty (N=40).

Inclusion criteria Group I (absent puberty)

1) Morning serum testosterone value by inclusion (07.30-09.00) and start (08.00-10.00) that is less 0.5 nmol/L
2) Pre-pubertal LHRH test
3) Testicular volume 1-3ml bilaterally
4) Bone age 11 years or older

Inclusion criteria Group II (delayed puberty)

1) Morning serum testosterone by inclusion (07.30-09.00) of 1 – 3 nmol/L and at start (08.00-10.00) less than 4 nmol/L
2) Testicular volume 4-6 mL bilaterally
3) Bone age more than 11 years or older

Safety

Physical examination at start and vital signs and standard clinical chemistry and hematology investigations before and after the study will be undertaken in order to document the health status of the subject.

Statistical methods

The primary objective will be studied with non parametric method s Fisher exact chi-square test. Descriptive statistics, parametric and non parametric tests will be used for the secondary objectives. Other statistical methods e.g. regression modelling might be used in an exploratory fashion but no formal interference will be made. Demographics, safety and self-assessed outcomes will be presented by means of descriptive statistics.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study protocol.

Abbreviation	Explanation
ADR	Adverse drug reaction. A response to a drug that is noxious and unintended and occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease
AE	Adverse event. Any unfavourable and unintended sign including an abnormal laboratory finding, symptom or disease associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure that occurs during the course of the study.
CRF	Case Report Form (electronic/paper)
DAE	Discontinuation due to Adverse Event
ECG	Electrocardiogram
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
MPA	Medical Products Agency (Läkemedelsverket)
OAE	Other Significant Adverse Event (i.e. adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment
SAE	Serious adverse event. Any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity or is a congenital anomaly/birth defect
SoC	Standard of Care
SUSAR	Suspected Unexpected Serious Adverse Reaction. A serious adverse reactions in subjects given a drug, that may or may not be dose related, but are unexpected, as they are not consistent with current safety information

STUDY STRUCTURE

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1. INTRODUCTION

1.1 Background

Development and maturation of the reproductive system begins in fetal life and is an active process throughout the first postnatal months. During late pregnancy and the first months after birth the testosterone levels are high due to high activity in the hypothalamic (GnRH)/ pituitary/ gonadotropin (LH/ FSH)/ testis (testosterone) axis. After 3-6 months the activity decreases and remains low until start of puberty when the gonadotropic activity increases again. Male puberty begins when hypothalamic GnRH impulses start to stimulate the pituary for release of gonadotropins (LH and FSH) during the night. This process normally starts at the age of 10-14 years.

The increased secretion of gonadotropins results in testicular enlargement from 1-2 mL to 3 mL and is the first physical manifestation of puberty. FSH stimulates the spermatogenesis by trophic effect on Sertoli cells and LH the Leydig cells to produce testosterone in the testis. The testis size of 3 ml represents transient phase from pre-puberty to puberty (1) and taking all the information on pattern and levels of testosterone together, the 3 mL testis can be seen to belong more to the pubertal stage than to the prepubertal stage. Testicular size continues to increase throughout puberty, reaching maximal adult size about 6 years after the onset of puberty. The volume of 4 mL or more is considered as the clinical landmark of puberty. Thus, delayed puberty is defined as no evidence of an increase in testicular volume (greater than or equal to 4 mL) by 15 years of age. The consequence of increased secretion of gonadotropins and testis enlargement is testosterone production which in turn leads to development of secondary male sexual characteristics. Testosterone plays an important role in the modulation of the male somatotropic axis in adulthood, as it appears to be the case in puberty, and that this effect is partly dependent on the aromatization of testosterone to estradiol. Thus, the testosterone is the driver of the growth spurt and involved in the masculinization process. Gonadotropins reach the highest level during late night and early morning as a consequence also the testosteron levels are elevated early in the morning. Indeed, testosterone in early morning above 0.7 nM seems to predict progression of puberty with start of the pubertal growth spurt within 12-18 months. Early morning plasma testosterone is an accurate predictor of imminent pubertal development in pre-pubertal young males (2).

Factors that determine the timing of pubertal onset remain poorly understood and the subject of intense investigation but general health, nutrition and genetic factors all are known to contribute. Approximately 2.5% of healthy adolescents are identified as having pubertal delay. Most are boys. Diseases that can delay the puberty of start are e.g. hypothyroidism, celiac disease, asthma and psychiatric disorders. Boys with late start of puberty or slow progression of puberty are rather common (3) and they may suffer both mentally and socially from this condition. In Sweden boys with delayed puberty are if they whish routinely treated with low dose Testoviron Depot® from the age of 14 unless no pathological disease is the cause the puberty delay.

1.2 Rationale for conducting this study

Boys with delayed or absent puberty have by tradition been treated with intra muscular injections of testosterone (Testoviron Depot®; Testosterone Enanthate) in low doses once a month. Although extensive clinical experience this therapy has not been evaluated in clinical trials, yet it has become the standard of care (SoC). Furthermore, Testoviron Depot® was unregistered 2006 and is now available only on licence in Sweden. During the last 6 to 8 years new testosterone replacement therapy has reached the market e.g. muscular injections of long acting testosterone (Nebido®), gels (Testogel®, Tostrex®, Testim®) and transdermal plasters Intrinsa®. These new treatments are labeled for adult men and women but not in children and adolescents. However, these products are being used off label today for the treatment of delayed or absent puberty in young males. The hormones have the possibility to be administrated in low doses by manipulation of the adult formulation, gel doses, transdermal patches or long acting intra muscular injections and thereby have the possibility to be used for treatment of delay of puberty in male adolescents. Intrinsa®, was however recently (May 2012) taken away at the European market.

A comparison between these products is important to make before Testoviron Depot® completely disappears from the market (not even available on license) which has been the SoC, based on clinical experience and not clinical trials.

In the present study, the treatment with Testoviron Depot® will be evaluated which would be an advantage since thorough clinical trials has never been performed. The treatment with Testoviron Depot® will be compared to low doses of solutions for intra muscular injections with Nebido®. Treatment with Nebido has the advantage to gels, both regarding the compliance of the treatment and it is easier to obtain the correct amount of the product for administration. With intra muscular injections of testosterone we have full control of how much testosterone is given. Furthermore we have limited clinical experience on what 200-250mg Nebedio can result in testosterone levels seen in early and mid puberty for 3 months. The body's natural testosterone has a rapid turnover rate which requires a continuous production to maintain steady levels in the blood. This means that also substitution therapy must meet the requirement of even and "high" levels to have an optimal therapeutic effect. Thus, only formulations having different depot functions have been considered when choosing treatment in the present study.

The reason for having Puregon (rhFSH) as co-treatment in boys with absence of puberty is to mimic spontaneous puberty and optimize conditions for puberty to get started. In spontaneous puberty both FSH and LH increases, and in the present study FSH is substituted by Puregon and LH by its effect on Leidig cells as testosterone. Furthermore, the levels seen with low dos Nebido is more physiological since the testosterone levels are even and possibility to obtain similar to levels seen in spontaneous early or mid puberty compared to Testoviron wich resulst in high testosterone similar or higher seen in adult males levels first 2-3 days after injection and thereafter decline to low levels last week befor next injection. We will evaluate a new and more physiological combination therapy by combining FSH with Nebido and compare with the traditional Testoviron treatment in boys with abscens of puberty. For boys with slow pace of puberty we will evaluate to principal forms of testosterone treatment for accelerating the pubertal development: Testoviron with high testosterone similar or higher seen in

adult males levels first 2-3 days after injection and thereafter decline to low levels last week befor next injection to Nebido treatment were the testosterone levels are even and increased from early to mid puberty levels.

The study population will be selected among young male adolescents seeking care for delayed puberty involving worries about their height, physical appearance or concerns about the development of genitals and the emotional distress associated to the puberty development.

Long-term safety, more than 1 year, has not been evaluated in double blind placebo controlled studies. The present study will last for maximum one year. Moreover, only medically healthy young male subjects will be included in the study.

1.3 Research hypothesis

Does administration of testosterone with monthly rhythm induce puberty better than testosterone administrated in more constant levels?

1.4 Benefit/risk and ethical assessment

Testoviron Depot® and Nebido® are anabolic steroids with high anabolic and androgenic effects. It is a long acting injectable testosterone that are used not only for medical purposes but also amongst athletes abuse since it increase strength, weight and muscle mass. Since Testoviron Depot® treatment is not evaluated there is no treatment benefit for boys in conventional group compared to the boys in the groups with new treatments.

Testosterone and estradiol will be monitored careful as too low levels will result in lack of effect and too high levels in adverse events e.g. aggressiveness, acne, Hb increase and potentially also impair long term growth due to early closure of growth zones. As testosterone and estradiol will be monitored careful during the study doses will be adjusted to give testosterone levels seen in early/mid puberty

1.5 Ethical assessment

The study will be conducted in compliance with ICH Good Clinical Practice and applicable regulatory requirements and in accordance with the ethical principles in the Declaration of Helsinki. For detailed information regarding ethics and regulatory review, informed consent, data protection and audits and inspections see **Appendix C** (Ethics).

Approval has been obtained from the ethic committee (IRB/IEC) in Göteborg (Dnr 506-12). Regulatory authorities will receive the clinical trial application (CTA) and required documents.

The investigator will ensure that the subject and parent/guardian are given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study and ensure that the subjects are notified that they are free to discontinue from the study at any time. Signed and dated informed consent should be obtained before conducting any study specific procedures and stored in the Study Master File. A copy of the signed Informed Consent Form is kept by the subject.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary objective

The primary objective of this study is to evaluate the effect of Testoviron Depot® and Nebido® on pubertal development in young males with delayed puberty and the effect of Testoviron Depot® and Nebido® in combination with Puregon in young males with absent of puberty.

2.1.1 Primary endpoint(s)

The primary endpoint in group I (boys with abscent puberty) will be increase in blood LH after GnRH test. Testis size ≥8 mL will be the primary endpoint for group II (boys with delayed puberty).

2.2 Secondary objective(s)

- Testis size (mL) in group I from baseline to 1 year (group I)
- Serum testosterone levels during treatment with Testoviron Depot® and Nebido®.
- Signs of masculinization from baseline to 3, 6 and 12 months (Body growth, hair growth measured as tanner stage and inspection of axillaries hair, deepening of voice, gender identity)
- Health-related Quality of life (KIDSCREEN, JTJÄ)

2.3 Exploratory variables

- LH and FSH
- Estradiol
- DHEAS
- SHBG, AMH, Inhibin B
- Mass spectrometric steroid profile of steroids

3. STUDY DESIGN AND PROCEDURES

3.1 Overall study design and flow chart

This is a prospective, randomized, controlled, open clinical study with parallel-group design. A total of 70 young males with delayed or absent puberty will be randomized to receive hormone replacement during 6 or 12 months. The study includes 9 or 10 visits at the clinic during a total period of 12 months.

To not expose boys for a new treatment (Nebido and Puregon in group I and Nebido in group II) that is inferior to Stand of Care Testoviron, a futility test will be performed after 10 evaluable patients in each arm in the groups.

The subjects will be divided to two groups depending on whether they have I) absences of puberty (n=30) or II) delayed/slow progress of puberty (n=40):

I) Boys with absence of puberty (testis 1-3 mL and morning testosterone less than 0.5nM) will be randomized to two alternative pubertal replacement therapies; 1) low dose of Testoviron Depot® (SoC) 50 mg i.m. injection once a month or 2) rhFSH (Puregon®) and low dose Nebido® (one injection of 200 mg every 3 months). Those randomized to receiving rhFSH (Puregon®) and low dose Nebido® will start both treatments concomitant. The rhFSH (Puregon®) dose will be adjusted to reach serum FSH levels of 3-5IU/ml (see appendix D). This is expected to happen within one month (first check after one week after start of treatment with Puregon) and if not reach within one month of start of Puregon treatment the patient will be withdrawn from the study. Boys who have been randomized to Puregon/Nebido but at inclusion have serum levels of FSH above 3 will not be treated with Puregon, but if the patient during the study on 2 concomitant visits have serum FSH below 3IU/L he will be treated with Puregon t to achive serum FSH levels 3-5 IU/L during the rest of the study (see appendix D). The treatment will continue for 12 months. The effect will be evaluated after 6 and 12 months respectively on the development of masculinization and on spontaneous start of puberty. If the spontaneous puberty has started, the pubertal hormone replacement treatment will be stopped. The Nebido treatment will be dose adjusted in patients after the 1st injection if the testosterone profile shows to high or low levels; the goal is to have similar levels during 2 months as in early puberty (1-3 nM). For individual Nebido® adjustments in group I see appendix D.

II) Boys with delayed/slow progress of puberty (testis 4-6 mL and morning testosterone less than 4 nM) will be randomized to receive either 1) Testoviron Depot® (SoC) 75 mg i.m. injection once a month (SoC) or 2) Nebido® one injection of 250 mg at start and after 3 month. The treatment will continue for 6 months, dose adjustments will be possible after the first 4 patients on Nebido® (250 mg) for the rest of the study. The aim for the Nebido® treatment is to have morning testosterone levels in the lower range of mid puberty (4-9 nmol/L). An interim analysis has to be done after the first 5 patients and thereafter keep or adjust the dose (see appendix D for adjustment of the Nebido® dose for group II). The treatment will be evaluated after 6 month on development of masculinization and on spontaneous start of puberty after 12 months.

Figure 1 and Table 1 show the study overview and activities.

Figure 1a ***Study design Group I (absence of puberty)***

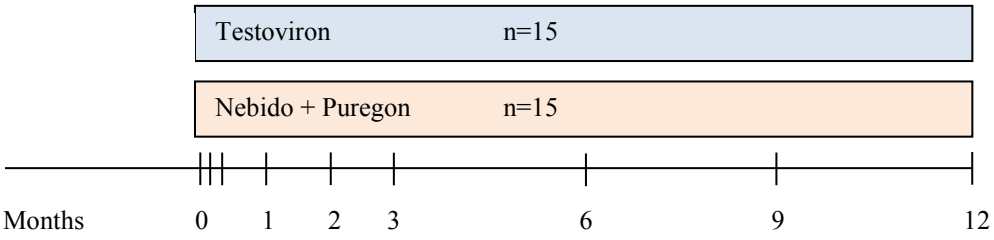

Figure 1b ***Study design Group II (delayed puberty)***

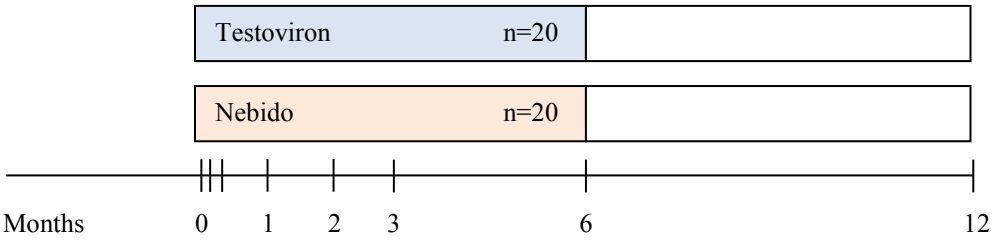

Table 1 ***Study activities***

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9*	Visit 10
	Pre-	0	2 days	7 days	1 month	2 months	3 months	6 months	9* months	12 months
Informed consent	X									
Demography	X									
Medical history	X									
Nicotine use	X									
Weight, height	X						X	X	X	X
Pulse, blood pressure	X									
Physical exam	X									
Bone-age	X									
LHRH test	X									X*
Pubertal stage	X						X	X	X	X
HQOL	X							X		X
Hb, liver enzymes	X						X	X		X
LH, FSH	X	X		X*	X		X	X	X	X
DHEAS,		X						X		X
Incl/excl criteria		X								
Study drug		X								
Testosterone, estradiol		X	X	X	X	X	X	X		X

SHBG, AMH, InhibinB		X			X			X		X
Signs of masculinization								X		X
Plasma for masspektometri of steroids	X							X		X
Adverse events	X	X	X	X	X	X	X	X	X	X
Serious Adverse event recording	X	– – –	– – –	– – –	– – –	– – –	– – –	– – –	– – –	→

*Visit 9: Group I only

3.1.1 Stopping criteria

To avoid that the treatment with Nebido and Puregon in group I and Nebido in group II is not inferior to SoC, a futility test will be performed after 10 evaluable patients in each arm in the groups. This futility analysis can result in stopping the study if the new treatment (Nebido and Puregon in group I and Nebido in group II) is statistically significant inferior or superior, or continue the study as planed or increase the numbers patients to have a possibility to reach statistical significance.

3.1.1.1 Stopping criteria for dose continuation

General stopping criteria for dose continuation are hypersensitivity reactions to the active substance or any excipients. However, mild skin reaction at the injection- or patch site will not be judged as stopping criteria.

The first dose of Puregon will be given at the clinic under medical supervision for approximately 2 hours to exclude any acute hypersensitivity reaction. Acute toxicity of Puregon is very low. No special treatment measures are required in case of overdose except that drug treatment should be stopped or the dosage reduced.

Suspected anaphylactic reactions have been reported after injection of Nebido. Subjects will be observed after each injection at the clinic and in case of any reaction routine medical care applies.

3.2 Rationale for study design, doses and control groups

The research group have clinical experience on testosterone levels from a few patients who have successfully been treated with Nebidoat Swedish hospitals (unpublished data). This is the base for the chosen doses in the present study. The dose of Testoviron Depot® is the off-label dose most commonly used in Sweden.

This study is open (not blinded) and does not use placebo-control design. It would not be, according to our view ethical acceptable to decline young males hormone replacement therapy in a placebo-controlled study design since the inclusion exlusion criteria are similar to the requirement in clinical routin in the Department of Pediatrics at the NÄL-hospital in Trollhättan and SU/Östra in Göteborg to obtain treatment. Every year during the relatively short period of puberty is valuable for the young males to receive treatment. Recruitment of subjects into this study will be among boys who actively

seek medical care for worries about delayed puberty. The psychological consequences of pubertal delay in boys are noteworthy because it involves emotional distress, poor body image and low self-esteem (4). These boys are also more likely to be teased or bullied. Mood and anxiety disorders are among the most prevalent mental health conditions affecting youth, boys as well as girls. Boys may be more reluctant to seek care for emotional problems, fearing that this may be perceived as a weakness. Therefore, it is of utmost importance for the clinician to bring up these topics with young men.

To not expose boys for a new treatment (Nebido and Puregon in group I and Nebido in group II) that is inferior to SoC, a futility test will be performed after 10 evaluable patients in each arm in the groups. This futility analysis can result in stopping the study if the new treatment (Nebido and Puregon in group I and Nebido in group II) is statistically significant inferior or superior, or continue the study as planed or increase the numbers patients to have a possibility to reach statistical significance.

4. SUBJECT POPULATION

A total of 70 young healthy males between 14 and 16 years with clinically verified absent or delayed puberty will be included. The study population will be identified among referrals to the Department of Pediatrics at the NÄL-hospital in Trollhättan and SU/Östra in Göteborg.

Subjects will be divided into 2 main groups; Group I and Group II depending on puberty status. Each group will be sub-divided into treatment groups (Table 2):

Table 2 Subjects and treatment

Subjects	**Treatment**	**N**
Group I (absent puberty):	1) Testoviron	15
	2) Puregon and Nebido	15
Group II (delayed puberty):	1) Testoviron	20
	2) Nebido	20

Investigator must keep a record of subjects who entered pre-study screening but were never enrolled (subject screening log). Subjects that do not meet the inclusion/exclusion criteria for a study must not be enrolled into the study. Subject population should be selected without bias.

Two groups of boys will be included; Group I with absent puberty and Group II with delayed puberty

4.1 Inclusion criteria Group I (absent puberty)

For inclusion in the study subjects must fulfil the following criteria.

1. Signed informed consent

2. Healthy young male subjects 14-16 years old
3. Morning serum testosterone value by inclusion (07.30-09.00) and start (08.00-10.00) that is less 0.5 nmol/L
4. Pre-pubertal LHRH test
5. Testicular volume 1-3ml bilaterally
6. Bone age 11 years or older
7. Otherwise clinically normal physical findings and laboratory values as judged by the investigator

4.2 Inclusion criteria Group II (delayed puberty)

For inclusion in the study subjects must fulfil the following criteria.

1. Signed informed consent
2. Healthy young male subjects 14-16 years old
3. Morning serum testosterone by inclusion (07.30-09.00) of 1 – 3 nmol/L and at start (08.00-10.00) less than 4 nmol/L
4. Testicular volume 4-6 mL bilaterally
5. Bone age more than 11 years or older

4.3 Exclusion criteria

Subjects must not enter the study if any of the following exclusion criteria are fulfilled

1. Growth spurt
2. Bone age < 11 years
3. Untreated hypothyroidism
4. Suspected growth hormone deficiency
5. Untreated celiac disease
6. Steroid or immune-suppression medicated disease
7. Training doses over 10 hours a week
8. Known or suspected allergic reaction towards the active substances or other components
9. Clinical judgement by the investigator that the subject should not participate in the study
10. Use of anabolic steroids or other drugs.

Subjects that do not meet the inclusion/exclusion criteria for a study should not, under any circumstances, be enrolled into the study.

4.4 Subject enrolment and randomization

The investigator will obtain signed informed consent from the potential subject as well as both parent(s)/guardian(s) before any study specific procedures are performed. The investigator will thereafter determine subject eligibility according to study criteria and finally assign eligible subject unique *randomization code* (i.e. subject number).

Randomization codes will be assigned strictly sequentially as subjects are eligible for randomization. If a subject discontinues participation in the study, his subject number cannot be re-used. New subject gets a new number in consecutive order.

A randomisation schedule, containing randomisation code and treatment, will be provided by one of the study leaders.

4.5 Restrictions during the study

There are no certain restrictions during the study.

4.6 Withdrawal of subjects and study stop

Subjects are free to discontinue their participation in the study at any time without prejudice to further treatment. The subjects may be withdrawn from the study at the discretion of the investigator due to safety concerns or if judged non-compliant with study procedures. Other reasons for discontinuing a subject are incorrect enrolment and subjects lost to follow-up.

A subject who discontinues will be asked about the reason(s) for discontinuation and the presence of any adverse events. If possible, they will be seen and assessed by the investigator. Adverse events will be followed up.

Also, the manufacturers of the study drugs may revoke their products, which may lead to the entire study may be interrupted.

4.6.1 Premature termination of the study

The Sponsor/Investigator may decide to stop the trial or part of the trial at any time. If a trial is prematurely terminated or suspended, the Investigator should promptly inform the patients and ensure appropriate therapy and follow-up. Furthermore, the Investigator should promptly inform the Ethics committee and provide a detailed written explanation. The regulatory authority should be informed according to national regulations.

5. TREATMENTS

Puregon®, Nebido® and Testoviron Depot® will be administrated in the study.

Table 3 Identity of investigational product(s)

Investigational product	Dosage form and strength	Administration dose	Manufacturer
Puregon®-Follitropin beta	Solution for injection s.c. 300 IE/0.36 mL	30-75 IE, 3 times a week	MSD
Nebido®	Solution for injection i.m. 1000 mg/4mL	200mg (at start and after 3 months) in group I and 250 mg in group II	Bayer
Testoviron Depot®	Solution for injection i.m. 250mg/mL	0.2mL=50 mg per month in group I 0,3ml=75mg per month in group II (total 6 injections)	Bayer Shering Pharma AG

Puregon (rhFSH) and Nebido are indicated in men with hypogonadotrop, hypogonadism and testosterone deficiency. Testoviron Depot is the most common testosterone ester for substitution therapy when testosterone deficiency has been confirmed.

Other common side effects are skin reactions at the application site, headache, gastro-intestinal, insomnia.

Testoviron Depot gives a pronounced variation in testosterone levels in the blood, with the highest values the day after injection and then gradually decreasing levels until the next scheduled injection.

Nebido provide a more steady level of testosterone in the blood between dosing sessions.

Testoviron Depot is currently not registered in Sweden and will be prescribed on license.

5.1.1 Doses and treatment regimens

Lowest possible starting doses will be administrated.

Puregon® solution (rhFSH) for injection subcutaneously in ampoules of 300 IE/0.36 mL, will be administrated as multiple doses (3 times per week) to boys randomized to Puregon/Nebio who have serum FSH levels below 3 IU/L Group I. The first dose of Puregon will be given at the clinic under medical supervision to rule out any hypersensitivity reactions immediately after dosing. Otherwise it will be used and handled according to manufacturer instruction. Boys who at inclusion have serum levels of FSH above 3, but during the study on 2 concomitant visits have serum FSH below 3 IU/L will be offered to start Puregon treatment to achive serum FSH levels reached 3-6 IU/L during the rest of the study.

Nebido® a solution for injection 1000 mg/4mL and 200 mg or 250 mg will be administrated every 3rd month s. For patients on Nebido® in group I, an evaluation of the testosterone profile will be done

after the first injection and possibility to adjust the dose. For patients in group II an interim analysis on testosterone levels will be done after 4 patients in each group for the possibility to titrate up or down for the remaining study period. For Puregon® (rhFSH) dosing see appendix D

Group I: Individual dosing on the basis that the testosterone level should be between 2-4 nM during 8 of 12 weeks. The dose may be adjusted up or down with intervals of 50 mg.

Group II: A dose check will be made after the first 4 boys. If the testosterone levels are between 4-9 nM durin 8 of 12 weeks, the dose may be adjusted up or down with intervals of 50 mg. See appendix D

The aim for Nebido® treatment is to have morning testosterone levels in the mid or upper early pubery range (1-3 nM) for boys with absence of puberty (group I) and the lower range of mid puberty (4-9 nmol/L) for boys with delayed puberty (Group II). See appendix D

Testoviron Depot® is given once a moth intra muscular and the dose will be 50 mg in group I and 75 mg in group II (SoC; no dose adjustment).

5.1.2 Labelling

The label will include the following information:

Study code, randomisation code, name of investigational product, pharmaceutical dosage form, strength, quantity, dosage instructions, name of investigator (to be filled in at study site), expiry date, order number, storage instruction, “for clinical study use only”, “Keep out of the reach of children”, name, address and telephone number of sponsor

5.1.3 Storage

All study drugs must be kept in a secure place under appropriate storage conditions. Puregon will be stored at 2 ºC- 8 ºC. Appropriate storage conditions as specified on the investigational product label will be followed.

5.2 Concomitant medication and post-study treatment(s)

Medication, which is considered necessary for the subject’s safety and well-being, may be given at the discretion of the investigator. The administration of all medication (including investigational product) must be recorded in the appropriate sections of the case report form (CRF).

At the end of the study decision of continued treatment will be made.

5.3 Treatment compliance

No uncertainty about compliance will exist when injections (Nebido and Testoviron) are administrated under the supervision of the study nurse at the clinic.

The unbroken ampoules (Testoviron) will be assessed against the dispensed amount. Subjects will be asked to collect empty patch envelopes and ampoules and bring to study visits. Any discrepancies should be discussed with the patient and commented upon in the CRFs.

5.3.1 Accountability

It is the investigator's responsibility to establish a system for handling investigational medicinal products to ensure that it is correctly received and recorded and only dispensed to trial subjects in accordance with this protocol and manufacturer instructions.

6. STUDY MEASUREMENTS AND VARIABLES

6.1 Screening and demography

Each subject will undergo a pre-entry medical examination 5-14 days prior to the first study day. This will consist of:

- Information and signed consent
- Demographic data - date of birth, birth weight and length, current height and weight,
- Habits of nicotine
- Past and present medical including family history
- Present medications
- Pulse, systolic and diastolic blood pressure
- Physical examination including cardiovascular and respiratory systems, abdomen, general appearance, skin, head and neck, lymph nodes, thyroid and reflexes and specification of abnormalities
- Bone age
- Health-related quality of life (KIDSCREEN, JTJÄ)
- Blood sampling (20 ml) for haematology assessments and clinical chemistry (Table 4)
- Puberty stage (testicular volume and pubic hairiness)

6.1.1 Methods of assessments

According to routine standards at the hospital.

6.1.2 Post-study

After the 12 month treatment period the patient will be followed and treated according clinical routine at the site.

6.2 Bone age

To estimate the maturity of the boy's skeletal system, a single X-ray of the left wrist, hand and fingers will be undertaken. The X-ray image will be compared with X-rays images in a standard atlas of bone development of children of the same gender and age. This is a routine investigation and not an extra research investigation. The bone maturation is measured by two models BP and TW. This shows the maturation of the bone and how much the child still has to grow.

6.3 LHRH-test

The LHRH-test will be performed in the Group I subjects at visits 1, 8 and 10.

At visit 8 (after 6 months) the LHRH-test will be performed in the morning, before the first Nebido injection is given. In the Testoviron- arm, the LHRH-test will be made same day as Testoviron administration but prior to the injection itself.

At visit 10 (after 12 months) the LHRH-test will be performed same day as the Nebido/Testoviron injections but prior to the injection itself.

The LHRH-test involves injecting synthetic LHRH into a vein and measuring blood for LH and FSH levels (which, if the pituitary is functioning normally, will rise). A blood sample is taken before the injection, then 20 and 60 minutes afterwards. A cannula is placed in a vein in the arm to enable the nurse to give the LHRH and take samples. Side-effects are rare, but can include nausea, headache and flushing.

6.4 Pubertal stage

Size of the testis and hairiness will be registered at visit 1, 7, 8, 9 and 10.

6.5 Signs of masculinization

Signs of masculinization will be evaluated by the study nurse at visit 8 and 10 after 6 and 12 months of hormone replacement. Testis size, pubic and axillary hair, growth and weight gain will be evaluated.

6.6 Blood sampling

For each patient blood will be collected for safety screen (6.5.3) and for the assessment of biomarkers relevant for the objectives in the study.

Samples will be labelled individually with pre-printed labels marked with study code, subject number, media (blood, plasma), visit number and date.

6.6.1 Study-specific blood samples

Venous blood samples will be taken at all follow-up visits according to Table 1. Venous blood will be collected in EDTA tubes, heparinized tubes and plain serum tubes. Some samples will be centrifuged

as soon as possible, transferred into cryovial tubes and immediately frozen. Some samples will be analysed immediately in connection to the visit and some will be stored until later analysis.

Capillary blood will be accepted in those cases when venous blood is not possible to draw. Depending on type of analysis approximately 250-3000 µL will be needed for the various analysis.

6.6.1.1 Haemoglobin (Hb)

Whole blood in EDTA tubes will be used for analysis.

6.6.1.2 LH and FSH

Venous blood for analysis of LH and FSH will be drawn at visits 1, 2, 5, 7, 8, 9 and 10 and analyzed as soon as possible at the central laboratory at each hospital.

6.6.1.3 DHEAS

Venous blood (2.5 mL) for analysis of DHEAS will be drawn at visits 2, 8 and 10. It will be frozen and stored stored in -20° C for later analysis.

6.6.1.4 Testosteron and Estrogen

Venous blood for testosterone (2.5 mL) and estrogen (5 mL) analysis will be drawn at visits 2, 3, 4, 5, 6, 7, 8 and 10, centrifuged, frozen and sent to tillväxtlab at Drottning Silvias barn- och ungdomsjukhus; Sahlgrenska University/Östra, Göteborg and stored in -20° C för later analysis.

6.6.1.5 SHBG, AMH, InhibinB

Approximately 10 mL venous blood will be drawn at visits 2, 5, 8 and 10 and centrifuged. Serum will be used for the analysis of SHBG (2.5 mL), AMH (5 mL) and Inhibin B (2.5 mL) .

Blood for the AMH analysis will be centrifuged within 4 hours, frozen and stored in -20° C for later analysis.

6.6.1.6 Mass spectrometric analysis of steroids

Approximately 5 mL blood will be drawn at visit 1, 8 and 10 and analyzed for 16 specific steroids with a mass spectromtric method. The analysis will result in a steroid profile which will be possible to compare against clinical status and treatment outcomes.

6.6.2 Questionnaires

6.6.2.1 Standardize procedures for administration of questionnaires

The questionnaires will be filled in at the study site. The patients should be allowed to sit alone in a reasonable quiet environment to answer the questions. It will be emphasized that patients complete the questionnaires prior to clinical measurements and before meeting a doctor. Questionnaires should be answered by the patient herself alone, however, the nurse/assistant will be informed about helping the patients to complete the questionnaires if necessary, however without influencing the patients'

responses. Ensure the patient confidentiality. Study nurse/assistant should check the questionnaires for completeness. The principal investigator will assure that appropriate training relevant to the study is given.

6.6.2.2 KIDSCREEN

Kids screen for children and adolescents (8–17 years) is a generic health-related quality of life instrument for both healthy and sic children. It measures physical well-being, psychological, well-being, social support, peers and financial resources. It takes 15 minutes to fill in by the patients themselves.

6.6.2.3 Jag Tycker Jag Är

"Jag Tycker Jag Är**" (**"Jag tycker jag är" (JTJÄ) - is a measure of well-being and self-esteem with the domains of physical abilities, mental health, and relationships with others. It is available for both 6-10 year old children and 11-16 years adolescents. The JTJÄ has been widely used in clinical trials in the Västra Götaland region and in the childrens clinic at Queen Silvia Hospital/SU/Ö in particular**).**

6.6.3 Safety measurements and variables

Blood (10 ml) for laboratory safety screen (Table 2) in a fasting condition will be taken at pre-entry visit (Table 4).

Table 4 Blood and urine screen

Clinical Chemistry	***Haematology***
S-Creatinine	B-Haemoglobin (Hb)
S-Bilirubin	B-Platelet count
SP-Alkaline phosphatase (ALP)	B-Leukocytes, total count
SP-Alanine aminotransferase (ALAT)	B-Leukocytes diff.absolute count:
S -Aspartate aminotransferase (ASAT)	
S-Albumin	***Urinanalyser***
S-Potassium	U-Erythrocytes (U-Ery)
S-Calcium	U-Albumin (U-Alb)
S-Sodium	U-Glucose (U-Glu)
P-Glucose	
CRP	
P-Cholesterol total	
P-TG, HDL, LDL	

The blood samples will be analysed with routine methods at the Department of Clinical Chemistry, SU/Östra, Göteborg and NÄL, Nu-sjukvården and valid reference values of all routine analyses will be obtained. Laboratory values outside the reference limit suspected to be of any clinical significance will be repeated.

6.6.4 Volume of blood

The total volume of blood that will be drawn from each subject in this study is as follows:

Table 5 Volume of blood (ml) to be drawn from each subject

Assessment	Visit	Total
Safety	1, 7, 8, 10	40
LH, FSH	1, 2, 5, 7, 8, 9, 10	35
Testosteron, estradiol	2, 3, 4, 5, 6, 7, 8, 10	60
DHEAS	1, 8, 10	10
SHBG, AMH, inhibin B	2, 5, 8, 10	40
Total		185

Approximately 185 mL blood will be drawn from each subject during the entire study. Some of the blood will be immediately analysed and some of the samples will be frozen for later analyses unidentified at Tillväxtcentrum Göteborg.

6.7 Handling, storage and destruction of biological samples

The safety blood samples at visit 1 will be used up or disposed after analyses.

Study-specific blood samples will be analysed during and after finalized study. These samples will be reported to the Sahlgrenska Univestity Hospital/Ö primary biobank and stored for a maximum time period of 15 years and used only as objected in this study protocol. Samples are coded and code keys will be kept by the care provider. The origin can be traced to the human from whom it was taken.

6.7.1 Withdrawal of informed consent for biological samples

If a subject withdraws consent to the use of biological samples donated the samples will be disposed/destroyed, if not already analysed and documented and the subject is withdrawn from further study participation.

The principle investigator ensures the central laboratory holding the samples is informed about the withdrawn consent immediately and that samples are disposed/destructed and the action documented returned to the study site.

7. SAFETY

It is of the utmost importance that the principal investigator and all staff involved in the study are familiar with the content of this section. The principle investigator is responsible for safety surveillance and for ensuring that procedures and expertise are available to handle medical emergencies during the study. Further details on definitions is described in Appendix B.

In the case of a medical emergency the investigator may contact **Dr. Martin Österbrand** martin.osterbrand@vgregion.se or at 010-4351836 or 070-2833177.

Physical examination, vital signs and safety blood screen will be made by the study physician at study start in order to document the health status of the subjects.

Adverse Events in the present study will be collected and registered from the time of signed informed consent throughout the treatment period including the follow-up period.

7.1.1 Definition of adverse events

An adverse event AE) is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms, signs or abnormal results of an investigation.

In clinical studies, an AE can occur at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs.

7.1.2 Definitions of serious adverse event

Serious AEs (SAEs) and discontinuations due to AEs must be collected and an assessment of causality of the SAE should be performed. An SAE is an experience that at any dose, during any study phase results in any of the following:

- Death
- A life-threatening experience
- In-patient hospitalization or prolongation of existing hospitalisation
- A persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

The following terms and definitions are used when assessing the causal relationship between each AE/SAE and the relevant trial product(s):

- Definite – There is no doubt that the incident is related
- Probably- Good reason and sufficient documentation to assume a causal relationship
- Possible- A causal relationship is conceivable and cannot be dismissed
- Unlikely- The event is most likely related to aetiology other than the trial product
- Unknown/Unclassifiable: a report suggesting an adverse event reaction, which cannot be judged because information is insufficient or contradictory and which cannot be supplemented of verified.

A **Suspected Unexpected Serious Adverse Reaction (SUSAR)** is a serious adverse reaction in subjects given a drug, which may or may not be dose related, but are unexpected, as they are not consistent with current safety information.

For further guidance on the definition of a SAE, **see Appendix B**.

7.1.3 Intensity rating

It is important to distinguish between serious and *severe* AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 0. An AE of severe intensity need not necessarily be considered serious. The following terms are used for definitions for severity/intensity rating:

- None – No symptoms
- Mild - Transient symptoms; awareness of sign or symptom, but easily tolerated. No interference with the subject's daily activities
- Moderate - Marked symptoms; discomfort sufficient to cause interference with normal activities. Moderate interference with the subject's daily activities
- Severe - Considerable interference with the subject's daily activities; unacceptable, incapacitating. Inability to perform normal activities
- N/A Not Applicable

7.1.4 Reporting of serious adverse events (SAEs and SUSARs)

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure. All SAEs will be recorded on separate AE pages in the CRF. The SAE Data Form and fax confirmation sheet must be processed in accordance with guidelines on retention of records at the site.

The Ethics Committees and Regulatory Authorities must be informed by the sponsor; in this study the principle investigator, as soon as possible or within **7 days**. A supplemented detailed report must be sent within another **8 days**. The SAE Report Form will be used together with relevant supporting documentation (eg, ECG, laboratory results, autopsy report) and relevant CRF modules. The sponsor shall ensure that a SUSAR which is not fatal or life-threatening is reported within **15 days**.

All SUSARs have to be electronically registered in the EMEAs database. For the current study, the sponsor/principle investigator will delegate registration of SUSARs in the EudraVigilance database to the Swedish Medical Products Agency (Läkemedlesverket). The CIOMS form will be used and sent electronically to registrator@mpa.se.

The current reference safety information contained in FASS and homepage (Testoviron) will apply. All SAEs will be recorded in both subject hospital record and in the CRF.

Yearly safety summary reports including notifications of SAEs and SUSARs will be provided to the Ethics Committee and the MPA according to local regulations and guidelines

Follow-up of unresolved adverse events

Any AEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator for as long as medically indicated, but without further recording in the CRF.

7.1.5 Safety committee

Safety committee will not be used in this study.

8. STATISTICAL METHODS AND SAMPLE SIZE

The aim of this hypothesis-generating study is to evaluate the development of puberty in young males with a delayed progress or absent puberty after various testosterone supplementations with or without rhFSH. The primary endpoint of this study is pubertal LH responses on GnRH test at 12 months (group I) and testis size 8 mL or more for young males with a delayed progress puberty (group II). LH ≥1 ug/mL is clinically relevant sign that puberty has started and 8mL in testis size relevant for the clinical signs of puberty (growth spurt). The primary endpoint is in both groups a dichotomy response (0/1 yes or no).

The sample size is not formally calculated with consideration to statistical power since Testoviron treatment is SoC today and has not been evaluated.

Since one of the aims in the study is to know if Testoviron treatment can be replaced by Puregon/Nebido in boys with ascents of puberty and by Nebido in boys with delay and slow progression of puberty a bioequivalence analysis and non-inferior interpretation will be performed on primary end points.

Bioequivalence analysis and Non-inferior interpretation: The new treatments will be clinically considered similar if the treatments are in 80 -125 % of the result of Testoviron in each group. Since the result on the primary endpoint cannot be expressed with confidence interval (the result on the primary end point is yes or no), the statistical analysis of the results will be done with Fisher exact chi-square test. Furthermore, accepting a criterion that the difference of numbers of boys becoming pubertal being at the most 25%, and further assuming, that 80% (p2, n=16) boys on the old drug, Testoviron Depot®, will become pubertal: using the normal approximation when calculating the lower 95% level for the difference between p1 (% success for boys on Nebido®) and p2 (80%), the number of boys becoming pubertal on Nebido® must exceed 15." (see Appendix E).

For young males with absence or delayed progress of puberty, the number of subjects (n=30 in Group I and n=40 in Group II) in a parallel-group designed study is considered sufficient for judgments on the primary hypothesis due to clinical experience of treating the present study population with hormone therapy. However, to not expose boys for a new treatment (Nebido and Puregon in group I and Nebido in group II) that is inferior to SoC, a futility test will be performed after 10 evaluable patients in each arm in the groups. This futility analysis can result in stopping the study if the new treatment (Nebido and Puregon in group I and Nebido in group II) is statistically significant inferior or superior, or

continue the study as planed or increase the numbers patients to have a possibility to reach statistical significance.

The primary objective will be studied with non parametric methods Fisher exact chi-square test,

Descriptive statistics, parametric and non parametric tests will be used for the secondary objectives.

Other statistical methods e.g. regression modelling might be used in an exploratory fashion but no formal interference will be made.

Demographics, safety and self-assessed outcomes will be presented by means of descriptive statistics.

8.1.1 Evaluation and calculations of variables

In Group I: For the boys with absent puberty we need to follow the testosterone values to evaluate the doses given. We also need to follow estrogen since testosterone is converted into estrogen and then terminate the growth spurt. This will give us new information on how to better induce puberty

9. DATA MANAGEMENT

9.1 Recording of data

Paper case record forms (CRF), will be used to record all data. Data entry, editing and analyses will be done by the responsible investigators and study nurse(s). The investigators will ensure that all data collected in the study will be provided. He/she ensures the accuracy, completeness, legibility and timeliness of the data recorded in the appropriate sections of the CRF and according to any instructions provided.

Clean File/database lock must be documented. The reason for any excluded data or protocol deviations will be described in the study report. Any changes in the database after Clean File/database lock must be documented.

Data will thereafter be entered into an Excel datasheet and analysed with S.P.S.S.19 statistical software.

CRF documentation and other source data will be retained for at least 10 years after finalization of the clinical study.

9.1.1 Source data

All patient source data such as analysis results from the hospital laboratories and other measurements made will be stored at the hospital according to routines.

The CRFs serves as the source for demographic data, medical history and physical examination and measurements. Original scorings of patient-reported outcomes (x) and xx analysis reports will be defined as source data.

9.2 Training of study site personnel

The local investigator at each site will ensure that appropriate training relevant to the study is given to all of these staff and that any new information relevant to the performance of this study is forwarded to the staff involved. All staff has undergone GCP training.

9.3 Monitoring of the study

An independent study nurse will be appointed for monitoring the study. The monitor will be appropriately trained and informed about the nature of the study, patient written information, GCP and applicable regulatory requirements. Monitor's qualifications will be documented.

The monitor will have regular contacts with the study sites to verify informed consents of participating subjects, to confirm that facilities remain acceptable, that the investigational team is adhering to the protocol that data are being accurately recorded in the Case Report Forms (CRF) and that therapy accountability is being carried out. The study nurse will also ensure source data verification (comparison of the data in the CRF with the hospital/practice and other records at the investigational site).

9.4 Study agreements

All agreements between the Principal Investigator and laboratory or other technical facilities in which the measurement or assessment of the study evaluation criteria are performed must be in place before any study-related procedures can take place, or subjects be enrolled.

9.5 Changes to the protocol and informed consent form

If there are any substantial changes to the study protocol, then these changes will be documented in a study protocol amendment anywhere required a new version of the study protocol (Amended Protocol).

The amendment must be approved by the Ethics Committee and if applicable, also the national regulatory authority, before implementation.

If a protocol amendment requires a change to the Informed Consent Form, the Ethics Committee must approve the revised Informed Consent Form before the revised form is used.

9.6 Insurances

Like in the general Swedish health care, the study subjects are covered by the Patient insurance and pharmaceutical insurance.

9.7 Rapport and publications

A summary report of study results will be sent to the Ethics Committee and the MPA within one year after entire study is finalized.

Results from the study may be published in national and international scientific journals.

10. STUDY TIMETABLE

First Subject In: March 2013. Last Subject Last Visit: December 2015 (group II) and December 2022 (group I)

Final Study Report: December 2016 or within 12 months from end of study.

10.1 Definition of end of study

The end of the entire study is defined as"the last visit of the last subject undergoing the trial".

11. LIST OF REFERENCES

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Study Protocol Appendix A

Study Code: Pub-01 version 3

EudraCT no. 2012-002337-11

Date: 2013-01-30

Appendix A – Signatures

INVESTIGATORS SIGNATURES

I agree to the terms of this study protocol, including all appendices:

Dr. Ensio Norjavaara, Sahlgrenska UniversityHospital SU/Ö, Göteborg	Date (Day Month Year)
Dr. Österbrand, NÄL, NU-sjukvården	Date (Day Month Year)
Dr Hans Fors, NÄL, NU-sjukvården	Date (Day Month Year)

Study protocol Appendix B

Definitions of Adverse Events and Procedures in case of Pregnancy

Definitions:

Adverse Events

Serious Adverse Events

A medical emergency usually constitutes an SAE and is to be reported as such

Intensity rating

Causal relationship

Action taken

Reporting in CRF

Adverse Events based on signs and symptoms

Final outcome assessment

Reporting of serious adverse events

Further guidance on Serious Adverse Events:

Life threatening

Hospitalisation

Important medical event or medical intervention

A guide to interpreting the causality QUESTION

Other significant Adverse Events

Procedures in case of pregnancy

Maternal exposure

Overdose

DEFINITIONS OF ADVERSE EVENTS AND PROCEDURES IN CASE OF PREGNANCY

It is of the utmost importance that all staff involved in the study is familiar with the content of this section. The Principal Investigator is responsible for ensuring this.

Adverse Events

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs

Serious Adverse Events and suspected unexpected serious adverse reactions (SUSAR)

It is important to distinguish between Serious Adverse Events (SAEs) and severe adverse events (AEs). Severity is a measure of intensity whereas seriousness is defined by the criteria listed below. An AE of severe intensity need not necessarily be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but not an SAE. On the other hand, a stroke which results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

A medical emergency usually constitutes an SAE and is to be reported as such

A serious adverse event is an AE occurring during any study phase (i.e. run-in, pre-entry, screening, treatment, wash-out, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfils one or more of the following criteria:

- results in death
- is immediately life-threatening
- requires in-patient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital abnormality or birth defect

- is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

For reporting purposes, any suspected transmission via a medicinal product of an infectious agent is also considered an SAE and is reported in an expedited manner. Any organism, virus or infectious particle (for example prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent.

Definitions for severity rating

0. None – No symptoms

1. Mild - Transient symptoms; awareness of sign or symptom, but easily tolerated. No interference with the subject's daily activities

2. Moderate - Marked symptoms; discomfort sufficient to cause interference with normal activities. Moderate interference with the subject's daily activities

3. Severe - Considerable interference with the subject's daily activities; unacceptable, incapacitating. Inability to perform normal activities

N/A Not Applicable

Causal relationship

The causality of (S)AEs (ie, their relationship to study treatment and/or the investigational procedure) will be assessed by the investigator(s), who in completing the relevant case report form must answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the drug/the investigational procedure?"

The following terms and definitions are used when assessing the causal relationship between each AE and the relevant trial product(s):

1. Definite - There is no doubt that the incident is related
2. Probable - Good reason and sufficient documentation to assume a causal relationship
3. Possible- A causal relationship is conceivable and cannot be dismissed
4. Unlikely - The event is most likely related to aetiology other than the trial product
5. Not related – The event is not related to the trail product
6. Unknown/Unclassifiable: a report suggesting an adverse event reaction, which cannot be judged because information is insufficient or contradictory and which cannot be supplemented or verified.

N/A Not Applicable

Causal relationship in cases where the disease under study has deteriorated due to lack of effect will be classified as no reasonable possibility.
For SAEs causal relationship will also be assessed for additional study drug and/or other medication and/or study procedure. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as "yes".

Action taken:

0 None
1 Dose of study drug changed
2 Study drug temporarily stopped
3 Study drug stopped
N/A Not Applicable

Reporting in the Case report Form

The following variables will be recorded in the CRF for each AE; description of the AE, the date and time when the AE started and stopped, maximum intensity, whether the AE is serious or not, causality rating (yes or no; if yes specify), action taken with regard to investigational product, AE caused subject to discontinue study and outcome.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the subject or reported in response to the open question from the study personnel: *"Have you had any health problems since the previous visit?"*, or revealed by observation will be collected and recorded in the CRF. When collecting AEs the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Follow-up – Outcome assessment

Any AEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator for as long as medically indicated, but without further recording in the CRF.

The following terms and definitions are used in assessing the final outcome of an AE:

- Recovered - The subject has fully recovered, or by medical or surgical treatment the condition has returned to the level observed at the first trial-related activity after the subject signed the informed consent.

- Recovering - This term is only applicable if the subject has completed the trial or has died from another AE. The condition is improving and the subject is expected to recover from the event.

- Recovered with sequelae - The subject has recovered from the condition, but with lasting effect due to a disease, injury, treatment or procedure. If a sequela meets an SAE criterion, the AE must be reported as an SAE.

- Not recovered - The condition of the subject has not improved and the symptoms are unchanged, or the outcome is not known at the time of reporting.

- Fatal - This term is only applicable if the subject died from a condition related to the reported AE. Outcomes of other reported AEs in a subject before he/she died should be assessed as “recovered”, “recovering”, “recovered with sequelae” or “not recovered”. An AE with fatal outcome must be reported as an SAE.

- Unknown - This term is only applicable if the subject is lost to follow-up

Reporting of serious adverse events

For studies in countries implementing the EU Clinical Trials Directive, informing Ethics Committees and Regulatory Authorities will be performed by the sponsor.

All Suspected Unexpected Serious Adverse Reactions (SUSARs) have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). The Clinical Study Serious Adverse Event Report Form will be used together with other relevant supporting documentation (e.g. ECG, laboratory results, autopsy report) and relevant CRF modules. All SUSARs have to be electronically registered in the EMEAs database.

FURTHER GUIDELINES ON THE DEFINITION OF A SERIOUS ADVERSE EVENT

Life threatening

‘Life-threatening’ means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject’s death. ‘Life-threatening’ does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Out-patient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease

existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life-threatening or result in death, hospitalisation, disability or incapacity but may jeopardize the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Examples of such events are:

- Angioedema not severe enough to require intubation but requiring iv hydrocortisone treatment

- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine

- Intensive treatment in an emergency room or at home for allergic bronchospasm

- Blood dyscrasias (eg, neutropenia or anaemia requiring blood transfusion, etc) or convulsions that do not result in hospitalisation

- Development of drug dependency or drug abuse

A guide to interpreting the causality QUESTION
The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR could the AE be anticipated from its pharmacological properties?

- Dechallenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?

- No alternative cause. The AE cannot be reasonably explained by aetiology such as the underlying disease, other drugs, other host or environmental factors.

- Rechallenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? A re-challenge would not normally be recommended or supported.

- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship?

A "reasonable possibility" could be considered to exist for an AE where one or more of these factors exist.

In contrast, there would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course but any dechallenge (if performed) is negative or ambiguous or there is another more likely cause of the AE.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?
- Is there a known mechanism?

Ambiguous cases should be considered as being a "reasonable possibility" of a causal relationship unless further evidence becomes available to refute this. Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

Other significant Adverse Events

An expert will identify other significant Adverse Events (OAEs) during the evaluation of safety data for the Clinical Study Report. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment, will be classified as OAEs. Examples of these are marked haematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment. For each OAE, a narrative will be written and included in the Clinical Study Report.

Procedures in case of pregnancy

Pregnancy itself is not regarded as an adverse event unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of a contraceptive medication. The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even if the subject was discontinued from the study. All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and

handled as SAEs. Elective abortions without complications should not be handled as AEs. All outcomes of pregnancy must be reported to PI on the pregnancy outcomes report form.

Part I of this form must be completed in full and returned to PI within 30 days. Part II of the form must be completed when the outcome of the pregnancy is known. Reports of normal outcomes should be sent within 30 days.

Overdose

- An overdose with associated AEs is recorded as the AE diagnosis/symptoms on the relevant AE modules in the CRF.

Study protocol Appendix C – Ethics and regulatory review

The final study protocol, including the final version of the Written Informed Consent Form and other information given to subjects eg, advertisements must be approved or given a favourable opinion by an Ethics Committee before enrolment of any subject into the study.

Before enrolment of any subject into the study, the final study protocol, including the final version of the Informed Consent Form, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

It is the responsibility of the Principal Investigator to apply to the Ethics Committee in writing. The application document should:

- contain the name and address of the Ethics Committee
- clearly identify, by title and date, the protocol and other documents submitted for review
- be dated.

In addition, the Principal Investigator should request the Ethics Committee to provide:

- their approval/opinion in a dated document identifying the Principal Investigator's application
- Ethics Committee composition for the meeting when the approval was given
- a statement confirming that the Ethics Committee is organised and operates according to GCP and applicable laws and regulations.

The Principal Investigator is responsible for informing the Ethics Committee of any modifications and amendments to the protocol as per local requirements.

Progress reports and notifications of serious and unexpected adverse drug reactions will be provided to the Ethics Committee according to local regulations and guidelines

All correspondence with the Ethics Committee should be filed by the Principal Investigator in the ISF.

The Principle Investigator is also responsible for obtaining approvals from scientific bodies (eg, to use radiolabelled substances) if necessary for the study.

SUBJECT INFORMATION AND WRITTEN INFORMED CONSENT FORM

The Principal Investigator(s) at each centre will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study.

The Principal Investigator(s) must store the original, signed Informed Consent Form in the Investigator's Study File. A copy of the signed Informed Consent Form must be given to the subject.

If a protocol amendment requires a change to the Informed Consent Form, the Ethics Committee must approve modifications that lead to a revised Informed Consent Form before the revised form is used.

SUBJECT DATA PROTECTION

The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

Extra precautions are taken to preserve confidentiality and prevent genetic or other study data being linked to the identity of the subject. In exceptional circumstances, however, certain individuals might see both the genetic, other study data and the personal identifiers of a subject. For example, in the case of a medical emergency or an investigator might know a subject's identity and also have access to his or her data. Also Regulatory authorities may require access to the relevant files, though the subject's medical information and the genetic files would remain physically separate

AUDITS AND INSPECTIONS

Authorized representatives of Sponsor, a regulatory authority, or an Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements.

Study protocol Appendix D - Dose adjustment of Puragon and Nebido

I) Boys with absence of puberty

a. The rhFSH (Puregon®) dose will be adjusted to reach serum FSH levels of 3.0 IU/mL or higher. The starting dose will be 50 IU. The FSH levels will be determined on visit 4. If serum FSH is lower than 3.0 IU/L the dose will be increased to 75 IU. If serum FSH are higher than 5.0 IU/L the dose will be reduced to 30 IU. Patients who have changed the Puregon® to 30IU or 75IU, an extra visit 7 -10 days after the change will be done to determine the serum FSH levels. For patients on 75IU Puregon® if serum FSH under 3.0 IU/L the Puregon® dose will be increased to 100IU and if serum FSH are higher than 5.0 the dose will be reduced to 50IU. For patients on 30IU Puregon® if serum FSH under 3.0 IU/L the Puregon® dose will be increased to 50IU and if serum FSH are higher than 5.0 the treatment with Puregon® will be stopped. If serum FSH in these patients are not 3.0 IU/mL or higher at visit 5, the patient will be taken out from the study.
b. Dose adjustments of starting dos of Puregon® is possible after the first 5 patients for the rest of the study. If the serum FSH levels are less than 3.0 IU/L at visit 4 in 3 or more of 5 patient, the starting dose will be increased to 75IU for the remaining patients randomized to Puregon® and Nebido®.
c. Boys who have been randomized to Puregon®)/Nebido but at inclusion have serum levels of FSH above 3 will not be treated with Puregon®, but if the patient during the study on 2 concomitant visits have serum FSH below 3.0 IU/L will be treated with (Puregon®) as described above
d. The Nebido treatment will be dose adjusted in patients after the first injection if the testosterone profile shows to high or low levels, the goal is to have the similar levels during 2 months as in early puberty (1-3 nM). If the serum testosterone level are less than 1.0 nM at visit 6, the dose will be increased to 250mg for the remaining part of the study. If the serum testosterone level is higher than 3.0 nM at visit 6, the dose will be decreased to 150mg.

II Boys with delayed/slow progress of puberty

a. Dose adjustments of Nebido® is possible after the first 5 patients on Nebido® (250 mg) for the rest of the study. The aim for the Nebido® treatment is to have testosterone levels in the lower range of mid puberty (4-9 nmol/L) during 2 months. An interim analysis will be done after the first 5 patients and thereafter keep or adjust the dose for the remaining study. If the serum testosterone level are less than 4.0 nM at visit 6 in 3 or more of 5 patient, the dose will be increased

to 300 mg for the remaining patients randomized to Nebido®. If the serum testosterone level are higher than 9.0 nM at visit Y in 2 or more in 5 patient, the dose will be decreased to 200 mg for the remaining patients randomized to Nebido®.

Study protocol Appendix E - Statistiska beräkningar

p2	nOK, gl drog	nOK, ny drog	p1	p1-p2	p1*(1-p1)/n1	p2*(1-p2)/20	under rot	rot
0,8	16	17	0,85	0,05	0,006375	0,008	0,014375	0,119896
0,8	16	16	0,8	0	0,008	0,008	0,016	0,126491
0,8	16	15	0,75	-0,05	0,009375	0,008	0,017375	0,131814
0,8	16	14	0,7	-0,1	0,0105	0,008	0,0185	0,136015
0,8	16	13	0,65	-0,15	0,011375	0,008	0,019375	0,139194
0,8	16	12	0,6	-0,2	0,012	0,008	0,02	0,141421

p2 %"lyckade" i testoviron gruppen
p1 %"lyckade" i grupp med ny behandling

16 pojkar måste "lyckas" i den nya behandlingen, dvs lika många som antas "lyckas" efter den gamla behandlingen för inte den nya behandlingen ska betraktas som inferior, dvs 25% färre lyckade med nya drogen