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Serum antimüllerian hormone levels remain stable through the menstrual cycle and after oral or vaginal administration of synthetic sex steroids

THESE

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Rapport de synthèse

Les concentrations sériques d'hormone antimüllérienne restent stables pendant le cycle menstruel et après administration orale ou vaginale de stéroïdes sexuels de synthèse

Objectif de l'étude : étudier si l'administration orale ou vaginale d'hormones contraceptives influence les concentrations sériques d'hormone antimüllérienne (AMH).

Design : essai prospectif chez des femmes recrutées par annonce. Les femmes désireuses d'avoir une contraception ont été randomisées entre une contraception orale et une contraception vaginale. Celles qui ne souhaitaient pas de contraception ont été incluses dans le groupe témoin.

Cadre de l'étude : unité de médecine de la reproduction d'un hôpital universitaire.

Patientes : vingt-quatre jeunes femmes en bonne santé avec des cycles menstruels réguliers qui n'avaient pas utilisé de contraception hormonale pendant les trois mois précédant l'étude.

Intervention : contraception orale ou vaginale du 5ème au 25ème jour du cycle menstruel dans les groupes contraception versus pas de contraception dans le groupe témoin.

Mesure d'issue : variations inter et intra-cycle des concentrations sériques d'AMH dans les trois groupes: groupe témoin en cycle spontané et groupes sous contraception oestroprogestative orale ou vaginale.

Résultats : les fluctuations d'AMH observées pendant le cycle menstruel (variations intra-cycle) restent dans les valeurs des variations entre deux cycles (variations inter-cycles) tant chez les femmes en cycle spontané que chez les femmes sous contraception orale ou vaginale.

Conclusions : nos résultats confirment que les concentrations sériques d'AMH restent stables pendant le cycle menstruel et indiquent qu'elles ne sont pas influencées par l'administration exogène de stéroïdes sexuels contraceptifs, que ce soit par voie orale ou vaginale.

CLINICAL ARTICLE

Serum antimüllerian hormone levels remain stable throughout the menstrual cycle and after oral or vaginal administration of synthetic sex steroids

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Objective: To investigate whether the oral or vaginal administration of contraceptive hormones might affect antimüllerian hormone (AMH) levels.
Design: Prospective trial with women recruited by advertisement. Women who wished contraception were randomized between oral or vaginal estroprogestative contraception, and those who did not choose contraception were included in the control group.
Setting: Fertility clinic of a tertiary university hospital.
Patient(s): Twenty-four young, healthy volunteer women with regular cycles who had received no hormonal contraception for at least 3 months before the study.
Intervention(s): Oral or vaginal estroprogestative contraception from day 5 to 25 of a menstrual cycle versus no contraception.
Main Outcome Measure(s): Intercycle and intracycle variations of serum AMH levels in normally ovulating volunteers and following the initiation of oral or vaginal estroprogestative contraception.
Result(s): Fluctuations of AMH levels observed during the menstrual cycle remained within cycle-to-cycle variability in cycling controls and in women receiving oral or vaginal contraception.
Conclusion(s): Our findings confirm that AMH levels remain steady during the menstrual cycle, indicating that they are unaffected by exogenous sex steroids used for contraception whether administered orally or vaginally. (Fertil Steril® 2007; ■: ■–■. ©2007 by American Society for Reproductive Medicine.)

Key Words: Antimüllerian hormone, AMH/MIS, hormonal contraception, sex steroids, menstrual cycle

Antimüllerian hormone (AMH), a member of the transforming growth factors superfamily, is solely synthesized in genital organs. Known for over five decades for its role in the organogenesis of internal sex organs, AMH has recently been rediscovered when ultrasensitive assays revealed that it is synthesized by adult gonads in both sexes. In females, AMH is produced by preantral and small antral follicles and may serve as a paracrine regulator of early follicular growth (1). Mounting evidence indicates that AMH levels, which reflect the size of the cohort of recruitable follicles, also predict the magnitude of the ovarian response to controlled ovarian hyperstimulation (COH). As the number of preantral and antral follicles directly reflects the size of the cohort of primordial follicles, AMH levels have been proposed as a marker of ovarian reserve.

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Several studies have supported the concept that AMH levels remain constant throughout the menstrual cycle in the adult female (2, 3), in pregnancy, and in the puerperium (4). Hence, AMH levels appear to be independent of gonadotropin levels. The stability of AMH levels throughout the various steps of reproductive physiology constitutes a definite practical advantage for using AMH measurements when investigating and managing infertility. The stability of AMH makes it the only marker of ovarian reserve that can be measured indiscriminately during the menstrual cycle for fertility workups. Yet, in our practice, incidental findings of low AMH levels encountered in women receiving contraceptive hormones vaginally led us to query whether AMH levels might be affected by exogenous estroprogestative hormones. Our randomized trial studied the effect of oral or vaginal administration of contraceptive hormones on AMH levels in women who previously had ovulated regularly.

MATERIALS AND METHODS

The study protocol was reviewed and accepted by the institutional review board. Written, informed consent was obtained from each woman participating in the study. We recruited 24

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healthy volunteers to participate in this trial. All volunteers were regularly cycling women, with menstrual cycles lasting from 25 to 35 days, who had received no hormonal contraception for at least 3 consecutive months before recruitment. The study took place between March and September 2006 at the infertility clinic of the Geneva University Hospital. The study protocol excluded women whose history revealed irregular cycles and/or was suggestive of polycystic ovary syndrome (PCOS) and/or constituted a contraindication to hormonal contraception (high blood pressure, family history of thrombosis, or sudden unexplained death).

The women were recruited by advertisement and were first asked whether they were interested in contraception. Those who wished contraception were randomized between two contraceptive methods. The women who did not want any contraception were included in the control group (group 1).

The 14 women accepted hormonal contraception were randomized to receive either 0.02 mg of ethinylestradiol (EE) plus 0.15 mg of desogestrel (DSG) orally (Mercilon; Organon Pharmaceuticals, Oss, the Netherlands) (group 2) or 0.015 mg of EE and 0.12 mg of etonogestrel, the active metabolite of DSG, through a vaginal ring (Nuvaring; Organon) (group 3). The hormones were administered from day 5 to 25 of the menstrual cycle. Group randomization relied on opaque, sealed envelopes. Ten women without contraception were included in the control group.

Blood samples for AMH measurement were taken on four occasions: days 2 to 4 (intercycle 1), days 16 to 18, days 23 to 25 of the first menstrual cycle, and days 2 to 4 of the next menstrual cycle (intercycle 2).

Serum AMH was measured by enzyme-linked immunosorbent assay (ELISA) using the AMH/müllerian-inhibiting substance (MIS) ELISA kit (Immunotech-Beckman, Marseilles, France). The detection limit of the assay was 0.1 ng/mL; the interassay coefficients of variation were 9.6% for a serum AMH concentration of 3.5 ng/mL and 13.7% for a serum AMH concentration of 9 ng/mL. The intra-assay coefficient of variation was 4.1% for a serum AMH concentration >0.4 ng/mL. These coefficients of variation served as a reference for judging AMH variations occurring during the menstrual or hormone contraception cycles.

On days 2 to 4 of the first cycle, an ultrasound with antral follicular count (AFC) was performed and serum follicle-stimulating hormone (FSH) levels were measured. Serum FSH levels were also measured on days 2 to 4 of the following cycle.

Statistical analysis was performed using SPSS software version 14.0 (SPSS, Inc., Chicago, IL). The power analysis for repeated measures was performed using Acluster Version 2.0 (World Health Organization, Geneva, Switzerland). Spearman's correlation test was used to analyze the relationships among AFC, age, and the AMH and FSH levels on days 2 to 4 of both cycles. An analysis of variance (ANOVA) for repeated measures was used to analyze AMH fluctuations occurring within the menstrual cycle in the control group (group 1) and after administering contraceptive hormones in groups 2 and 3. Differences in AMH values among the three groups were tested using ANOVA. Data are expressed as mean ± standard deviation (SD). In all analyses, $P<.05$ was considered statistically significant.

RESULTS

All three study groups had comparable characteristics, with overall mean of 24.1 years (SD ± 3.5) for age, 21.5 kg/m^2 (SD ± 2.1) for body mass index, 29.3 days (SD ± 2.7) for cycle length, and 13 years (SD ± 1.2) for age at menarche (Table 1). None of the differences among the groups reached statistical significance.

The FSH levels were measured in all of the women on days 2 to 4 of both studied cycles. All of the women had FSH levels <10 IU/L in both cycles, with an overall mean of 5.34 (SD ± 1.58) IU/L and 5.32 (SD ± 2.23) IU/L in the first and second cycles, respectively. The AFC, evaluated on days 2 to 4 of the first cycle, was normal in all of the women, ranging from 17 to 41 follicles and with an overall mean of 28.5 follicles (SD ± 7). There were no differences in FSH levels or AFC among the three groups. The FSH and AMH measures showed a statistically significant inverse correlation on days 2 to 4 of both cycles with Spearman's correlation coefficients of −0.57 and −0.53 for the first and second cycles, respectively. There was no correlation of AFC with age or the baseline AMH or FSH levels, with Spearman's correlation coefficients of 0.15, −0.01, and −0.3, respectively.

In the control group, mean AMH levels on days 2 to 4 were 4.4 (SD ± 1.2) and 4.2 (SD ± 1.4) ng/mL for the first and second cycles, respectively. Mean intracycle levels on days 16 to 18 and 23 to 25 were 4.2 (SD ± 1.7) and 4.3 (SD ± 2.29) ng/mL, respectively. In women who started oral contraception on day 5 (group 2), the mean AMH levels on days 2 to 4 were 5.0 (SD ± 2.0) and 6.2 (SD ± 3.0) ng/mL for the first and second cycles, respectively. The mean intracycle levels on days 16 to 18 and 23 to 25 were 5.2 (SD ± 2.2) and 5.4 (SD ± 2.3) ng/mL, respectively. In the women who started vaginal contraception on day 5 (group 3), the mean AMH levels on days 2 to 4 were 4.9 (SD ± 3.3) and 5.5 (SD ± 4.1) ng/mL for the first and second cycles, respectively. The mean intracycle levels on days 16 to 18 and 23 to 25 were 4.5 (SD ± 2.8) and 4.8 (SD ± 3.1) ng/mL, respectively (Fig. 1).

The longitudinal changes of AMH levels in individual women in the three groups are described in Figure 2.

In the control group, the intercycle variability of individual AMH values was 28.5 ± 26.4% (group 1). This did not differ in a statistically significant fashion from the values of 26.8 ± 30.8% and 23.8 ± 18.8% observed for intercycle variability of individual values following oral and vaginal contraception (groups 2 and 3), respectively.

In all cases, the changes in AMH values measured during the menstrual cycle and while receiving oral and vaginal

TABLE 1

Patient characteristics in the three groups (mean and standard deviation).

	Control group	Contraceptive pill group	Contraceptive ring group	Total
Number of women	10	7	7	24
Age (years)	24.3 (4.9)	24.6 (1.3)	23.3 (2.7)	24.1 (3.5)
Cycle length (days)	28.4 (1.6)	30.3 (2.4)	29.7 (4.0)	29.3 (2.7)
Body mass index (kg/m^2)	21.3 (2.1)	21.4 (1.2)	21.9 (3.0)	21.5 (2.1)
Number of pregnancies	0.2 (0.4)	0	0.1 (0.4)	0.13 (0.3)
Number of deliveries	0	0	0	0
Antral follicular count	30.7 (8.2)	27.7 (5.1)	26.3 (6.8)	28.5 (7.0)
FSH day 2–4 (cycle 1)	5.5 (1.9)	5.5 (1.2)	5.0 (1.5)	5.3 (1.6)
FSH day 2–4 (cycle 2)	5.6 (2.3)	4.8 (2.8)	5.3 (1.9)	5.3 (2.2)

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contraception remained well within the intercycle variability observed in untreated controls. None of the intracycle variations within each group and compared among the three groups reached statistical significance.

The intraclass correlation coefficients were 0.72 between groups 1 and 2, 0.85 between groups 1 and 3, and 0.89 between groups 2 and 3. Our study had enough participants to detect differences corresponding to ±1.5 SD of AMH levels (3 ng/mL), a clinically meaningful difference. In this case the study power varies between 77% and 90% for intraclass correlation coefficients between 0.9 and 0.7. Our study is underpowered, however, for detecting differences corresponding to ±1 SD of AMH levels (2 ng/mL), with a power in the latter case ranging between 44% and 55% for intraclass correlation coefficients between 0.9 and 0.7.

DISCUSSION

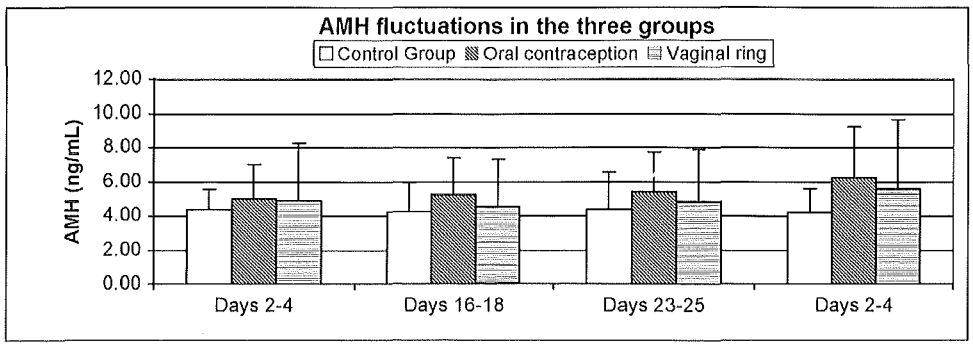
Our study confirms prior reports indicating that AMH levels do not fluctuate significantly during the menstrual cycle. In our study, variations in AMH levels seen during the menstrual cycle fell well within the intercycle variability observed in our participants. Our results also suggest that hormonal contraception, whether administered orally or vaginally, has no effect on AMH levels.

The mean AMH levels tended to be higher in group 2, who were receiving oral contraception, intermediate in the group 3, who were receiving vaginal contraception, and lowest in the controls. However, these differences among the three groups had existed before treatment was initiated and were not statistically significant. As illustrated in Figure 2, longitudinal changes of AMH levels observed in each individual were comparable among the three groups.

Our study had enough power to detect a clinically meaningful difference of 3 ng/mL, equivalent to ±1.5 SD of the overall mean of AMH levels. Further studies are needed to detect possible fluctuations of AMH levels of smaller amplitude taking place during the menstrual cycle or as a result of oral or vaginal contraception. Such smaller differences might be informative

FIGURE 1

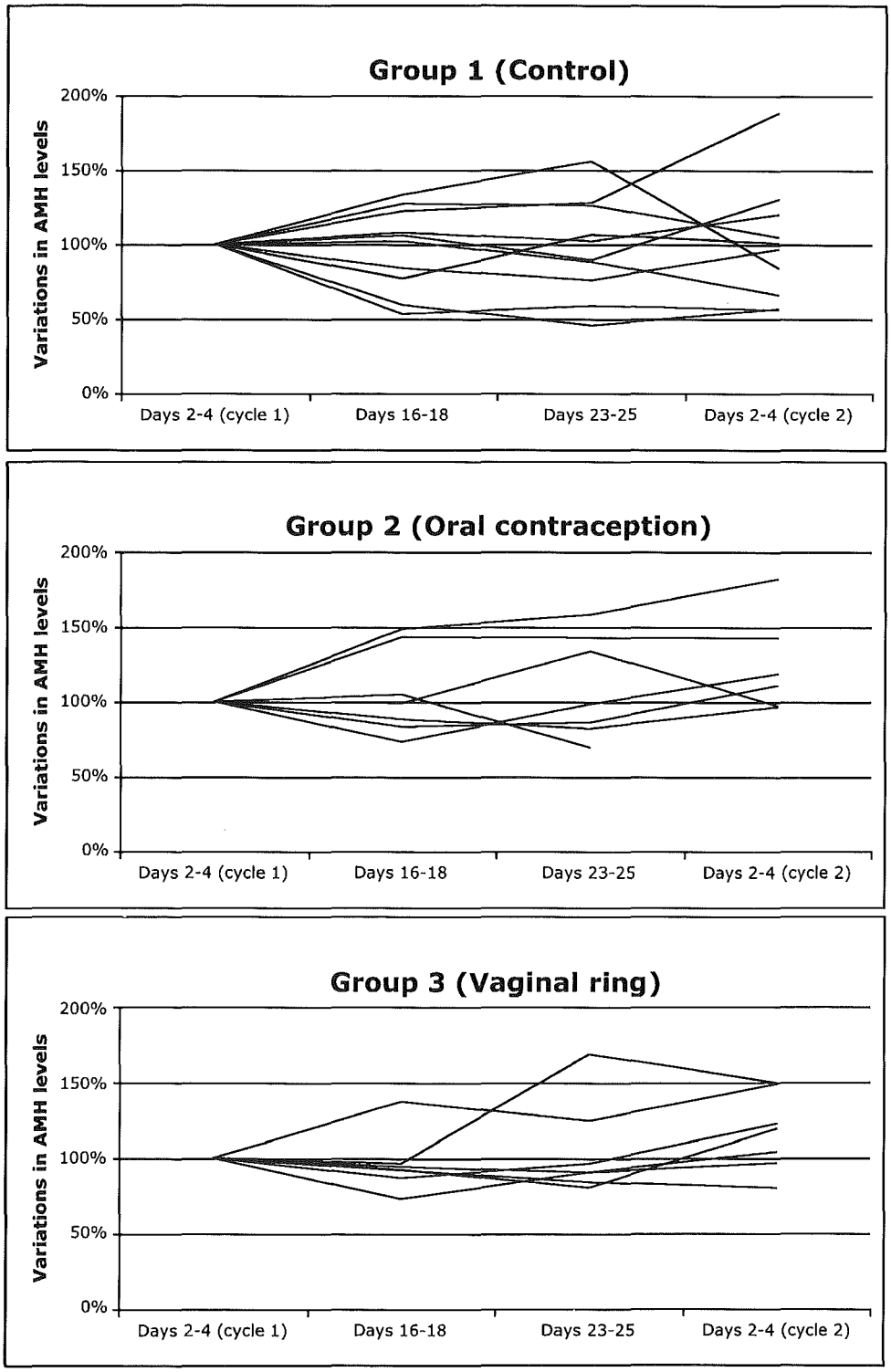
Antimüllerian hormone (AMH) fluctuations in the three groups. Serum AMH levels measured at four different times (mean ± SD) in the three groups. The AMH levels remained stable in cycling controls (group 1, open bars, n = 10) and in women receiving hormone contraception administered orally (group 2, diagonal hatching, n = 7) or vaginally (group 3, horizontal hatching, n = 7).



Streuli. AMH and hormonal contraception. Fertil Steril 2007.

FIGURE 2

Longitudinal changes of antimüllerian hormone (AMH) levels in individuals. Each patient's AMH level on days 2 to 4 of the first cycle serves as a baseline. Levels of AMH on days 16 to 18 and 23 to 25 of the first cycle and on day 2 to 4 of the second cycle are expressed as variations from the baseline level.



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on possible regulatory mechanisms affecting circulating AMH levels, but they would be of minor clinical meaningfulness when interpreting AMH levels for assessing ovarian reserve.

All study participants were normally cycling women in their twenties with no contraception for at least 3 months before the study, no signs or history of PCOS, and a normal ovarian reserve as assessed by FSH levels and AFC on days 2 to 4. Randomization only concerned women who desired contraception; it was considered ethically undesirable to randomize between contraception and no contraception because of the risk of undesired pregnancies. Otherwise, the three groups had comparable characteristics, as illustrated in Table 1, and we believe that this limitation in the study design was unlikely to affect our results and conclusions. Our findings show a wide variation in follicle numbers in women of similar age and no correlation of AFC with AMH and FSH levels or age.

These results seem surprising at first, but we realize that there is scarce information on AFC in cycling women in their twenties who have no history of infertility or PCOS. Two studies that reported high AFC values with wide variations in women of this age group are supportive of our findings. Ruess et al. (5) reported a mean AFC of 36.6 (SD $\pm$ 12.1) with a range of 23 to 63 in 10 women in their early twenties. A study by Scheffer et al. (6) reported a high variation in AFC within each age group. A recently published study by Somunkiran et al. (7) described a positive correlation between AFC and baseline AMH levels in 30 women with PCOS. Yet the same correlation was absent in 15 normally cycling controls who were matched for age and body mass index.

Our findings are comparable with those reported by La Marca et al. (3) who found stable AMH levels measured every other day in 12 cycling women with a mean age of 23 $\pm$ 3 years. Another study by Hehenkamp et al. (2) conducted in 44 women with proven fertility, aged 25 to 46 years (mean age: 38 years) also concluded that AMH does not follow a cyclic pattern and is independent of hormonal fluctuations in the hypothalamo-ovarian crosstalk system.

The only data suggesting a small but statistically significant periovulatory rise in AMH levels were reported by Cook et al. (8). However, these investigators concluded that the small fluctuations observed were probably of no clinical relevance and did not suggest AMH dependency on the hypothalamo-ovarian feedback mechanism.

Our study extends these findings by indicating that AMH levels also are unaffected by exogenous estrogens and progestins used for contraception, whether administered orally or vaginally. This information is of great practical value as it indicates that AMH measurement retains its entire value as a predictor of ovarian reserve even in women taking hormonal contraception.

Our results are consistent with the concept that AMH reflects the continuous noncyclic growth of small follicles in the ovary (1). Schematically, two steps are recognized in follicular growth depending on whether it is dependent of gonadotropins. The initial recruitment and growth of primordial follicles, which appears relatively gonadotropin independent is referred as the noncyclic recruitment phase. The second or cyclic phase leads to the recruitment, selection, and maturation of the ovulatory follicles under the influence of FSH and LH. Weenen and al. (1) studied AMH expression patterns in human granulosa cells at different stages of follicular development. AMH was absent in primordial follicles and reached its highest expression levels in preantral and small antral follicles, disappearing in larger follicles undergoing recruitment and in atretic follicles. These results strengthen the hypothesis that AMH, produced by the pool of growing follicles, acts as a paracrine feedback signal inhibiting initial recruitment of primordial follicles. Thus, it is logical to assume that AMH levels do not fluctuate during the menstrual cycle and should therefore not be affected by hormonal changes such as those induced by pregnancy or exogenous hormonal administration, including hormonal contraception.

La Marca et al. (4) evaluated AMH levels during gestation and the early puerperium and studied the relationship between FSH and AMH levels during pregnancy. The values of AMH remained stable during gestation, suggesting that early follicular development, and therefore AMH production, continues during pregnancy without being affected by decreasing FSH levels.

Oral or vaginal administration of contraceptive hormones profoundly affects gonadotropin levels and in turn ovarian function. Our results showed no differences in AMH levels after regularly cycling women initiated oral or vaginal hormonal contraception. This further supports the concept that AMH levels are not affected by changes in gonadotropins induced by exogenous estroprogestative hormones and that the noncyclic recruitment of follicles is not altered during hormonal contraception. One limitation of our study, however, is its duration. For methodological reasons, we selected women who had not been on hormone contraception for at least 3 months and studied the impact of 1 month of treatment.

After submission of our study, Somunkiran et al. (7) reported on the effects of hormonal contraception (35 μg of EE and 2 mg of cyproterone acetate) on AMH levels in 30 women with PCOS and in 15 normally cycling controls matched for age and body mass index, and found no changes in either group over a period of 6 months. The investigators concluded that AMH can be used as a diagnostic marker of PCOS in patients taking contraception. Our study confirms that conclusion and extends it to vaginally administered hormonal contraception.

Our observation that contraceptive hormones have no effect on AMH levels further supports the concept that AMH is not affected by FSH levels, at least not within the range of changes commonly encountered in reproductive physiology.

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