PRELIMINARY STUDY ON ACTIVATION OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS IN INFANCY (MINIPUBERTY) AND ITS EFFECTS ON CATCH-UP GROWTH AND NEUROCOGNITIVE OUTCOME IN MALE PRETERM NEONATES BORN AT <32 WEEKS OF GESTATIONAL AGE

Running title:	**Minipuberty and its effects on preterm neonates**
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This study will be performed according to the Research Governance Framework for Health and Community Care (Second edition, 2006) and WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI Ethical Principles for Medical Research Involving Human Subjects 1964 (as amended).

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STUDY SYNOPSIS

Title of Study:	Preliminary study on activation of the hypothalamic-pituitary-gonadal axis in infancy (minipuberty) and its effects on catch-up growth and neurocognitive outcome in male preterm neonates born at <32 weeks of gestational age
Study Centre:	Royal Hospital for Children, Glasgow
Duration of Study:	18 months
Primary Objective:	- To describe when urinary hormones (follicle stimulating hormone and luteinising hormone) start to raise and when they reach their peak among male preterm neonates born with a gestational age <32 weeks. - To investigate if there is a difference in hormone levels and their peak timing between extremely preterm babies (<28 weeks of gestational age) and very preterm babies (28-32 weeks of gestational age).
Secondary Objective:	- Identify a link between hormone levels and linear growth in the first months of life in preterm neonates. - Identify a link between hormone levels and the development of external genitalia (testicular volume and position, penile length). - Identify a link between hormone levels and short-term neurocognitive development.
Rationale:	The knowledge about the activation of the hypothalamic-pituitary-gonadal axis (HPG) in the first months of life is relatively recent. It is scientifically proven that in this period, called "minipuberty", there is the same activation that occurs during puberty. This is responsible for changes in terms of growth, pubertal development and neurobehavioral patterns. Understanding all the factors involved in children's physical and neurocognitive development has a significant scientific impact and could provide useful information in case of future problems. As prematurity survival rates continue to improve, clinicians need to be familiar with the hormonal changes in these patients and the first months of life provide a 'window of opportunity' for functional studies of the HPG axis prior to pubertal development
Methodology:	This is a preliminary prospective and crossectional study.
Sample Size:	20 male preterm neonates with a gestational age ≤32 weeks
Main Inclusion Criteria:	• Male preterm infants born ≤32 weeks of gestational age • Written informed consent provided
Main Exclusion Criteria:	• Female sex assignment at birth • Male preterm infants born >32 weeks of gestational age • Male infants born at term
Statistical Analysis:	Results shall be expressed as median (range) and analysed using Minitab. Some data may require log transformation. Pearson's correlation shall be used for univariate analysis of association of and multivariate analysis shall be performed using backward stepwise linear regression analysis including factors significant from univariate analysis (Pearson's

	correlation) as independent factors. The Mann Whitney U test shall be used for comparison of nonparametric data. Statistical significance shall be set at $p < 0.05$.

Full title: PRELIMINARY STUDY ON ACTIVATION OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS IN INFANCY (MINIPUBERTY) AND ITS EFFECTS ON CATCH-UP GROWTH AND NEUROCOGNITIVE OUTCOME IN MALE PRETERM NEONATES BORN AT <32 WEEKS OF GESTATIONAL AGE

Short title: Minipuberty and its effects on preterm neonates

1. Introduction

The activation of the hypothalamic-pituitary-gonadal axis (HPG) develops through three main moments: foetal life, first postnatal months (usually first 6 months of life) and finally during puberty.[1] During the foetal period, there is a peak of gonadotropin secretion starting from the second trimester of gestation, and a subsequent suppression in the last weeks until birth, due to the negative feedback of placental estrogens.[2] In male foetuses, LH levels exceed those of FSH. The foetal testis secretes testosterone and anti-müllerian hormone (AMH) from the 8th week of gestation and this is essential for masculinization. Formation of the active metabolite of testosterone, dihydrotestosterone, is required for the development of the prostate, penis and scrotum.[3] Initial testicular development is intra-abdominal and the descent of the testes into the scrotum occurs in two phases. The first, transabdominal, phase is completed by 15 weeks of gestation. The second, inguinoscrotal, phase is usually completed by the end of the 35th week of gestation and this phase is androgen dependent. Testosterone levels are high in male foetuses between 10 and 20 weeks of gestational age, reaching adult values, and decrease thereafter towards term.[4] In the same way LH and FSH levels decrease towards the end of gestation and are low at term in both sexes, due to the negative feedback of placental oestrogens.

A second post-natal HPG activation, also called "minipuberty", occurs at around 1 week of age, when placental hormones are cleared from the circulation. From the first weeks of life onwards there is a progressive increase in gonadotropins levels with a greater increase of FSH in females and of LH in males. During the "minipuberty" period gonadotropins levels have a peak at 3 months of life and a progressive exhaustion at around 6 months, except for FSH levels in females that can remain high even up to 3 or 4 years. Particularly in males, testosterone and androgen levels are associated with development and maturation of the reproductive system (penis and testis) as well as the androgynous cutaneous manifestations and a different neurobehavioral structure.[5] Postnatal testosterone levels have been associated with male-type behaviour in 14-month-old infants, suggesting a role in neurobehavioral development.[6] Recently, it has also been suggested how the increasing testosterone level during the first 6 months of life, as well as during puberty, translates into an increase of linear growth, regardless of the levels of growth hormone (GH) or insulin-like growth factor (IGF1).[7]

Preterm birth does not seem to influence post-natal HPG activation, since that gonadotropin levels seem to raise up at the same time as term infants after the birth. The difference is that in preterm neonates these values last higher and longer than full-term newborns. According to the most recent longitudinal data, the post-natal activity of this axis declines at about the same in term neonates compared to premature infants with the same corrected gestational age, suggesting that the HPG activity is evolutionarily regulated.[8,9] This higher increase of gonadotropin levels in premature males has been associated with a faster penile and testicular growth after birth compared to full-term boys. At last, in neonates small for gestational age (SGA) there are some evidence that post-natal FSH and testosterone levels are higher compared to infants with an adequate weight (AGA), although it is not known

whether this correlates or have any influence on the catch-up growth of this subjects.[10,11] Understanding how "minipuberty" can influence linear growth, genitalia development and neurocognitive processes in preterm neonates has a significant scientific impact and could provide us more information in understanding auxological and neurobehavioral developmental patterns of this population.

2. Aim/Primary and Secondary Objectives

This is a preliminary prospective and crosssectional study aiming to describe "minipuberty" in male preterm children at the Royal Hospital for Children in Glasgow. The research project has been discussed by the Developmental Endocrinology Research Group at the University of Glasgow.

- **Primary Endpoint**

- To describe when urinary hormones (follicle stimulating hormone and luteinising hormone) start to raise and when they reach their peak among male preterm neonates born with a gestational age <32 weeks.
- To investigate if there is a difference in hormone levels and their peak timing between extremely preterm babies (<28 weeks of gestational age) and very preterm babies (28-32 weeks of gestational age).

- **Secondary endpoints**

- Identify a link between hormone levels and linear growth in the first months of life in preterm neonates.
- Identify a link between hormone levels and the development of external genitalia (testicular volume and position, penile length).
- Identify a link between hormone levels and short-term neurocognitive development.

3. Study Design

This is a preliminary prospective and crosssectional study on male preterm children at the Royal Hospital for Children in Glasgow. The study will not include any investigational medical product. The research project has been discussed by the Developmental Endocrinology Research Group at the University of Glasgow.

This study will be performed according to the Research Governance Framework for Health and Community Care (Second edition, 2006).

3.1 Study Population

We aim to enroll 20 neonates born under 32 weeks of gestational age at the Royal Hospital for Children in Glasgow. Neonates will be enrolled during the first 3 days of life with written informed consent signed by their parents.

3.2 Inclusion criteria

- Male preterm infants born ≤32 weeks of gestational age
- Written informed consent provided

3.3 Exclusion criteria

- Female sex assignment at birth
- Male preterm infants born >32 weeks of gestational age
- Male infants born at term

3.4 Identification of participants and consent

The family of a newborn eligible for this study will be approached in the neonatal unit after the birth. Information material about the study shall be provided with an initial private discussion and we will make the family aware that participation is voluntary and they can leave the study at any time without their baby's standard care being affected. We will ask for contact details during this first meeting in order to contact them later checking whether they are interested in participating and start with the study. Consent will be taken in the Neonatal Intensive Care Unit in a private counselling room by the research team (Dr Martina Rodie or Dr Alessandra Boncompagni or Dr Andrew Brunton). Parents will be given a minimum of 24 hours to consider participation and they could take a decision until 72 hours of newborn's life. Neonates of parents who will provide written informed consent will be enrolled in this study.

3.5 Withdrawal of subjects

Participants have the right to withdraw from the study at any point for any reason.

4 Study Outcome Measures

Our study will include 3 types of different evaluations on each neonate:

- Multiple urine collections to evaluate urinary gonadotrophin levels
- Multiple clinical evaluations of auxological parameters and external genitalia
- Neurocognitive assessment at 3 months of corrected gestational age

Timing of sample analysis and simultaneously clinical evaluation will be as follow:

T1: 1-3 days of life
T2: 7 days of life
T2a – T2z: one per week until 40 weeks of post-conceptional age or until discharge from Neonatal Unit
T3-TX: urinary sample analysis and clinical evaluation during the routine follow up clinics until 12 months of corrected gestational age

4.1 Primary Outcome Measure

Urine collection will be done with a plastic bag with a sticky strip on one end, made to fit over baby's genital area. In extremely preterm babies (<28 weeks of gestational age) urine collection will be from cotton wool in the nappy because of their sensitive skin.
Samples will be analyzed on fresh urine for urinary gonadotropin levels (FSH, LH) as detailed in laboratory tests section.

4.2. Secondary Outcome Measure

- Auxological parameters for each evaluation will include: length, weight, head circumference. They are routinely checked both during the baby's hospital stay and the routine follow-up clinics therefore we will not add any to the standard baby's care.
- External genitalia evaluation will include: testicular volume and position, penile length. This is not a part of the routine monitoring of preterm babies but it is not invasive and it will be done at the same time of plastic bag's positioning for urine collection in order to minimize handling of the baby.
- Short term neurocognitive outcome: during the hospital stay in the Neonatal Unit parents will be asked if they agree with video recording their baby using their mobile phone at home after discharge. We would like to assess the Fidgety Movements at 3 months of corrected gestational age using the Prechtl's method[12,13]. If the parents agree, we will give an instruction sheet before they go home briefly explaining how they have to record their baby and for how long. Fidgety Movements are a spontaneous pattern of movements which parents can record without the need for a healthcare professional. Parents will upload their videos onto a secure video messaging system (www.vcreate.tv) which already has governance approval in GGC and is endorsed by the NHS. Laura Lucaccioni is an Italian researcher at the University Hospital of Modena (Italy). She is a highly trained expert of Prechtl's Method and a member of the General Movements Trust. With an expert team she will review all the videos giving an evaluation of the Fidgety Movements pattern. We will

store the videos for a maximum of 10 years and will delete them after this time.

4.3 Laboratory Tests

Measurement of LH and FSH urinary levels will be made on fresh samples using electro-chemiluminescence immunoassay (ECLIA). Sample analysis will be performed by the Biochemistry Department of the Royal Hospital for Children in Glasgow using Abbott Architect i1600 instrument.

5. Assessment of Safety

We do not expect particular problems in this study. Urine collection will be made in the same way of routine collection in infants. Auxological evaluations are part of the routine care of preterm neonates and we will add a non-invasive evaluation of external genitalia without particular risks for the babies. Neurobehavioural development will be evaluated using parents' video only if they agree with video recording their baby. This will allow parents to stay at home without bringing their baby to the hospital.

6. Statistics and Data Analysis

Results shall be expressed as median (range) and analysed using Minitab. Some data may require log transformation. Pearson's correlation shall be used for univariate analysis of association of and multivariate analysis shall be performed using backward stepwise linear regression analysis including factors significant from univariate analysis (Pearson's correlation) as independent factors. The Mann Whitney U test shall be used for comparison of nonparametric data. Statistical significance shall be set at $p < 0.05$.

7. STUDY CLOSURE / DEFINITION OF END OF TRIAL

Planned start date: February 2019
Planned end date: August 2020
Total duration: 18 months
The enrolment is expected to last 6 months starting from the approval.
The study will be finished at the last visit (12 months of corrected gestational age) of the last newborn enrolled.

8. Ethical Consideration

The study will be carried out in accordance with the World Medical Association Declaration of Helsinki (1964) and its revisions (Tokyo [1975], Venice [1983], Hong Kong [1989], South Africa [1996] and Edinburgh [2000]).
Favourable ethical opinion will be sought from an appropriate REC before patients are entered into this clinical trial. Patients will only be allowed to enter the study once their

parents have provided written informed consent. The CI will be responsible for updating the Ethics committee of any new information related to the study.

9. Finance and Indemnity

This study is sponsored by NHS Greater Glasgow & Clyde. The sponsor will be liable for negligent harm caused by the design of the trial. NHS indemnity is provided under the Clinical Negligence and Other Risks Indemnity Scheme (CNORIS).

11. Publications

We may report and disseminate the results of our study through peer reviewed scientific journals, internal report and conference presentation.

12. References

1. Kuiri-Hänninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. Horm Res Paediatr. 2014;82(2):73-80
2. Reyes F, Winter JS, Faiman C. Studies on human sexual development. Fetal gonadal and adrenal sex steroids. J Clin Endocrinol Metab 1973;37:74–78.
3. Debieve F, Beerlandt S, Hubinont C, Thomas K. Gonadotropins, prolactin, inhibin A, in-hibin B, and activin A in human fetal serum from midpregnancy and term pregnancy. J Clin Endocrinol Metab 2000;85:270–274.
4. Takagi S, Yoshida T, Tsubata K, Ozaki H, Fujii TK, Nomura Y, Sawada M. Sex differences in fetal gonadotropins and androgens. J Steroid Biochem 1977;8:609–620.
5. Winter JS, Faiman C, Hobson WC, Prasad AV, Reyes FI. Pituitary-gonadal relations in infancy. I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. J Clin Endocrinol Metab 1975;40:545–551.
6. Lamminmäki A, Hines M, Kuiri-Hänninen T,Kilpeläinen L, Dunkel L, Sankilampi U. Testosterone measured in infancy predicts subsequent sex-typed behavior in boys and in girls. Horm Behav 2012;61:611–616.
7. Kiviranta P, Kuiri-Hänninen T, Saari A, Lamidi M-L, Dunkel L, Sankilampi U. Transient postnatal gonadal activation and growth velocity in infancy. Pediatrics. 2016; 138
8. Kuiri-Hänninen T, Seuri R, Tyrväinen E, Turpeinen U, Hämäläinen E, Stenman UH, Dunkel L, Sankilampi U. Increased activity of the hypothalamic-pituitary-testicular axis in infancy results in increased androgen action in premature boys. J Clin Endocrinol Metab 2011;96:98–105
9. Kuiri-Hänninen T1, Haanpää M, Turpeinen U, Hämäläinen E, Seuri R, Tyrväinen E, Sankilampi U, Dunkel L.Postnatal Ovarian Activation Has Effects In Estrogen Target Tissues In Infant Girls. J Clin Endocrinol Metab. 2013 Dec;98(12):4709-16

10. Panu Kiviranta, Tanja Kuiri-Hänninen, Antti Saari, Marja-Leena Lamidi, Leo Dunkel, Ulla Sankilampi. Transient Postnatal Gonadal Activation and Growth Velocity in Infancy. Pediatrics 2016 Jul 9;138

11. Ibáñez L, Valls C, Cols M, Ferrer A, Marcos MV, De Zegher F. Hypersecretion of FSH in infant boys and girls born small for gestational age. J Clin Endocrinol Metab 2002;87:1986–1988.

12. Einspieler C, Prechtl HFR, Ferrari F, Cioni G, Bos AF. The qualitative assessment of general movements in preterm, term and young infants - review of the methodology. Early Hum. Dev. 50 (1997) 47-60

13. Einspieler C, Peharz R, Marschik PB. Fidgety movements - tiny in appearance, but huge in impact. J. Pediatr. (Rio J) 92 (2016) S64-70