**Protocol of ANNA: ANte-Natal Auditory system assessment.**

**Background:**In May 2020, my wife entered the 5th month of her first pregnancy. As all parents, we wondered: when will our baby start to hear us? After a short literature search (i.e. after reading the first three questionable blogs that popped up on Google), we learnt that Anna probably started hearing her first sounds around the 18th-20th week of pregnancy. It was much less clear to us, though, whether and how she would be responding to these sounds.   
In the following days, I started talking to my wife’s belly and I initially thought the baby was indeed responding to my words by kicking and punching. After few moments of enthusiasm, the statistician inside me started complaining: it was impossible to know whether the kicks were in fact *caused* by myself, or whether she would have kicked anyway just for fun even if I was watching the curling world cup. Afterall, it must be incredibly boring to stay in the womb for 9 months.   
The aim of this trial is therefore to evaluate whether there is any difference in the movement patterns of Anna due to her father talking to her.

**Eligibility and randomisation:**  
Inclusion criteria were: living in the PI’s wife belly and being older than 20 weeks. There is a single participant in this trial, Anna, after whom the trial is named. She is of course eligible to be enrolled and her guardian, father and PI of this study has signed the consent form for her. There is no conflict of interest.   
This is a N-of-1 trial, though for simplicity we will treat it as a simple two-arm trial.  
The number of fetal movements in multiple 30 seconds windows will be measured at different time points. At each time point, Anna will be randomised to receive the active treatment (her father talking like an idiot to her mother’s belly for 30 seconds) or the control (30 seconds of awkward silence). The whole randomisation list will be drawn at the beginning of the study running a very sophisticated R code (sample(c(“Silence”,”Talking”),N,rep=TRUE)). The study is of course open label, as it would be kind of difficult to do placebo talking, but the experimenter will only see the randomised treatment after deciding to do a measurement (to avoid selective choice of times when to do the measurement). To achieve this, the whole randomisation list will be copied to the Excel data spreadsheet, but initially hidden with a very sophisticated technique (painting the corresponding cells black).  
Times at which to measure the number of movements will be chosen randomly throughout the day.  
Protocol amendment #1: we had not anticipated hiccups would screw our results. On the 19th June, after 95 observations had been recorded, two of which completely off because of hiccups, we decided to amend the protocol. If the baby has hiccups, no measurement will be taken.  
  
**Outcomes:**   
The primary outcome will be the number of distinct clear movements felt by the father in a 30 seconds window. As it is sometimes difficult to distinguish between two separate movements when the baby goes wild, a secondary outcome will be binary movement/no movement.   
  
**Procedures:**Measurements will be taken in the position where the mother stands when the father and PI asks to take them. The father will put both hands on the belly and either start talking immediately or remain silent while the mother times 30 seconds with her phone. The mother swears that she will never reveal to the outside world the deep intellectual content of the “belly talks”.   
  
**Data to be recorded:**   
On top of randomised treatment and number of movements detected, for each measurement we will collect the following variables: date, time, position and distance from last meal. These are the factors that are considered most likely to influence the outcome.   
  
**Sample size:**We assumed that the average number of movements per 30 seconds would be 0.75 in the control arm and we estimated that 200 observations would give us 95% power to detect a difference if the average number of movements in the active arm was 1.25. The 95% power was chosen both because (i) I don’t like choosing 80 or 90 just because everybody else does so and (ii) it’s the year when the movie Babe was released.   
Note that, since the analyses will be adjusted for pre-specified covariates, power will actually be higher than this, but I have no intention to complicate my sample size calculation further, so just get on with it.

**Statistical analysis plan:**  
The main analysis model will be a simple Poisson regression model with number of movements as outcome, adjusting for pregnancy week, time, distance from last meal and position. The estimand of interest is the treatment effect parameter in the fully adjusted model.   
A secondary analysis will use a logistic regression model, adjusted for the same variables, with binary move/no move as the outcome.   
  
**Subgroup analyses:**   
We plan to additionally report the estimates from an unadjusted model, together with subgroup analyses by pregnancy month, time (8-13 vs 13-20 vs 20-8), distance from meal (<=2 vs >2) and position.

**Adherence and Missing Data:**  
As the PI has worked in Missing Data for a while, he knows how important it is to collect all the intended data. For this reason, whenever some data will be lost, he will make them up completely with the sole goal of favouring his own research hypothesis.   
There is no anticipated adherence issue, as the sole participant does not have much options to run away from the study.

**Safety:**  
There is no anticipated safety issue associated with hearing the PI’s awesome voice. However, in case he decided to sing some awkward song completely out of tune, the mother will be responsible to stop the experiment in the interest of the baby’s physical and mental health.

**Data management:**  
Data will be recorded in an Excel spreadsheet on the PI’s laptop straight after each measurement. There will be no backup, so that if something happens we’ll be screwed.   
The Independent Data Monitoring Committee will make sure there are no safety issues throughout the trial and will analyse the data independently at the end of the trial, in order to check whether they can replicate the results.

**Sponsor and Funding:**  
The trial sponsor will be the PI, father, data collector and trials statistician. The trial is funded by him and his wife and the Bill and Melinda Gates Foundation.   
(Not really, this is just to see whether I can attract some conspiracy theorists).