Norwegian Institute of Public Health - Centre for Fertility and Health – impact case number 3

Institution: Norwegian Institute of Public Health
Administrative unit: Centre for Fertility and Health
Title of case study: The role of chronological and biological aging in fertility
Period when the underpinning research was undertaken: 2018-2023
Period when staff involved in the underpinning research were employed by the submitting institution: 2017-2023
Period when the impact occurred: 2019-2023

1. Summary of the impact

Age is undoubtedly one of the most important factors in fertility. People age with different speed, and both the ability to become pregnant and health vary greatly within people of the same chronological age. Some genetic measures, including DNA methylation and telomere length, are surprisingly good indicators for biological aging. Accurate estimation of biological age can advance our understanding of biological mechanisms linking age to fertility, development, health and disease. Also, we have shown that gestational age can be predicted by genetic measures, which is particularly valuable in gauging fetal and newborn's development.

2. Underpinning research

Leveraging genetic, epigenetic and telomere data from the Norwegian Mother, Father, and Child Cohort Study (MoBa), we present here a selection of key findings from our research on the importance of chronological age, epigenetic aging and telomere length on fertility [dates in brackets]:

1) With Norwegian registry data we could show that the risk of miscarriage increases steeply with maternal age over 35 years, and around half of all pregnancies in women above age 40 end in a miscarriage. [2018 – 2019]
2) Building on previous work we show that an epigenetic gestational age clock built on DNA-methylation data from the more recent Illumina MethylationEPIC BeadChip (EPIC) platform estimates gestational age more precisely than previously published clocks based on data from earlier Illumina platforms. [2018-2022]
3) There were strong correlations between DNA-methylation and gestational age across seven main cell-types in cord blood, most of which was attributable to nucleated red blood cells (nRBCs). These correlations were closely related to genes involved in erythropoiesis, immune response, and the transition from fetal to adult hemoglobin. [2018-2022]
4) Our gestational age clock showed a similar performance when applied to samples from children born after assisted reproductive technologies and after natural conceptions. [2018-2022]
5) We found that telomere length is associated with 823 CpG sites using an epigenome-wide association study (2019) and have submitted a paper that shows that polygenic scores for telomere length predict the observed telomere length equally well for newborn children and adults.
6) We found a significant difference in the epigenetic age acceleration in adults calculated by the DunedinPoAm clock between in vitro fertilization (IVF) and non-ART mothers after adjustment for potential confounders. [2020 – 2022]

The above findings warrant further investigations and are already seeding new initiatives and exciting applications. Examples include the use of the Cytometry by time of flight (CyTOF) instrument at NIPH to disentangle specific contributions from different cell types to gestational

age. This will enable us to map the roles of different cell types in biological processes related to the postnatal period. Another application pertains to measuring DNA methylation levels of extracted cell-free fetal DNA in maternal blood as an alternative to cord-blood DNA, given the invasiveness and ethic-legal limitations of sampling biologic specimens before a baby is born.

The bulk of the work underpinning the impact of our research into gestational age and biological aging is based on funding from several research grants, including the following:

1. The Norwegian Research Council of Norway's Centre of Excellence funding Scheme (grant no. 262700)
   OUTPUT: Researcher time, PhD, Costs for analyses of DNA methylation
2. NIH: The National Institutes of Health (NIH; grant no. R01 1HL134840-01NIPH): "Telomeres and female fecundity."
   OUTPUT: Researcher time, PhD, Costs for analyses of telomere measurements
3. The Research Council of Norway's FRIPRO call (grant no. 262043): "Telomere length, epigenetic age and T cells in women who give birth at an older age."

Names of the key researchers and what positions they held at the administrative unit at the time of the research (where researchers joined or left the administrative unit during this time, these dates must also be stated).

NAME OF RESEARCHER	POSITION	DATE JOINING	DATE LEAVING
Siri Eldevik Håberg	Director	Nov 1, 2017	-
Per Magnus	Deputy Director	Nov 1, 2017	-
Astanand Jugessur	Senior scientist	Nov 1, 2017	-
Jon Bohlin	Senior scientist	Jan 1, 2020	-
Yunsung Lee	PhD/Researcher	Feb 1, 2018	-
Kristine L. Haftorn	PhD research fellow	Feb 1, 2019	June 30, 2023
Håkon Gjessing	Principal Investigator	Nov 1, 2017	-
William Denault	Postdoc	Jan 1, 2018	August 31, 2022
Julia Romanowska	Researcher	Nov 1, 2017	-
Maria C Magnus	Senior scientist	Aug 1, 2018	-
Haakon Nustad	Researcher	Jul 1, 2020	-
Christian Page	Researcher	Nov 1, 2017	-
Robert Lyle	Senior scientist	Apr 3, 2020	-

3. References to the research - Researchers in our unit in bold

1. **Magnus MC, Wilcox AJ,** Morken NH**,** Weinberg CR, **Håberg SE.** (2019). Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study. *BMJ,* 364, l869. https://doi.org/10.1136/bmj.l869
2. **Lee Y,** Sun D, Ori APS, Lu AT, Seeboth A, Harris SE, Deary IJ, Marioni RE, Soerensen M, Mengel-From J, Hjelmborg J, Christensen K, Wilson JG, Levy D, Reiner AP, Chen W, Li S, **Harris JR, Magnus P,** Aviv A**, Jugessur A,** Horvath S**.** (2019). Epigenome-wide association study of leukocyte telomere length. *Aging (Albany NY)*, 11(16), 5876-5894. https://doi.org/10.18632/aging.102230
3. **Haftorn KL, Denault WRP, Lee Y, Page CM, Romanowska J, Lyle R**, Næss ØE, Kristjansson D, **Magnus PM, Håberg SE, Bohlin J, Jugessur A**. (2023). Nucleated red blood cells explain most of the association between DNA methylation and gestational age. *Commun Biol,* 6(1), 224. https://doi.org/10.1038/s42003-023-04584-w

4. **Lee Y, Haftorn KL, Denault WRP, Nustad HE, Page CM, Lyle R**, Lee-Ødegård S, Moen GH, Prasad RB, Groop LC, Sletner L, Sommer C, **Magnus MC, Gjessing HK, Harris JR, Magnus P, Håberg SE, Jugessur A, Bohlin J**. (2020). Blood-based epigenetic estimators of chronological age in human adults using DNA methylation data from the Illumina MethylationEPIC array. *BMC Genomics,* 21(1), 747. https://doi.org/10.1186/s12864-020-07168-8
5. **Lee Y**, Choufani S, Weksberg R, Wilson SL, Yuan V, Burt A, Marsit C, Lu AT, Ritz B, **Bohlin J, Gjessing HK, Harris JR, Magnus P**, Binder AM, Robinson WP, **Jugessur A**, Horvath S. (2019). Placental epigenetic clocks: estimating gestational age using placental DNA methylation levels. *Aging (Albany NY),* 11(12), 4238-4253. https://doi.org/10.18632/aging.102049
6. **Lee Y, Bohlin J, Page CM, Nustad HE, Harris JR, Magnus P, Jugessur A, Magnus MC, Håberg SE, Hanevik HI**. (2022). Associations between epigenetic age acceleration and infertility. *Hum Reprod,* 37(9), 2063-2074. https://doi.org/10.1093/humrep/deac147

4. Details of the impact

Our research has mainly focused on generating new knowledge to advance the field of chronological aging and biological aging in growth, development, fertility and health. Although it has been well known that women's age affects their ability to become pregnant, we were able to provide solid evidence quantifying the risk of miscarriage with maternal age. This study has proven valuable for other research groups around the world and has been highly cited in international publications since 2019.

We are in the forefront in developing and using biological aging clocks. Our team developed the first gestational age clock and have refined this gestational age clock and excelled the work around using DNA methylation in gestational age prediction. It has added significant value to both clinical and research settings by providing crucial insights into a newborn's developmental stage. Previous research has shown a link between preterm birth and several negative outcomes in neonates, extending into later life. The precise determination of gestational age is critical for effective perinatal care. Traditional methods, such as calculations based on the last menstrual period or ultrasound estimates, are fraught with limitations. With focus on cell-type specific relationships between gestational age and DNA methylation in cord blood we have identified strong correlations across seven main cell types found in cord blood, particularly in nucleated red blood cells (nRBCs). The DNA methylation markers (CpGs) we discovered were linked to genes crucial in the development of red blood cells, various developmental processes, and the preparation for birth and adaptation to life outside the womb. These findings not only contribute to our scientific understanding of these vital processes, but also highlight the potential for practical applications in neonatal care and developmental research. Promising new research based on our work in this field includes the potential of generating new methods for gestational age determination based on blood samples.

Our team has also worked extensively on refining and using DNA methylation clocks in adults as markers of accelerated biological aging and its implications for lower fertility and how the rate of biological aging in adults is relevant for later risk of disease. Prior to our work, a variety of epigenetic clocks had been developed and associated with various environmental exposures and diseases in the elderly, but these were mostly based on older methylation platforms. Our research, based on the newer methylation platform has provided numerous insights regarding the importance of the additional epigenetic marks included on this platform.

Several studies have suggested that epigenetic age acceleration in mothers who conceived using ART may be associated with low oocyte yield and poor ovarian response. However, the difference in epigenetic age acceleration between non-ART and ART mothers (or fathers) had not been examined previously. We filled this gap in knowledge by comparing epigenetic age derived from

various epigenetic clocks between non-ART and ART mothers and fathers. We found a significant difference in the epigenetic age acceleration between in vitro fertilization (IVF) and non-ART mothers after adjustment for potential confounders. A plausible biological mechanism for the observed association is that mothers who undergo IVF may be nearer to menopause compared to mothers who do not use ART.

We are now at a stage where it is possible to delve deeper into the significance of the findings in terms of their relevance for translational applications. Accordingly, we will seek funding to investigate cell-specific methylation profiles using state-of-the-art instruments (CyTOF) in-house at the NIPH, in addition to extracting cell-free fetal DNA in the maternal circulation to determine methylation profiles at an earlier stage than at birth.

5. Sources to corroborate the impact

Apart from peer-reviewed journal articles (a selection of which is provided above), we have also disseminated information about our research to the scientific community as oral or poster presentations at national and international venues, as well as popularized reports in news outlets to reach out to a non-technical audience. These include:

1) Epigenomics of Common Diseases 2022 conference at the Wellcome Trust in the UK (**Haftorn KL** *et al.* "Nucleated red blood cells explain most of the association between DNA methylation and gestational age").
2) The EPEC 2023 meeting in Stockholm, Sweden (**Haftorn KL** *et al.* "The epigenetic landscape of gestational age")
3) The 2023 European Society of Human Genetics (ESHG) Conference in Glasgow, UK (**Haftorn KL** *et al.* "Stability selection enhances feature selection and enables accurate prediction of gestational age using only seven DNA methylation sites").
4) The 2019 Annual Meeting of the Epigenomics of Common Diseases conference (Cambridge, United Kingdom)
5) The 2022 Annual Meeting of the European Society of Human Genetics in Vienna, Austria (**Haftorn KL** *et al.* "Epigenome-wide association study of gestational age at birth using DNA methylation data measured on the Illumina MethylationEPIC BeadChip microarray").
6) Science News DK. Interview of Kristine Haftorn by the Danish Novo Nordic, available at https://www.sciencenews.dk/en/newborns-dna-reveals-their-gestational-age