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Exploring the Ovarian Reserve within Health Parameters: A Latent Class Analysis.

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Abstract

The process of ovarian aging is influenced by a complex and poorly understood interplay of endocrine, metabolic and environmental factors. The purpose of this study was to explore the feasibility of using Latent Class Analysis to identify subgroups based on cardiometabolic, psychological and reproductive parameters of health and to describe patterns of anti-Müllerian hormone levels, a biomarker of the ovarian reserve, within these subgroups. Sixty-nine lean (BMI 25kg/m²) and severely obese (BMI 40kg/m²) postpartum women in Edinburgh, Scotland were included in this exploratory study. The best fitting model included 3 classes; Class 1, n = 23 (33.5%); Class 2; n = 30 (42.2%); Class 3; n = 16 (24.3%). Postpartum women with lower ovarian reserve had less favorable cardiometabolic and psychological profiles. Examining the ovarian reserve within distinct subgroups based on parameters of health that affect ovarian aging may facilitate risk stratification in the context of ovarian aging.

Keywords

Ovarian reserve; latent class analysis; cardiometabolic; psychological

The ovarian reserve--the number of remaining ovarian follicles--declines gradually over a women's reproductive lifespan and natural menopause occurs when the ovarian reserve is depleted (Daan & Fauser, 2015). Anti-Müllerian hormone (AMH) plays a role in ovarian follicle growth, specifically in regulating the pace of follicle recruitment and selection (Visser et al., 2007). Serum levels of AMH reflect the continuous non-cyclic growth of small ovarian follicles, and therefore mirror the size of the remaining follicle pool (Dewailly, Alebi, Duhamel, & Stojanovi, 2014; Jeppesen et al., 2013). Considerable variation in the

age at natural menopause suggests that factors other than chronological age contribute to the depletion of the ovarian follicle pool over time (van Disseldorp et al., 2008).

While numerous studies have examined the effect of biobehavioral factors on AMH concentrations (Dolleman et al., 2013; La Marca et al., 2012), few have explored patterns within and between women with proven fertility as a means of identifying subgroups. Accurate group classification would improve the clinical interpretation of serum AMH concentrations and facilitate risk stratification in the context of ovarian aging (Pal, Bevilacqua, & Santoro, 2010). As cardiovascular, metabolic, and psychological factors have each been shown to have an effect on the ovarian reserve (Balkan, Cetin, Usluogullari, Unal, & Usluogullari, 2014; Bleil et al., 2012a, 2012b; de Kat, Broekmans, Laven, & van der Schouw, 2015), we hypothesized that latent class analysis (LCA) could be used to develop subgroup classifications using these factors and that these subgroups would provide a useful context within which to examine the complex interplay of variables involved in the process of ovarian aging.

LCA is a clustering method used to identify patterns in factor configurations and has been effectively used to examine co-occurrence patterns of risk factors in chronic disease development (Dewailly, Alebi, Duhamel, & Stojanovi, 2014; Leventhal, Huh, & Dunton, 2014). Rather than providing prescriptive clinical guidelines regarding the nature of class structures, we sought to elucidate the complex process of ovarian aging by exploring AMH within the context of cardiometabolic, psychological and reproductive factors (Henry, Dymnicki, Mohatt, Allen, & Kelly, 2015; Leventhal et al., 2014). As the underlying mechanisms contributing to premature ovarian aging are not well understood, LCA may be a useful method for clarifying how co-occurrence patterns of risk factors contribute to subtle changes in ovarian functioning over time (Leventhal et al., 2014). We conducted an exploratory study with the purpose of testing the feasibility of this hypothesis in a sample of postpartum women in whom cardiovascular, metabolic, and psychological factors had been measured during pregnancy and postpartum.

Methods

Design and Sample

This study used data from a prospective cohort study conducted in Edinburgh, Scotland from 2008–2013 examining associations between mood and weight changes in lean (BMI 25kg/m^2) and very severely obese (BMI 40kg/m^2) women with singleton pregnancies (Mina et al., 2015). In the parent study, cardiovascular, metabolic, and psychological measures were recorded during pregnancy and 3 months postpartum. For this exploratory cross-sectional study, only data collected during the study visit three months postpartum were used. This time point was selected to avoid the ovarian suppression that occurs during pregnancy and to coincide with the expected recovery of AMH levels (Köninger et al., 2015). Women diagnosed with gestational diabetes or polycystic ovary syndrome were excluded as these conditions are known to influence AMH levels (Iliodromiti, Kelsey, Anderson, & Nelson, 2013; Łebkowska et al., 2016). All samples and data were collected with ethical approval (references 08/S1101/39 and 13/ES/0126), and with fully informed and written consent from all women.

Measures

Cardiometabolic risk factors included fasting lipids (total cholesterol, high density lipoprotein, triglycerides), fasting glucose, fasting morning cortisol and BMI (Mina et al., 2015). BMI was used to group women as lean ($\text{BMI} < 25\text{kg/m}^2$) or severely obese (SO) ($\text{BMI} \geq 40\text{kg/m}^2$).

Psychological measures included the Hospital Anxiety and Depression Scale (HADS), the State-Trait Anxiety Index (STAI) and the General Health Questionnaire (GHQ). The HADS evaluates anxiety and depression symptoms (range: 0–21) and has been reported to help in differentiating transient and enduring stress during pregnancy (Matthey & Ross-Hamid, 2012). The STAI evaluates both state and trait anxiety (range: 20–80 each), and has been previously validated in severely obese pregnant women (Gunning et al., 2010). The GHQ-12 uses binary scoring (range: 0–15) and has been shown to reliably differentiate stress levels between pregnant and non-pregnant controls (Goldberg, 1973; van Bussel, Spitz, & Demyttenaere, 2006).

Reproductive measures included LH, FSH, estradiol (E2), AMH and breastfeeding status. Serum levels of LH, FSH, E2 and AMH were measured in stored serum samples in a single batch by electrochemiluminescence immunoassay using the Roche Elecsys assay system (West Sussex, UK) (reference numbers: 11732234122, 11775863122, 06656021190 and 06331076190 respectively). The intra-assay coefficients of variation were 6.2–6.9% for LH, 7.3–8.1% for FSH, 1.5–3.2% for E2, and 5.8–6.9% for AMH. Breastfeeding status at both time points was assessed with a single yes/no question: ‘Are you breastfeeding your baby now?’. A ‘yes’ answer included both exclusive and non-exclusive breastfeeding.

Data Analyses

To describe the data, means and standard deviations were calculated for clinical and demographic variables in the whole sample. A one-way ANOVA was used to explore differences in variables between lean vs. very severely obese women, and in breastfeeding vs. non-breastfeeding women. The ANOVA was conducted to identify meaningful differences present in the data. Bonferroni adjustment was used, dividing α of 0.05 by the number of variables ($n = 12$), to account for multiple statistical testing with only differences resulting in a p-value lower than 0.004 considered statistically significant.

Anxiety and depression were analyzed using the methods outlined in Mina et al. (2015). Briefly, maternal mood outcomes were grouped into ‘anxiety symptoms’ and ‘depression symptoms’. Anxiety symptoms were represented by Hospital Anxiety (HA from the HADS) and both the state and trait components of the STAI. Depression outcomes were represented by Hospital Depression (HD, from the HADS) and the GHQ. To avoid multiple testing and the need to include a Bonferroni correction, the z-score was calculated for each outcome and averaged z-scores were used for each symptom group in the analysis.

Latent Class Analysis (LCA) was used to classify the subjects of the whole sample (heterogeneous) into smaller homogeneous classes/groups in which members are similar to each other and differentiated from subjects in other groups using cardiometabolic, psychological and reproductive factors. The objective is to identify groups naturally

occurring in the data, when the number of underlying groups is unknown (DiStefano, 2012; Kaplan, 2014). LCA assumes that the data comes from a mixture of populations that have different probability distributions and that the population consists of homogeneous subgroups, in which the groups are discrete and mutually exclusive (DiStefano, 2012).

The results of LCA provide probabilities for the proportion of the population expected in each group. Groups are not defined *a priori* but are rather probabilistic (Dewailly, Alebi, et al., 2014); individuals are allocated to groups based on observed values in the indicator variables used to determine class membership. Individuals of the same group are similar such that their observed values are assumed to come from the same probability distribution (Tein, Coxe, & Cham, 2013). Conditional probabilities describing the mean or likelihood of each indicator variable are provided for each class (Henry et al., 2015). LCA has demonstrated the ability to accurately determine class membership in samples as small as 50 (Henry et al., 2015). As this was an exploratory study to examine the feasibility of using LCA to identify subgroups, we used a small sample. The LCA model included the following indicator variables: age, fasting lipids (triglycerides, cholesterol, and HDL), fasting glucose, fasting cortisol, lean/obese, breastfeeding status, anxiety, depression, FSH, LH, E2, and AMH levels. All variables were continuous with the exception of lean/obese and breastfeeding status; LCA is capable of reliably identifying class structures using variables with mixed scales (DiStefano, 2012).

All analyses were conducted using R 3.3.3 (R Core Team, 2017), with the package flexmix (Leisch, 2004). This software allowed us to estimate LCA with different numbers of classes and compare the fit of each number of classes to identify how many classes should be estimated. The LCA was estimated for 1 to 10 classes, comparing the models with the Bayesian Information Criterion (BIC) and Integrated Completed Likelihood Criterion (ICL) (Burnham & Anderson, 2003). These information criteria penalized the log-likelihood of the model. The BIC and ICL are used to select the model from a set of candidate models that provides the best balance of model fit, complexity and parsimony, where models with lower BIC and ICL present better fit (Burnham & Anderson, 2003). Once the number of classes was defined, we looked at the characteristics of each class and the patterns of the variables of interest relative to the ovarian reserve. As the primary focus of this study was to establish the feasibility of using LCA to identify subgroups, we provide only a brief discussion of class characteristics.

Results

Sixty-nine lean ($n=38$) and severely obese ($n=31$) postpartum women with mean age 33.97 ($SD = 4.1$) years were included in the analysis. Forty-seven women were breastfeeding and 22 were not breastfeeding. Clinical and demographic data and group differences (lean/very severely obese and breastfeeding/non-breastfeeding) are summarized in Table 1. As expected, there was a significant negative correlation between age and logAMH ($r = -0.312$, $p = 0.002$).

Information criteria (BIC and ICL) and entropy were used to compare the LCA solutions from a different number of classes. As the number of classes increases, the log-likelihood

decreases, and entropy increases. The BIC and ICL decreased from 1 to 2 classes, increased minimally from 2 to 3 classes, and clearly increased from 4 or more classes. Given that the difference in information criteria between the 2 and 3 classes solution was small, we examined the parameter estimates for each of these solutions to identify which one presented results that were more theoretically fitting.

The best fitting LCA model with meaningful parameter characteristics for each class included 3 classes. For the 3-class LCA, 33.5% (n=23) of the sample was in Class 1, 42.2% (n=30) of the sample was in Class 2, and 24.3% (n=16) of the sample was in Class 3. Table 2 shows the variable characteristics for each class. For the continuous variables, the characteristic is presented as the average score of that variable within each class, for the binary variables (breastfeeding status and lean/obese), the characteristic is presented as the probability of presenting that characteristic. Comparisons between the classes by cardiometabolic, psychological and reproductive health parameters are shown in Table 3. For the 3-class solution, the entropy is 0.93. Entropy is a measure of uncertainty in the classification procedure, indicating how well the model predicts membership, with values close to 1 representing better prediction. The high entropy (0.93) for this LCA solution shows that the model accurately predicts membership. Each class is summarized briefly below:

Class 1: Highest mean AMH levels, lowest probability of breastfeeding, lowest mean cholesterol levels, lowest mean HDL levels, lowest probability of being lean, lowest mean fasting morning cortisol levels, lowest mean anxiety scores, lowest mean FSH and LH levels, and highest mean E2 levels

Class 2: Highest probability of breastfeeding, lowest mean triglyceride levels, highest mean HDL levels, highest probability of being lean, lowest mean depression score, highest mean FSH and LH levels, and lowest mean E2 levels

Class 3: Lowest mean AMH levels, highest mean triglyceride and cholesterol levels, highest mean anxiety and depression scores, and highest mean cortisol levels

Discussion

In this study, we used latent class analysis to explore possible subgroups of cardiometabolic, psychological and reproductive parameters of health in relation to ovarian reserve in a convenience sample of post-partum women. Three subgroups were identified. As expected, there was a negative correlation between age and AMH; however, age was not a strong determinant of class membership in the LCA. The subgroups identified through LCA provide insight into the complex interactions of various health related parameters in relation to ovarian reserve and contribute to our understanding of factors other than age involved in the process of ovarian aging. LCA offers a feasible approach for examining risk factors in the context of ovarian aging.

Class 1 had the highest mean AMH levels and the lowest mean cholesterol levels. Class 3 had the lowest mean AMH levels and the highest mean cholesterol and triglyceride levels. Several studies have observed the trend toward a less favorable cardiovascular risk profile in women with lower age-specific AMH levels (Bleil, Gregorich, McConnell, Rosen, &

Cedars, 2013; Tehrani, Erfani, Cheraghi, Tohidi, & Azizi, 2014). Cardiovascular disease risk factors have been suggested to accelerate the process of ovarian aging by impairing vascularization of the ovaries and accelerating ovarian decline (de Kat et al., 2015). It may be that these processes occur simultaneously and share similar underlying mechanisms, such that accelerated ovarian aging increases cardiovascular disease risk and increased cardiovascular disease risk accelerates ovarian aging (de Kat et al., 2015; Kok et al., 2006).

In the present study, the probability of being lean or severely obese did not demonstrate a strong class trend in relation to AMH. Given the strong class trend of cardiovascular risk factors in relation to AMH, this was an unexpected finding since severe obesity is a well-defined risk factor for early onset cardiovascular disease. This finding may be explained by the lack of variability in cardiometabolic factors observed in the sample, irrespective of BMI. Despite their obesity, these women are still relatively young and display a narrow range of variability in cardiometabolic factors. This lack of class trend may also be explained by the use of a postpartum sample of women; breastfeeding has been shown to have a protective effect on cardiovascular health regardless of pre-conception risk factors (McClure, Catov, Ness, & Schwarz, 2012).

The suppressive effect of obesity on reproductive function is well established (Klenov & Jungheim, 2014; Nelson, Stewart, Fleming, & Freeman, 2010; Vryonidou, Paschou, Muscogiuri, Orio, & Goulis, 2015). Obesity alters the ovarian follicular environment and contributes to anovulation (Klenov & Jungheim, 2014; Moy, Jindal, Lieman, & Buyuk, 2015). The effect of obesity on ovarian reserve is less well understood (Moy et al., 2015; Sahmay et al., 2012) with conflicting results (Malhotra, Bahadur, Singh, Kalaivani, & Mittal, 2013; Moy et al., 2015; Sahmay et al., 2012; Steiner, 2013). Whether obesity also accelerates follicle loss and perhaps contributes to diminished ovarian reserve remains unclear (Klenov & Jungheim, 2014).

Class 1 had the lowest mean fasting morning cortisol levels and was the only class to have cortisol levels within the normal range. Both the mean cortisol levels in class 2 and class 3 were higher than normal. Class 2 had the highest probability of breastfeeding. While few studies have examined hypothalamic pituitary adrenal (HPA) activity in breastfeeding, one study found that morning salivary cortisol levels were higher in women who predominantly breastfed (Ahn & Corwin, 2014). Class 3 had the highest fasting morning cortisol levels. This finding may be explained by the increased mean anxiety and depression scores as well as less favorable lipid profiles observed in this class (Veen et al., 2009). Dysregulation of the HPA axis impairs reproductive function by suppressing steroidogenesis and inhibiting gonadotropin release (Schliep et al., 2015). The effect of HPA activity on ovarian reserve is less well understood, however preliminary studies suggest associations between cortisol and abnormal AMH levels (Hardy et al., 2016).

In addition to the lowest mean AMH levels and a less favorable cardiovascular risk profile, class 3 also had the highest mean depression and anxiety scores (z-scores). Few studies have examined the association between psychological factors and biomarkers of the ovarian reserve (Bleil et al., 2012a; Pal et al., 2010). Psychological disorders such as depression and anxiety are associated with impaired reproductive function (Williams, Marsh, & Rasgon,

2007) and are also associated with greater cardiovascular and metabolic disease risk (Bleil, Bromberger, et al., 2013; Lamers et al., 2012; Nikkheslat et al., 2015).

Class 2 had the highest probability of breastfeeding and of being lean, the lowest mean triglyceride levels, and the highest mean HDL levels. This is consistent with a protective effect of breastfeeding on cardiovascular health regardless of pre-conception risk factors (McClure et al., 2012). The probability of breastfeeding did not, however, demonstrate a strong class trend in relation to AMH. Several studies have reported a suppressive effect of pregnancy on the ovarian reserve (Gerli et al., 2015; Königer et al., 2013; Nelson et al., 2010). The effect of breastfeeding is not previously described, although AMH was not reduced in women with hyperprolactinaemia-induced amenorrhea (Li, Anderson, Yeung, Ho, & Ng, 2011). In this study, there was no significant difference in AMH concentrations between breastfeeding and non-breastfeeding postpartum women.

Consistent with this class also having the highest probability of breastfeeding, class 2 had the lowest E2 levels (McNeilly, 1993). Class 2 also had the highest gonadotropin levels, a finding that was unexpected and the basis for which is unclear. Class 1 had the highest E2 levels and the lowest FSH and LH levels. Class 1 also had the highest AMH levels. While AMH does not fluctuate significantly across the menstrual cycle, levels are at their highest in the late follicular phase, corresponding with rising estradiol levels (Wunder, Bersinger, Yared, Kretschmer, & Birkhäuser, 2008). Reproductive hormone levels were not able to be timed according to menstrual cycle phase in our postpartum sample, so these findings should be interpreted with caution.

This was an exploratory study with a small sample size. However, this was appropriate given that the study was exploratory in nature, it was the first to use latent class analysis to explore subgroups of risk profiles for ovarian reserve, and was meant to determine the feasibility of this type of analysis for this population. It is important to note that the sample consisted of a population of postpartum women with known fertility; most studies examining factors associated with AMH concentrations have been conducted in infertile populations.

While preliminary, the findings from this study can be used as the foundation for future analyses of subgroups in relation to the ovarian reserve. The findings from this exploratory study should be replicated in larger samples to allow for independent validation, as well as in different reproductive contexts to examine how these variables behave in other populations. Current evidence suggests that the rate of change in AMH concentrations improves the accuracy of the biomarker to predict age at menopause (Freeman, Sammel, Lin, Boorman, & Gracia, 2012). Thus, longitudinal studies will be necessary to identify additional factors contributing to variation in the process of ovarian aging.

The process of ovarian aging spans many years, and at present, there are few clues, other than chronological age, that indicate where a woman is in her reproductive lifespan (Ottinger, 2011). In this study, we explored patterns in cardiometabolic, psychological and reproductive factors in relation to ovarian reserve and found less favorable cardiometabolic and psychological profiles in women with lower ovarian reserve. The findings from this study demonstrate that LCA is a feasible and useful approach for examining subgroups

based on various parameters of health, and sheds light on factors that may contribute to variation in serum AMH concentrations, providing a window into what may be the earlier stages of ovarian aging. With validation in larger samples, this information could be used to develop reliable subgroup classifications to aid in the early detection and prevention of premature ovarian aging.

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Table 1:

Clinical and Demographic Data

	Overall (n = 69) Mean ± SD	Lean (n = 38) Mean ± SD	Obese (n = 31) Mean ± SD	p-value	Breastfeeding (n = 47) Mean ± SD	Not Breastfeeding (n = 22) Mean ± SD	p-value
Age (years)	33.97 ± 4.1	34.54 ± 3.64	33.27 ± 4.56	0.203	34.6 ± 3.93	32.66 ± 4.24	0.07
Anxiety	0.04 ± 1.08	−0.2 ± 1.04	0.33 ± 1.06	0.039	−0.05 ± 1.07	0.24 ± 1.08	0.302
Depression	−0.08 ± 0.95	−0.38 ± 0.86	0.29 ± 0.94	0.003	−0.2 ± 0.91	0.17 ± 1.01	0.132
FSH (mIU/ml)	7.02 ± 3.56	7.79 ± 3.35	6.08 ± 3.64	0.046	7.4 ± 3.34	6.19 ± 3.95	0.188
LH (mIU/ml)	5.19 ± 3.93	4.9 ± 4.12	5.53 ± 3.73	0.513	5.21 ± 4.39	5.14 ± 2.82	0.952
E2 (pg/ml)	40.02 ± 38.06	31.02 ± 26.6	51.04 ± 46.71	0.029	27.9 ± 26.12	65.91 ± 46.52	<0.0001 *
AMH (ng/ml)	2.79 ± 2.5	3.19 ± 2.81	2.3 ± 2.0	0.143	2.98 ± 2.57	2.4 ± 2.36	0.375
Serum cortisol (nmol/L)	855.7 ± 556.5	963.7 ± 587	742.5 ± 504.9	0.168	867.88 ± 537.53	758.96 ± 577.62	0.446
Cholesterol (mmol/L)	5.28 ± 1.47	5.57 ± 1.51	4.92 ± 1.36	0.064	5.29 ± 1.36	5.26 ± 1.71	0.937
HDL (mmol/L)	1.57 ± 0.46	1.68 ± 0.46	1.44 ± 0.43	0.027	1.62 ± 0.4	1.46 ± 0.56	0.196
Triglycerides (mmol/L)	1.12 ± 0.81	1.01 ± 0.77	1.25 ± 0.85	0.234	1.01 ± 0.79	1.34 ± 0.82	0.115

* Alpha level .0004 considered significant

Note: Anxiety and depression are z-scores. Abbreviations: AMH, Anti-Müllerian Hormone; HDL, High-Density Lipoprotein; FSH, Follicle Stimulating Hormone; LH, Luteinizing Hormone; E2, Estradiol

Table 2:

Latent Class Analysis: Three-class model characteristics

Continuous Variable	Class 1 (32.2%) (n=22) Mean	Class 2 (43.4%) (n=30) Mean	Class 3 (24.3%) (n=17) Mean
AMH (ng/ml)	3.83	2.88	1.86
Age	33.25	35.01	33.08
Triglycerides (mmol/L)	1.04	0.64	1.85
Cholesterol (mmol/L)	4.1	5.05	6.53
HDL (mmol/L)	1.22	1.77	1.56
Glucose (mmol/L)	4.73	4.48	4.61
Anxiety (z-score)	-0.18	-0.05	0.33
Depression (z-score)	0.02	-0.26	0.1
Cortisol (nmol/L)	437.15	955.91	974.67
FSH (mIU/ml)	5.1	8.13	6.99
LH (mIU/ml)	4.34	5.9	4.85
E2 (pg/ml)	74.19	22.85	37.04
Binary Variable	Class 1 (32.2%) (n=22) Probability	Class 2 (43.4%) (n=30) Probability	Class 3 (24.3%) (n=17) Probability
Lean	0.29	0.73	0.51
Breastfeeding (Yes)	0.47	0.94	0.49

Note: For the continuous variables, the characteristic is presented as the mean of that variable within each class, for the binary variables (breastfeeding status and lean/obese), the characteristic is presented as the probability of presenting that characteristic. Abbreviations: AMH, Anti-Müllerian Hormone; HDL, High-Density Lipoprotein; FSH, Follicle Stimulating Hormone; LH, Luteinizing Hormone; E2, Estradiol

Table 3:

Comparisons between classes by health parameters

Class (mean AMH level)	Class 1 (3.83 ng/mL)	Class 2 (2.88 ng/mL)	Class 3 (1.86 ng/mL)
Cardiometabolic	<ul style="list-style-type: none"> • Lowest cholesterol and HDL • Lowest probability of being lean • Highest glucose • Lowest cortisol 	<ul style="list-style-type: none"> • Lowest triglycerides • Highest HDL • Highest probability of being lean • Lowest glucose 	<ul style="list-style-type: none"> • Highest triglycerides and cholesterol • Highest cortisol
Psychological	<ul style="list-style-type: none"> • Lowest anxiety 	<ul style="list-style-type: none"> • Lowest depression 	<ul style="list-style-type: none"> • Highest anxiety and depression
Reproductive	<ul style="list-style-type: none"> • Highest AMH • Lowest probability of breastfeeding • Lowest FSH and LH • Highest E2 	<ul style="list-style-type: none"> • Highest probability of breastfeeding • Highest FSH and LH • Lowest E2 	<ul style="list-style-type: none"> • Lowest AMH