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Ovarian reserve parameters: a comparison between users and non-users of hormonal contraception

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Abstract   It remains controversial whether anti-Müllerian hormone (AMH) concentration is influenced by hormonal contraception. This study quantified the effect of hormonal contraception on both endocrine and sonographic ovarian reserve markers in 228 users and 504 non-users of hormonal contraception. On day 2–5 of the menstrual cycle or during withdrawal bleeding, blood sampling and transvaginal sonography was performed. After adjusting for age, ovarian reserve parameters were lower among users than among non-users of hormonal contraception: serum AMH concentration by 29.8% (95% CI 19.9 to 38.5%), antral follicle count (AFC) by 30.4% (95% CI 23.6 to 36.7%) and ovarian volume by 42.2% (95% CI 37.8 to 46.3%). AFC in all follicle size categories (small, 2–4 mm; intermediate, 5–7 mm; large, 8–10 mm) was lower in users than in non-users of hormonal contraception. A negatively linear association was observed between duration of hormonal-contraception use and ovarian reserve parameters. No dose–response relation was found between the dose of ethinyloestradiol and AMH or AFC. This study indicates that ovarian reserve markers are lower in women using sex steroids for contraception. Thus, AMH concentration and AFC may not retain their accuracy as predictors of ovarian reserve in women using hormonal contraception.

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KEYWORDS: anti-Müllerian hormone, antral follicle count, antral follicle size, female age, hormonal contraception, ovarian reserve

Introduction

Oral contraceptives suppress the hypothalamic—pituitary—ovarian axis and thereby potentially influence ovarian reserve markers. Hormonal contraception is not only used as contraception but also as a symptomatic treatment of various endocrine disorders such as polycystic ovarian syndrome and endometriosis. Among infertile patients, pretreatment with oral contraceptives is often used for programming the cycle in anovulatory women prior to ovulation induction and prior to ovarian stimulation for IVF (Andersen et al., 2011).

Among established endocrine markers of the ovarian reserve, the serum concentration of anti-Müllerian hormone (AMH) seems to be a better parameter of the age-related ovarian follicle depletion than FSH, inhibin B or oestradiol (van Rooij et al., 2005). AMH is primarily expressed in the granulosa cells of small antral follicles (Weenen et al., 2004), whereas larger antral follicles secrete lower amounts of AMH. Serum AMH concentration is strongly correlated to the total antral follicle count (AFC), i.e. AFC 2—10 mm (de Vet et al., 2002; Fanchin et al., 2003; van Rooij et al., 2002), and positively correlated to the number of non-growing primordial follicles (Kelsey et al., 2012).

Among sonographic markers of the ovarian reserve, the AFC has generally been studied in infertile populations (Bancsi et al., 2002; Haadsma et al., 2007; Hendriks et al., 2007; Jayaprakasan et al., 2010), and there are only limited data on populations of healthy women (La Marca et al., 2011; Rosen et al., 2010). Clinically, ovarian reserve assessment with AMH concentration and AFC is primarily performed in infertile patients in order to: (i) predict the ovarian responsiveness to ovarian stimulation in assisted reproduction technology (Broekmans et al., 2006); (ii) to decide the type of stimulation protocol to be used (Nelson et al., 2009; Yates et al., 2011); (iii) to evaluate the risk of developing ovarian hyperstimulation syndrome (Nardo et al., 2009); and (iv) to contribute to the prediction of reproductive lifespan and the age at menopause (Broer et al., 2011; Tehrani et al., 2011). As oral contraceptives are frequently used both in fertile and infertile women, it is important to clarify whether serum AMH concentration and AFC are changed by administration of oral contraceptives.

Studies assessing sonographic changes have unanimously found a reduction in ovarian volume and/or AFC during hormonal contraception use (Arbo et al., 2007; Deb et al., 2012; Kristensen et al., 2012; Somunkiran et al., 2007; van den Berg et al., 2010). However, oral contraceptives have been reported either to insignificantly influence AMH concentration (Li et al., 2011; Somunkiran et al., 2007; Streuli et al., 2008; Deb et al., 2012) or to reduce AMH concentration (Arbo et al., 2007; Kristensen et al., 2012; van den Berg et al., 2010). Accordingly, this study quantified the effect of hormonal contraception on endocrine and sonographic markers of ovarian reserve in terms of AMH, AFC and ovarian volume.

Materials and methods

Study population

The current study comprises data obtained from participants in a prospective cohort study consisting of 863 healthy, female healthcare workers aged 21—41 years and employed at Copenhagen University Hospital, Rigshospitalet. Participants were enrolled in the study between September 2008 and February 2010. Participants completed an internet-based questionnaire including: data on reproductive history, physical parameters (i.e. bodyweight and height) and lifestyle factors including smoking habits.

From the prospective cohort, a subgroup of 228 participants was identified who at study entry was using combined oral contraceptives or the contraceptive vaginal ring. Further, a subgroup of 504 non-users of hormonal contraception was included as controls. Among the users of hormonal contraception, 217 (95.2%) used combined oral contraceptives and 11 (4.8%) used a contraceptive vaginal ring. Among the users of oral contraceptives, 101 (44.3%) used monophasic preparations with 20 µg ethinyloestradiol, 96 (42.1%) used monophasic preparations with 30—35 µg ethinyloestradiol and 20 (8.8%) used biphasic/triphasic oral contraceptives or oral contraceptives with an unknown dose of ethinyloestradiol.

Ovarian sonography

On day 2—5 of the menstrual cycle or during withdrawal bleeding, a transvaginal sonography was performed. The number of antral follicles was counted and grouped according to three predefined size categories: 2—4 mm (small), 5—7 mm (intermediate) and 8—10 mm (large). The ovarian volume was measured as previously described by Rosendahl et al. (2010) and calculated as the mean value of the left and right ovary. Ultrasound scan was performed with a BK pro focus scanner with a 4—9 MHz transducer. To minimize observational bias, all examinations were performed by the same investigator (JBG) using the same scanner. Furthermore, the participants' hormonal profiles as well as the responses to the internet-based questionnaire were blinded to the sonographer at the time of the sonographic examination.

Other covariates

Smoking was stratified as current, former and never smokers. Current smokers were stratified into no daily exposure, 1—10 cigarettes daily and above 10 cigarettes daily.

Endocrine parameters

Blood samples were taken on cycle Day 2—5 for measurement of serum AMH concentration. Fresh blood samples were centrifuged at 3000*g* for 12 min and serum samples were stored in Nunc tubes at −24°C. The serum AMH concentrations were measured by an enzyme-linked immunosorbent assay using the AMH/MIS kit (Immunotech, Beckman Coulter, Marseilles, France). The sensitivity was 0.7 pmol/l, and the intra- and inter-assay coefficients of variation were 12.3% and 14.2%, respectively. Serum concentrations of FSH were measured by electrochemiluminescence immunoassays (Roche Diagnostics, Mannheim, Germany) with corresponding analytical sensitivity of <0.1 IU/l, and the intra- and inter-assay coefficients of variation were 2.8% and 4.5%, respectively.

Statistical analysis and modelling

Baseline characteristics

Baseline characteristics were summarized as either median and interquartile range (IQR) or number and percentage.

Demographic, endocrine, and sonographic data were compared between users and non-users of hormonal contraception by application of the Mann–Whitney U-test to the continuous outcomes and the chi-squared test to the categorical variables. The overall association between serum AMH and AFC was assessed by Spearman's rank correlation coefficient (r_s).

AFC of the sizes small, intermediate, large and total (2–10 mm) was compared in users and non-users of hormonal contraception across the age strata 20.0–29.9 years ($n = 129$), 30.0–34.9 years ($n = 67$), and 35.0–41.9 years ($n = 32$), respectively. Due to tied zero-counts, the permutation two-way ANOVA was the most appropriate for testing the differences (Pesarin and Salmaso, 2010). The 95% confidence intervals for the mean follicle counts were computed by bootstrapping (Davison and Hinkley, 1997). Similar analyses were performed with proportions of follicles in the three size groups as outcomes.

Age-related decline patterns and differences in serum AMH concentration, AFC and ovarian volume

The age-related decline in serum AMH concentration, AFC and ovarian volume was visualized in scatter plots. All three variables had skewed distributions, so logarithmic transformations were applied. To test whether the age-related rates of decline accelerated with increasing age, a non-linear regression model was applied (Hansen et al., 2008), $\log(Y) = a - b \times \text{age}^c + \varepsilon$, with Y being the outcome, a, b and c the model parameters and ε the error term. When $c = 1$, the rate of decline is constant (linear model), when $c > 1$, it increases with age, and when $c < 1$, it decreases. Median serum AMH, AFC and ovarian volume, respectively, as functions of age were estimated by non-linear least squares and compared with the corresponding linear fit. The parameter estimates displayed strong non-linear interdependence; accordingly, tests and confidence intervals could not be obtained from the conventional least-squares theory. Instead, P-values were obtained by resampling residuals, and confidence intervals were computed by bootstrapping (Davison and Hinkley, 1997).

No significant deviations from linearity were found, so further analyses were conducted as multiple linear regressions. The rates of decay of AMH, AFC and ovarian volume were compared with the corresponding rates for non-users of hormonal contraception, and the overall differences in concentration of AMH, AFC and ovarian volume were assessed.

Effect of duration and type of hormonal contraception

Whether the duration of hormonal contraception use (years) and the type of oral contraception (monophasic preparation 20 versus ≥30 μg of ethinyloestradiol) were associated with AMH, AFC and ovarian volume, respectively, was tested by a multiple linear regression model adjusting for age.

Tests were considered statistically significant for a two-sided P-value <0.05. Descriptive statistics were computed using the Statistical Package for Social Sciences version 19.0 (SPSS, Chicago, IL, USA). Statistical analyses were performed with R version 2.13.2 (R Project for Statistical Computing, Vienna, Austria).

Ethics approvals

The Ethics Committee of the Capital region of Denmark approved the study (reference no. H-B-2007-129, approval granted 10 March 2008) and verbal and written informed consent was obtained from all participants before study inclusion. Approval was procured from the Danish Data Protection Agency (journal number 2008-41-1881).

Results

Demographic characteristics

As seen in **Table 1**, hormonal contraception users were significantly younger (median 29.2 years, IQR 26.9–32.6 years) than the non-users (median 33.7 years, IQR 30.9–36.9 years). No significant differences in demographic characteristics regarding body mass index and active smoking exposure were observed between users and non-users of hormonal contraception.

Serum FSH concentration was significantly higher in users of hormonal contraception (median 7.2 IU/l, IQR 5.8–9.6 IU/l) compared with non-users (median 6.7 IU/l, IQR 5.8–7.9 IU/l, $P = 0.002$).

Comparison of age-adjusted AMH concentration, AFC and ovarian volume

The serum AMH concentration was positively correlated to total AFC in both users ($r_s = 0.82$, $P < 0.001$) and non-users ($r_s = 0.86$, $P < 0.001$) of hormonal contraception.

Table 2 presents the sonographic and endocrine findings based on multiple linear regression analyses. Compared with non-users, users of hormonal contraception had a 29.8% (95% CI 19.9 to 38.5%) lower serum AMH concentration, 30.4% (95% CI 23.6 to 36.7%) lower AFC and 42.2% (95% CI 37.8 to 46.3%) lower ovarian volume. Furthermore, **Table 2** shows that the rate of decline for AMH, AFC and ovarian volume did not differ between users and non-users of hormonal contraception. The common rate of decline and differences in serum AMH, AFC and ovarian volume in the two populations are depicted in **Figure 1**.

Table 3 shows that when follicle size was further categorized into subclasses (small, intermediate and large), the number of antral follicles was significantly lower in all three antral follicle size categories in users compared with non-users of hormonal contraception. Accordingly, hormonal contraception users had lower age-adjusted AFC than non-users: small, 4.2 (95% CI 2.7 to 5.7, $P < 0.001$); intermediate, 2.0 (95% CI 1.4 to 2.7, $P < 0.001$); large, 0.18 (95% CI 0.04 to 0.32, $P = 0.01$). Furthermore, the proportion of small follicles was higher (2.8%, 95% CI −0.6 to 6.1%) and the proportions of intermediate (−2.4%, 95% CI −5.3 to 0.6) and large follicles (−0.5%, 95% CI −1.6 to 0.8%) were lower

Table 1 Demographic characteristics of users and non-users of hormonal contraception.

Variable	Users of hormonal contraception (n = 228)	Non-users of hormonal contraception (n = 504)	P-value
Age (years)	29.2 (26.9 to 32.6)	33.7 (30.9 to 36.9)	<0.001[a]
BMI (kg/m^2)	22.2 (20.8 to 24.3)	22.4 (20.8 to 24.5)	NS[a]
Smokers			
Current	48 (21.1)	103 (20.4)	0.02[b]
Former	46 (20.2)	151 (30.0)	
Never	134 (58.8)	250 (49.6)	
Current smoking exposure			
Not daily	20 (41.7)	49 (47.6)	NS[b]
1—10 cigarettes/day	18 (37.5)	44 (42.7)	
>10 cigarettes/day	10 (20.8)	10 (9.7)	

Values are median (interquartile range) or n (%).
BMI = body mass index; NS = not statistically significant.
[a]Mann—Whitney U-test.
[b]Chi-squared test.

Table 2 Differences in ovarian reserve parameters (anti-Müllerian hormone, antral follicle count and ovarian volume) and their decline with increasing age in users (n = 228) and non-users (n = 504) of hormonal contraception.

	Anti-Müllerian hormone	Antral follicles 2—10 mm	Ovarian volume
Crude assessment (median, IQR)[a]			
Non-users	20.1 (10.7 to 32.8)	20.0 (13.0 to 30.0)	5.1 (3.9 to 6.5)
Users	19.5 (10.7 to 30.6)	17.0 (11.0 to 25.0)	3.2 (2.2 to 4.4)
P-value	NS	0.001	<0.001
Age-adjusted assessment (median%, 95% CI)[b]			
Difference	29.8 (19.8 to 38.5)	30.4 (23.6 to 36.7)	42.2 (37.8 to 46.3)
P-value	<0.001	<0.001	<0.001
Decline per year (median%, 95% CI)[c]			
Non-users	7.0 (5.4 to 8.5)	5.0 (3.8 to 6.1)	1.7 (0.7 to 2.6)
Users	9.2 (7.0 to 11.3)	6.1 (4.5 to 7.7)	2.9 (1.4 to 4.4)
P-value	NS	NS	NS

CI = confidence interval; IQR = interquartile range.
[a]Units are pmol/l for anti-Müllerian hormone, n for antral follicle count and ml for ovarian volume. Mann—Whitney U-test. [b]Multiple linear regression adjusting for age.
[c]Multiple linear regression.

in users compared with non-users of hormonal contraception within the same age group, although this result was not statistically significant.

The duration of hormonal contraceptive use was negatively associated with all three ovarian reserve parameters. Thus, for every year of hormonal contraceptive use, corresponding decreases were observed for serum AMH of 2.3% (95% CI −0.6 to 5.0%, not statistically significant), AFC of 2.5% (95% CI 0.3 to 4.5%, P = 0.02) and ovarian volume of 2.7% (95% CI 0.8 to 4.4%, P = 0.005).

Non-statistically significant differences in serum AMH (1.3%, 95% CI −17.6 to 24.6%) or AFC (0.7%, 95% CI −15.1 to 16.0%) were observed between users of oral contraceptives

using monophasic preparations of 30—35 μg versus 20 μg ethinyloestradiol.

Discussion

As far as is known, this is the largest study to demonstrate a significant negative effect on ovarian reserve parameters from the use of hormonal contraception in a population of healthy women. The ovarian reserve parameters serum AMH concentration, AFC and ovarian volume were all markedly lower in users than in non-users of hormonal contraception. Serum AMH concentration was approximately 30% lower in users of hormonal contraception. Furthermore,
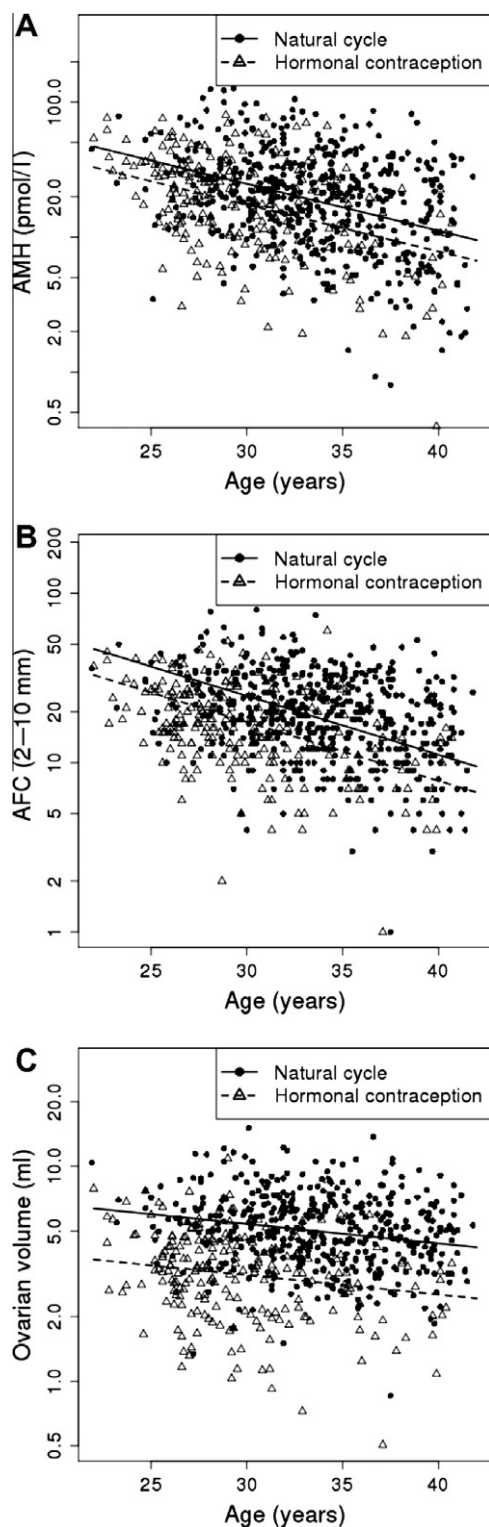
Figure 1 Relation between chronological age and ovarian reserve markers: (A) serum anti-Müllerian hormone (AMH); (B) antral follicle count (AFC); and (C) ovarian volume. Linear regression lines are grouped according to contraceptive status (users and non-users of hormonal contraception). AMH, AFC and ovarian volume are displayed in logarithmic scale. A significantly lower concentration in all ovarian reserve parameters was observed in users compared with non-users of hormonal contraception (see also Table 2).

elaborative analyses demonstrated that when follicle size was stratified into size categories, the use of hormonal contraception was significantly associated with a decreased number of antral follicles in all subclasses of follicles.

Additionally, this study found a significant decrease in AFC and ovarian volume with increasing duration of hormonal contraception use. The AMH concentration tended to decrease with increasing duration of hormonal contraception use, although statistical significance was not reached, presumably due to large variance of AMH concentration and an accordingly increased statistical uncertainty.

In this study, the overall duration of hormonal contraception use was reported retrospectively as the sum of years in which a woman had used hormonal contraception. However, it may have been more appropriate had the most recent period of use been recorded. Another limitation of this study was that data were retrospectively collected in regard to duration and type of hormonal contraception which may have induced recall bias. In addition, the use of questionnaire as a method of data collection may have caused bias. Lastly, bias may occur due to confounding by indication. Thus, specific data on the indication for oral contraceptives would have been appropriate.

To date, studies on sonographic changes have unanimously shown a reduction in ovarian volume and/or AFC during hormonal contraceptive use (Arbo et al., 2007; Deb et al., 2012; Kristensen et al., 2012; Somunkiran et al., 2007; van den Berg et al., 2010). However, ambiguous results exist in the relatively sparse literature regarding the impact of hormonal contraception on serum AMH concentration. In line with the current results, it was recently reported in a large cross-sectional study of 256 women that AMH concentration and AFC were significantly lower in users of oral contraceptives than in non-users (Kristensen et al., 2012). Further, van den Berg et al. (2010) reported that endocrine (AMH and FSH) and ultrasound markers (AFC and ovarian volume) measured at the end of the hormone-free interval in users of hormonal contraception did not seem to represent subsequent natural early follicular-phase values. Lastly, Arbo et al. (2007) studied 20 women and showed that administration of oral contraceptives in the luteal phase in one menstrual cycle may lead to a lower AMH concentration in the follicular phase in the following menstrual cycle.

Conversely, three studies with small sample sizes (23—34 patients) and one study with a relatively large sample size (143 patients) showed that, in selected groups of infertile patients including polycystic ovarian syndrome, serum AMH concentration did not change significantly during oral contraceptive treatment (Li et al., 2011; Somunkiran et al., 2007; Deb et al., 2012; Streuli et al., 2008; Andersen et al., 2011). Li et al. (2011) measured serum AMH concentration before and 3—4 months after use of hormonal contraception, administered as combined oral contraceptive pill (*n* = 23), combined injectable contraceptives (*n* = 23), progestogen-only injectables (*n* = 20) or levonorgestrel intrauterine devices (*n* = 20): no significant differences in serum AMH were found between pre- and post-treatment measurements within all treatment groups. Somunkiran et al. (2007) measured early follicular serum AMH, AFC and ovarian volume in 30 women with polycystic ovarian syndrome and in 15 women with regular cycles, before

Table 3   Characteristics of antral follicle subclasses according to age groups.

AFC group	Users of hormonal contraception[a]				Users compared with non-users of hormonal contraception	
	20.0—29.9 years (n = 129)	30.0—34.9 years (n = 67)	35.0—41.8 years (n = 32)	P-value[b]	Difference in AFC[c]	P-value[c]
2—10 mm	21.5 (19.8 to 23.2)	17.7 (15.4 to 20.2)	10.4 (8.6 to 12.3)	0.001	−6.4 (−8.2 to−4.6)	0.001
2—4 mm	15.9 (14.5 to 17.4)	12.4 (10.5 to 14.4)	7.3 (5.9 to 8.7	0.001	−4.2 (−5.7 to −2.7)	0.001
5—7 mm	5.3 (4.6 to 6.0)	4.8 (3.9 to 5.8)	2.7 (1.8 to 3.6)	0.002	−2.0 (−2.7 to −1.4)	0.001
8—10 mm	0.27 (0.16 to 0.40)	0.46 (0.28 to 0.66)	0.44 (0.16 to 0.75)	NS	−0.18 (−0.32 to −0.04)	0.01

Values are mean follicles (95% confidence interval).

AFC = antral follicle count.

[a]10,000 bootstrap samples were used to compute the 95% confidence intervals.[b]Non-parametric permutation test based on 10,000 permutations.

[c]Non-parametric permutation two-way ANOVA adjusting for age.

and 6 months after treatment with an oral contraceptive pill; the study found that AMH was unchanged, both in women with polycystic ovarian syndrome and those with regular cycles. The strengths of these studies are the repeated measurements in the individual woman, although the sample sizes were small.

Also, Deb et al. (2012) conducted a prospective case—control study to examine the effect of prolonged use (>1 year) of combined oral contraceptive pills on serum AMH concentration and AFC. They included 34 volunteers (oral contraceptive usage >1 year) as cases and 36 volunteers (oral contraceptive usage <1 year) as controls. No differences in serum AMH were found between the two groups. As in the current study, Deb et al. (2012) differentiated between smaller and larger antral follicles and found that hormonal contraception suppressed larger (6—10 mm) antral follicles and ovarian volume. In a large multinational study of normally menstruating patients entering ovarian stimulation for IVF or intracytoplasmic sperm injection, Andersen et al. (2011) showed that short-term administration (14—21 days) of oral contraceptives did not induce changes in serum AMH concentration.

According to the classical hypothesis by Gougeon (1996), normal folliculogenesis takes approximately 3 months, during which the early phase of follicle development is non-gonadotrophin dependent while the follicles become gonadotrophin responsive at the antral stage. During treatment with hormonal contraception, the exogenous sex steroids inhibit FSH secretion; consequently, FSH reaches normal intercycle values during only a very short period in the hormone-free interval (Andersen et al., 2011). This was also observed in the current study. Accordingly, only basal tonic gonadotrophin concentrations are present to stimulate early follicular growth during most of the cycle, consistent with the finding that induction of multifollicular growth requires larger doses of exogenous gonadotrophins when ovarian stimulation is performed after a withdrawal bleeding following oral contraceptive use (Rombauts et al., 2006). Thus, if it is posited that the full impact of hormonal contraception is achieved after up to 3 months of use, short-term administration of oral contraceptives as single-cycle, or even shorter, administration may have a

very limited impact on ovarian reserve parameters. In contrast, long-term use of hormonal contraception may lead to a reduced number of developing antral follicles and to a smaller size of the antral follicles. This is consistent with the current findings of lower serum AMH concentration, lower AFC and smaller ovarian volume among users of hormonal contraception.

Nevertheless, the duration of hormonal contraception does not elucidate the ambiguous results in the existing literature on this topic. Studies on short-term administration of oral contraceptives have revealed disparities regarding their influence on serum AMH concentration (Andersen et al., 2011; Arbo et al., 2007; Streuli et al., 2008). Similarly, prolonged administration of oral contraceptives has been associated with an inconsistent effect on serum AMH concentration (Deb et al., 2012; Kristensen et al., 2012; Li et al., 2011; Somunkiran et al., 2007).

The current study was unable to show a dose effect of ethinyloestradiol on ovarian reserve parameters. Although 20 μg ethinyloestradiol is considered a low dose and 30—35 μg ethinyloestradiol an average dose, the 10—15 μg difference may, nevertheless, be too small to detect any statistical significance between the doses.

The short-term effect of hormonal contraception on ovarian function is assumed to be reversible, which is consistent with the finding of van den Berg et al. (2010), who showed a significant increase in serum AMH concentration after termination of oral contraceptives.

As the early stages of follicular recruitment for growth are believed to be gonadotrophin independent, hormonal contraception should not permanently influence age at menopause (Bromberger et al., 1997; Cramer et al., 1995; Nagel et al., 2005; Torgerson et al., 1994). However, in one large study (n = 4523), women using oestrogen for a long period (≥3 years) in a high dose (>50 μg) were reported to enter menopause at a slightly younger age than women who did not use oral contraceptives or women using lower doses of oral contraceptives (de Vries et al., 2001). The current finding of a 30% reduction in ovarian reserve parameters may not be viewed as influencing the long-term ovarian follicular depletion. However, the reductions in serum AMH concentration and AFC, which are probably

temporary, should be taken into account when counselling women on their reproductive lifespan as these parameters might improve after termination of hormonal contraception use. Secondly fertility specialists should be aware of the influence of hormonal contraception when using AMH and AFC to determine protocols and gonadotrophin doses for ovarian stimulation.

Taken together, consistencies exist in the literature on the sonographic changes related to hormonal contraceptive use (Arbo et al., 2007; Deb et al., 2012; Kristensen et al., 2012; Somunkiran et al., 2007; van den Berg et al., 2010). However, regarding the effect of hormonal contraception on serum AMH, the literature to date is inconsistent. Further clarifying, longitudinal, large-sample studies are needed to explore the impact of dose—response and duration of hormonal contraception on serum AMH concentration. Accordingly, there is a need for large prospective longitudinal studies in which ovarian reserve parameters (AMH, AFC, ovarian volume) are measured repeatedly in the same individual before, during and after the use of oral contraceptives.

In conclusion, this study indicates that the ovarian reserve markers, i.e. serum AMH concentration, AFC and ovarian volume, are negatively affected by exogenous sex steroids used for contraception. These findings add weight to the notion that serum AMH concentration and AFC may not retain their value as predictors of ovarian reserve in women using hormonal contraception.

Acknowledgements

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