Title Form

"Salivary cortisol as stress marker in newborns subjected to double weighing with physiological body weight"

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PILOT STUDY PROTOCOL

Salivary cortisol as stress marker in newborns subjected to double weighing with body weight loss of 3±1,5% at 24 hr from birth

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1. **Introduction**

After the phase of early adaptation, newborn must learn quickly to live out of the mother uterus. In this phase of life, physiological mechanisms are activated (1). Newborn loss body weight because loss liquid by respiration, feces, urine and not adequate feeding (2). Body weight loss after birth is physiological when is lower than 10% but is also important the evaluation of body weight daily trend that must be lower than 5% (3,4). If newborn shows physiological body weight loss, no treatment will be necessary and rooming-in can continue together with exclusively breastfeeding (5,6)

Furthermore, if newborn lose more than 10% of body weight during 48hr of life or more than 5% of body weight in a day, a dehydration state will occur that will influence negatively the physiological cardio-circulatory and kidney adaptation. In these cases, is important execute controls and a specific therapy (7). When body weight loss is between 7%-10%, usually in Neonatology wards it is used double weighing before and after breastfeeding (8). This practice evaluates colostrum quantity that is assumed from infant during feeding and the necessity of milk formula integration to prevent an excessive body weight loss. Diuresis is also monitored, and parents are informed on the benefits of breastfeeding.

If body weight loss is greater than 10%, diuresis is monitored weighing the diaper and haematological examinations are performed. In this case, it is counselled an artificial milk integration after breastfeeding. Discharge can be influenced from the results of the controls and must be executed for the security of the newborn (9).

Health care providers must not be underestimated the possible allergies to formula milk and the mothers that give up breastfeeding after the use of formula milk. The newborn should receive breastfeeding from the delivery room (10,11). It is good practice, when the milk secretion is enough, to attach the infant to a single breast for each feeding and let it suck for 15-20 minutes (12). Quantity of milk that newborn assume for each feeding is object of careful evaluation. Double weighing consists in weighing newborn before and after feeding: the body weight difference is the quantity of milk assumed. In the first day of life, newborn should suck 10 gr of colostrum or milk for each feeding. Then starting from the second day of life, the quantity of milk assumed must be increased of 10 gr/die for the first week.

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According to scientific revisions, salivary cortisol is a valid method for measuring adrenocortical activity in newborns because it is non-invasive in fact the measurement of salivary cortisol has been used as a stress marker in newborns since 1992 (13,14). The mechanism by which cortisol induces stress, concerns the hypothalamic-pituitary-adrenal axis. The hypothalamus produces the corticotropin-releasing hormone, which stimulates the production of adrenocorticotropin by hypophysis. Adrenocorticotropin induces the secretion of glucocorticoids such as cortisol by stimulating the cortex of the adrenal gland. An appropriate release of cortisol due to stress factor is vital for surviving and the loss of this mechanism increase the risk of morbidity and mortality for newborns. However, prolonged exposure to a high level of cortisol may increase the risk of cognitive and behavioural disorders, hypertension, hyperlipidaemia, insulin resistance, immune deficiency and hippocampal impairment (15,16). The separation from the mother, the exposure to painful procedures but also routine interventions such as physical examination, diaper changes and bathing increase the salivary cortisol level of the newborn compared to the basal state (17). The measurement of salivary cortisol in the newborn therefore has the aim of understanding and preventing those stressful conditions that may have long-term side effects. For this reason, the lose weight in the first days and double weighing should be a stress practice for the newborn.

2. **Objective**

Evaluation of salivary cortisol level as stress marker in newborns with body weight loss of 3±1,5% at 24 hr from birth subjected to double weighing or to body weight control at 36 hr and 48 hr of life. Furthermore, evaluation of body weight trend between newborns subjected to double weighing or to body weight control at 36 hr and 48 hr of life.

3. Study Location

U.O. Neonatology, Department of mother and child's health, Poliambulanza Foundation, Brescia.

4. Population

Group A (Case): Newborns subjected to double weighing before and after breastfeeding, with body weight loss >3±1,5% at 24 hrs or 6% at 48 hrs from birth

Group B (Control): Newborns with body weight loss >3±1,5% at 24 hrs and 6 % at 48 hrs from birth and subjected to weight control at 36 hr and 48 hr of life

5. Inclusion Criteria

- Gestational age: 37-42 wks
- Body Weight: >2500 g
- Body weight loss >3±1,5% at 24 hr from birth
- First-born
- Eutocic delivery
- Mother BMI:19-24
- Glycaemia >50 mg/dl

6. Exclusion criteria

- Mother disease
- Admission in NICU
- Neonatal pathologies
- Utilization of human milk
- Body weight loss > 10%

7. Procedure

Body weight of newborns will be collected at birth(T0), at 24 hr of life (T1) and at 48 hr of life (T2) by Eura Mod.AS/1 OMIP, Milano.

Group A will be subjected to double weighing, before and after the next 6 breastfeeding. Breastfeeding will be carried out each 4 hr.

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Group B will be subjected to monitoring of body weight only at 24, 36 hr and 48 hr of life.

Saliva sample of the newborns will be collected in oral cavity at 48 hr of life. Samples collected will be storage at -80 C for up to 4 months and analysed by analysis laboratory of Poliambulanza Foundation.

Measurement

Weight scale used to obtain body weight is Eura Mod.AS/1 OMIP, Milano.

Data collected will be reported on a database by Microsoft Office Excel 2016.

SalivaBio Infant's Swab (SIS) is the kit used to collect salivary cortisol.

Statistical analysis will be carried out by IBM SPSS Statistics for Windows, v.25 (Armonk,NY:IBM Corp.)

8.Primary Outcome

Evaluation of salivary cortisol levels at 48 hr of life after the practice of double weighing to analyse stress levels of the newborns

H0: There is not difference in salivary cortisol level between group A and B.

H1: Salivary cortisol level in group A is greater than group B.

9.Secondary Outcome

Evaluation of body weight increase between group A and B.

H0: There is not difference in the body weight increase between group A and B

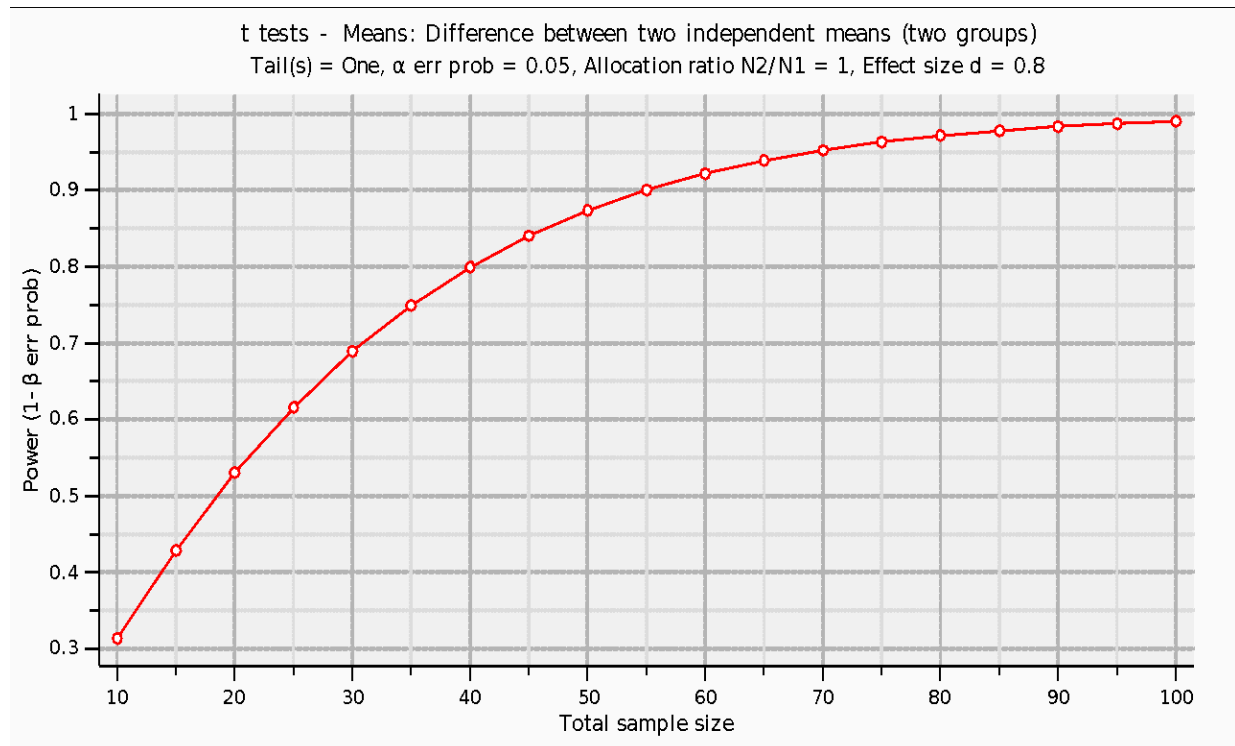**H1:** Group A shows a greater body weight increase than group B

10.Sample Size

Primary outcome will be evaluated by independent two samples t-test. Sample size will be computed setting:

Alfa error: 0.05

Beta error: 0.90

Effect size: 0.8

Sample size will be of 56 patients (28 group A + 28 group B)

Secondary outcome will be analysed by independent two samples t-test. Trend of body weight will be computed evaluating percentage increase from birth to 48 hr of life.

11. Randomization

Patients will be enrolled to group A or B by statistical software.

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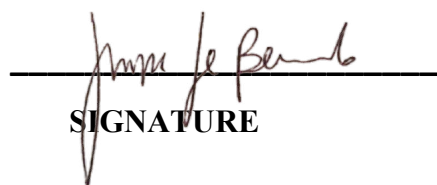
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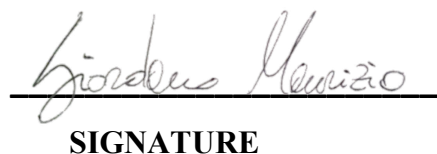
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