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Inter-cycle variability of anti-Müllerian hormone: implications for predicting controlled ovarian stimulation cycle outcomes

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Abstract

Background Anti-Müllerian hormone (AMH) is a widely used marker for estimating ovarian reserve, and it may predict response to ovarian stimulation. While AMH is considered a stable, cycle-independent marker, studies have shown it can exhibit significant fluctuations based on factors like age, reproductive stage, and menstrual cycle phase. The fluctuations in AMH levels can make it challenging to predict individual responses accurately, particularly when the AMH is not measured in the COS cycle. The aim of this study was to assess the inter-cycle variability of serum AMH levels in two consecutive menstrual cycles and their correlation with response to controlled ovarian stimulation outcome in the latter.

Methods In this single-centre retrospective cohort study, data of normal and low responder patients who underwent intracytoplasmic sperm injection following a GnRH antagonist cycle at a university hospital infertility clinic between January 2022 and December 2023 were reviewed. Serum AMH levels were measured in the early follicular phase of two consecutive menstrual cycles with Elecsys-AMH Roche® system (Roche Diagnostics, Meylan, France). Correlations between AMH levels and controlled ovarian stimulation outcomes, including total oocyte and mature oocyte (MII) counts, were assessed. The study included normal and poor responder women to maintain data integrity.

Results A total of 79 patients were included in the final analyses. Significant cycle-to-cycle variation in serum AMH levels was observed, with a median variation of 44.3%. Normal responders exhibited a mean change of 0.60 ± 0.46 ng/ml, while poor responders had a mean change of 0.28 ± 0.28 ng/ml. Approximately 20% of patients were reclassified between normal and poor responder categories based on the second AMH measurement. The controlled ovarian stimulation cycle AMH levels showed a stronger correlation with both total oocyte count ($r=0.871$, $P<0.001$) and MII oocyte count ($r=0.820$, $P<0.001$) compared to preceding cycle AMH levels.

Conclusion AMH levels can exhibit significant variations between consecutive cycles, potentially leading to misclassification of patients. Measuring AMH in the early follicular phase of the COS cycle provides a more accurate prediction of the numbers of total and MII oocytes collected. Consistent and repeated AMH measurements can help clinical decision-making.

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Keywords Anti-Müllerian hormone, Infertility, Inter-cycle variation, Ovarian reserve test

Introduction

Anti-Müllerian hormone (AMH) is one of the most common hormonal markers used to estimate ovarian reserve. It is a transforming growth factor- β family member, a glycosylated and dimeric protein secreted by granulosa cells of preantral and small antral follicles [1]. Previous studies have shown positive correlations between the serum levels of AMH and histologically determined primordial follicle pool and antral follicle count (AFC) which can be observed by ultrasound imaging [2]. The general acceptance is that AMH, as a stable, cycle-day-independent marker, is a reflection of small antral follicles and also can be a predictor of the response to controlled ovarian stimulation (COS) [3, 4].

Over the years, some researches have advocated and encouraged personalized COS regimens [3, 4]. Most of those studies have suggested that AMH could be used to predict the COS cycle outcome. The studies investigating the consistency of AMH levels within and between menstrual cycles have shown that AMH does not conform to the conventional endocrine cycle pattern and maintains a consistent profile over an extended duration [5–7]. Subsequent studies began to focus on intra-cycle and inter-cycle variation of AMH levels, and it has been highlighted that AMH levels can exhibit significant fluctuations, which depend on factors such as reproductive stage, age category, or menstrual cycle phase [8–11]. Furthermore, AMH levels are subject to various influences, such as body mass index, smoking habits, genetic variables, polymorphisms of AMH and the AMH receptor, vitamin D status, and ethnicity [11, 12].

Although much research on AMH has been published over the last two decades, it's not clearly recommended in the diagnoses of poor or high ovarian response. However, it's a commonly performed test in daily infertility practice. Although we usually measure AMH time-independently, intra- and inter-cycle changes may make it difficult to predict COS response for some specific patients. The aim of this article was to compare changes in AMH levels between two consecutive menstrual cycles and to evaluate the correlation of two different cycle AMH levels with COS response. This information can provide accurate timing and assessment of AMH measurement and has the potential to contribute to clinical strategy.

Materials and methods

In this single-centre retrospective cohort study, data of normal and low responder patients who underwent intracytoplasmic sperm injection following a GnRH antagonist cycle at the Infertility Centre, Ankara University Cebeci Hospital, between January 2022 and December

2023 were reviewed. The study protocol was approved by the Institutional Review Board of Ankara University School of Medicine (approval no: E-12405952-050.01.04-1373265). All patients to be included had two separate serum AMH measurements: one in the early follicular phase of controlled ovarian stimulation cycle just before commencement of gonadotropins and one in the early follicular phase of the preceding non-stimulated menstrual cycle. All data related to COS were extracted from the patient files and hospital database.

The inclusion criteria were female age 20–42 years, GnRH antagonist cycle, a gonadotropin starting dose of 225–300 IU/day. The exclusion criteria were utilization of long or natural cycle protocols, progestin-primed ovarian stimulation, presence of any untreated thyroid dysfunction or hyperprolactinemia, polycystic ovary syndrome according to Rotterdam Criteria, previous ovarian surgery, an ovarian cyst, exposure to cytotoxic drugs or pelvic radiation, hormonal contraceptive use within 3 months before sample collection and/or history of hyper-response in a previous COS cycle.

Before initiation of treatment, all patients underwent vaginal ultrasound to eliminate presence of > 10 mm follicles on day 2 of the cycle. Baseline hormonal profile was also assessed. Ovarian stimulation was carried out with recombinant FSH (Gonal-F; Merck-Serono, Geneva, Switzerland) and/or human menopausal gonadotropin (hMG; Menopur; Ferring GmbH, Wittland, Kiel, Germany) beginning from the second day of the menstrual cycle with a starting dose of 225–300 IU/day. Dose adjustment was performed individually according to ovarian response, as assessed by estradiol levels and ultrasound. The maximum dose of rFSH was 375 IU/day. The GnRH antagonist cetrorelix 0.25 mg/day (Cetrotide; Merck-Serono, Geneva, Switzerland) was initiated in a fixed manner on the sixth day of stimulation and continued throughout ovarian stimulation. Transvaginal ultrasound guided double-lumen oocyte retrieval was performed 36 h after final oocyte trigger (Ovitrelle 250 μ g, Merck Serono, Modugno, Italy).

The primary outcome measures were the change of serum AMH levels between index and preceding menstrual cycles and their correlations with the number of total and MII oocytes collected. A secondary outcome measure was the cycle-to-cycle variation of serum AMH levels indicated by percentage change and its impact on the clinical patient stratification regarding POSEIDON criteria. In order to make comments based on objective criteria, AMH and age-based evaluation was made when creating POSEIDON groups [13]. The expected poor responders are defined as those with AMH < 1.2 ng/ml.

Sample collection and AMH assessment

Serum samples were collected in the early follicular period of two consecutive menstrual cycles (day 2 or 3). All the AMH assays were obtained with the Ectys-AMH Roche® system (Roche Diagnostics, Meylan, France), whose measurement limits range from 0.03 to 23 ng/ml, with a sensitivity of 0.03 ng/ml [14]. It's a sandwich assay based on electrochemiluminescence technology. The total duration of the assay is 18 min, and the sample volume is 50 µL. The assay is calibrated against the Beckman Coulter AMH Gen II ELISA (unmodified version without predilution) assay with a measuring range of 0.01–23 ng/mL [14].

Statistical methods

Data analyses were performed by using SPSS Version 21.0 (IBM Corporation, Armonk, NYC, USA). Samples were tested with Shapiro-Wilk to determine normality of distributions. According to the results, non-parametric tests were preferred. Continuous variables are presented as median (minimum-maximum) values, and categorical variables are presented as frequency (percentage). Pearson correlation analysis was performed to test relationship between different AMH values and outcome parameters. A *P* value of <0.05 was considered statistically significant. A correlation coefficient >0.70 is defined as strong correlation.

Results

A total of 109 patients who underwent serum AMH measurements during the early follicular phase of two consecutive cycles were assessed for eligibility. The reasons for repeated AMH measurements included the preference of multiple physicians assessing the same patients at different visits, unintentional repeated measurements, and inconsistent AMH and AFC measurements leading

to additional AMH testing. Among them, 96 patients aged between 20 and 42 years who underwent COS with GnRH antagonist suppression were included in the study. However, 2 patients were excluded due to untreated thyroid dysfunction or hyperprolactinemia, and 15 patients were excluded due to a diagnosis of PCOS. Consequently, 79 patients were included in the final analyses. Table 1 presents the demographics and cycle parameters of the study cohort.

The median cycle-to-cycle variation of serum AMH level was 44.3% (interquartile range {IQR} 42.2%; range 0–1100%) between the two consecutive cycles. The median cycle-to-cycle variations in normal and poor responder subgroups were 27.1% (IQR 38.8%; range 0–62.3%) and 53.8% (IQR 48%; range 0–1100%), respectively. The mean change in serum AMH level was more significant in the normal responders when compared to the POSEIDON expected poor responders (0.60 ± 0.46 vs. 0.28 ± 0.28 , respectively; $P < 0.001$).

Figure 1 illustrates the changes in the serum AMH levels of each subject between the preceding and COS cycles. According to preceding cycle AMH values 32 patients were classified as normal responders and 47 patients were classified as POSEIDON expected poor responders. Although there was no significant change in the poor responder rate, 17.7% of all patients were reclassified into the other subgroup after the second measurement. Based on the AMH values during the COS cycle, 8 of 47 (17%) expected poor responders were identified as normal responders, while 6 (18.8%) normal responders were reclassified as expected POSEIDON poor responders. Transitions between normal and poor responder subgroups were observed within the serum AMH level range of 0.4–2.1 ng/ml.

Figures 2 and 3 present the correlations between the preceding and COS cycle AMH levels and the numbers of total and MII oocytes retrieved. The COS cycle AMH level exhibited a strong correlation with both the total number of oocytes and the number of MII oocytes (Pearson *r*: 0.871, $P < 0.001$ and Pearson *r*: 0.820, $P < 0.001$; respectively). However, the preceding cycle AMH level exhibited a strong correlation with the total number of oocytes, but a moderate correlation with the number of MII oocytes (Pearson *r*: 0.745, $P < 0.001$ and Pearson *r*: 0.651, $P < 0.001$; respectively). The COS cycle AMH and retrieved oocyte number correlation was significantly stronger than the preceding cycle AMH and oocyte number correlation (*r* difference 0.126, 95% CI 0.182–0.512; two-sided $P < 0.001$). In addition, the COS cycle AMH and MII oocyte number correlation was significantly stronger than the preceding cycle AMH and MII oocyte number correlation (*r* difference 0.169, 95% CI 0.205–0.501; two-sided $P < 0.001$).

Table 1 The demographics and cycle parameters of the study cohort

Variable	Median (min-max)
Age, years	35 (25–42)
Preceding cycle AMH at early follicular phase, ng/ml	1.07 (0.12–3.65)
COS cycle AMH at early follicular phase, ng/ml	0.88 (0.03–4.31)
Total number of retrieved oocytes	6 (0–21)
Number of metaphase II oocytes	4 (0–18)
Duration of stimulation, days	9 (7–14)
Baseline FSH, mIU/mL	7.9 (3.8–14.9)
Baseline LH, mIU/mL	5.5 (1.22–14.7)
Baseline estradiol, pg/mL	39 (22–64.6)
Baseline progesterone, ng/mL	0.39 (0.28–1.21)
Total dose of gonadotropins, IU	2650 (1050–4500)

Note AMH: Anti-Müllerian hormone; COS: controlled ovarian stimulation; FSH: follicle stimulating hormone; LH: luteinizing hormone

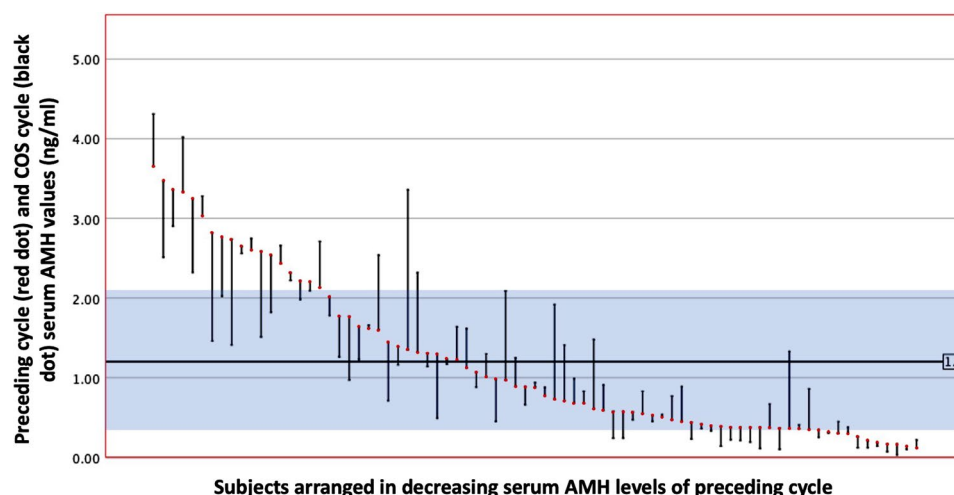


Fig. 1 Individual variation of AMH between two consecutive cycles. The blue-shaded area shows the range of first AMH measurement of patients who were reclassified in terms of ovarian response after the second measurement

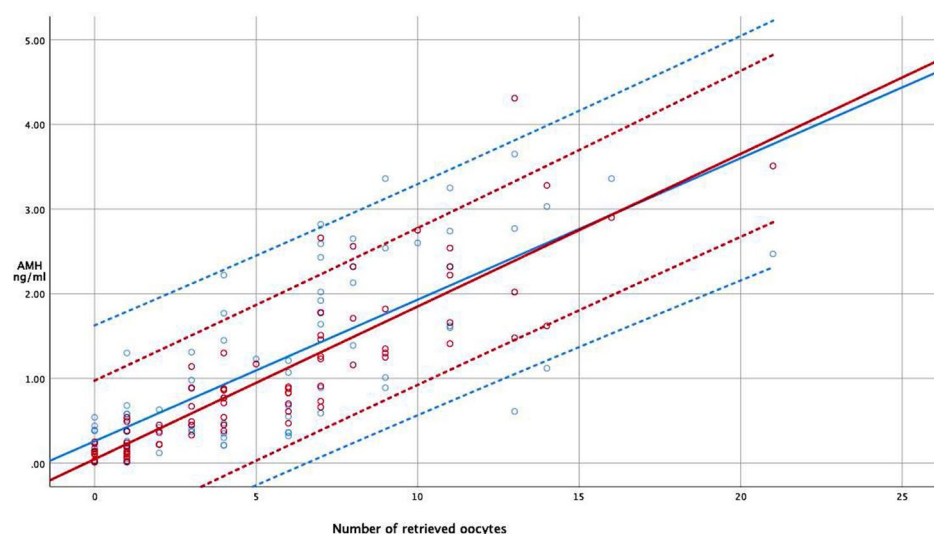


Fig. 2 Correlations between the total number of oocytes retrieved and serum AMH levels. Blue: Estimated linear regression line for the correlation between preceding cycle AMH and number of total oocytes retrieved. Area between dotted blue lines: 95% prediction interval for the blue line. Red: Estimated linear regression line for the correlation between COS cycle AMH and number of total oocytes retrieved. Area between dotted red lines: 95% prediction interval for the red line. The R^2 values for preceding and COS cycles are 0.555 and 0.759, respectively

Discussion

The present study was designed to assess the relationship between inter-cycle variation of serum AMH values and clinical response to ovarian stimulation. According to the results obtained from our study, serum AMH levels can show significant changes between two cycles, particularly in patients classified as poor responders. The change remains at a more reasonable level in normal responders. The response category of patients may change after the second AMH measurement in approximately 20% of patients who are defined as both normal and poor responders according to the first measurement. As expected, of the two sequential measurements, the

measurement at the beginning of the COS cycle shows a stronger correlation with the number of total and MII oocytes retrieved.

Anti-Mullerian hormone has become widely accepted as a reliable and consistent ovarian reserve test, reflecting ovarian function and response. Several studies have compared AMH with AFC and FSH in assessing ovarian response, consistently showing that AMH yields comparable or superior outcomes [15, 16]. Unlike FSH, there are no specific recommendations on the timing of AMH measurement, and it is typically assessed regardless of the menstrual cycle day. This study provides significant insights into the short-term intra-individual and

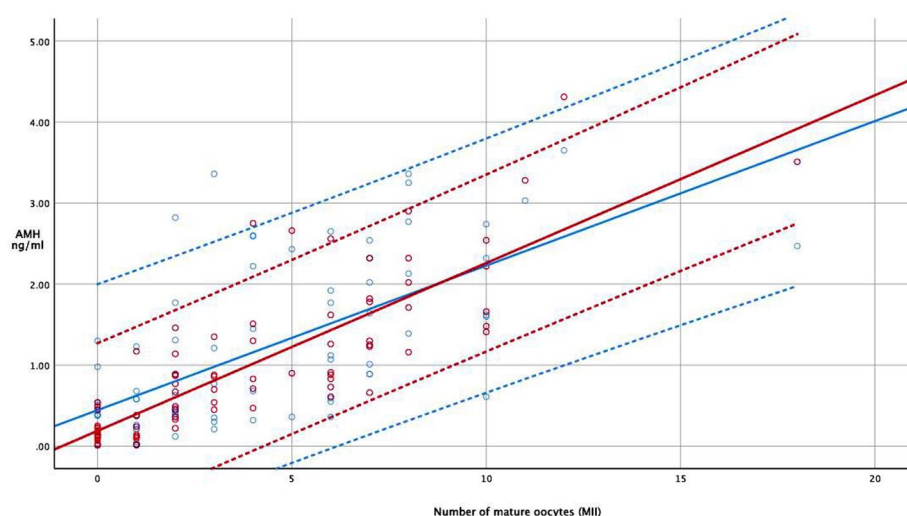


Fig. 3 Correlations between the number of MII oocytes retrieved and serum AMH levels. Blue: Estimated linear regression line for the correlation between preceding cycle AMH and number of MII oocytes retrieved. Area between dotted blue lines: 95% prediction interval for the blue line. Red: Estimated linear regression line for the correlation between COS cycle AMH and number of MII oocytes retrieved. Area between dotted red lines: 95% prediction interval for the red line. The R^2 values for preceding and COS cycles are 0.424 and 0.672, respectively

inter-cycle variability of AMH measurements in women undergoing COS. One of the most important findings of the study was the extent and rates of change in serum AMH levels. While the absolute change in serum AMH levels was greater in normal responder patients, the proportional change was more significant in poor responder patients. Smaller absolute changes can correspond to higher proportional rates, especially in patients with extremely low AMH levels.

Previously, Rombauts et al. assessed inter-cycle variability of the ovarian response across three consecutive IVF cycles with the same protocol and found that 25% of the patients changed their response category (low, normal, or high) [17]. The authors failed to show a significant correlation between response category change and FSH and AFC changes. The inter-cycle variability of AMH can cause misclassification of ovarian response category in approximately 20% of patients, indicating that a single AMH value may not be sufficient to accurately categorize patients as normal or poor responders. Clinicians should exercise caution when interpreting a single AMH concentration, taking into account the potential variability in results that may occur in an individual woman. In a previous study, a 28% inter-cycle variability was observed between two consecutive cycles [18]. In that study, at the time of the first measurement, four participants were initially classified as having low AMH (<1.2 ng/ml) according to the Bologna criteria. However, at the measurement in the subsequent cycle, two participants no longer met this classification, which is consistent with our findings. The BICYCLE study suggested that intra-cycle variability is higher than inter-cycle variation [19]. The authors

concluded that AMH level variability may reflect changes in antral follicle availability during the cycle. These results show that the baseline evaluation should be made using repeated and consistent AMH measurements, or it would be more accurate to use AMH at the beginning of the cycle in which COS will be started. Additionally, one study analysed intra- and inter-cycle fluctuations of AMH and AFC [20]. The lower intra- and inter-cycle fluctuation of AMH compared to AFC suggested that AMH is a more reliable and independent measure for evaluating ovarian reserve. In addition, in a large trial, Nelson et al. reported strong correlation between screening and COS cycle AMH values and the number of oocytes retrieved [21]. Similar to Nelson et al., we found strong correlation with previous cycle AMH and number of oocytes retrieved. However, the correlation was moderate between previous cycle AMH and number of MII oocytes retrieved. Similarly, we found that the AMH level during the current COS cycle showed stronger correlations with the total number of retrieved oocytes and the number of mature oocytes, compared to the AMH level in the preceding cycle. Moreover, the correlations between COS cycle AMH and both retrieved oocyte count and mature oocyte count were significantly stronger than the correlations between preceding cycle AMH. A screening AMH value from a previous cycle can fail to predict the number of MII oocytes collected following COS.

The biology, exposure, laboratory, cycle period, circadian rhythm, smoking, and autoimmune diseases can cause variations of AMH in between measurements. Different AMH values can be observed during different

periods of the menstrual cycle and folliculogenesis [22]. A significant decrease is observed especially during follicle selection [23]. Minor variations in AMH levels (about 10%) can arise from circadian rhythm variations and seasonal fluctuations [24]. Contraceptive use, pregnancy, and the use of chemotherapeutics such as methotrexate may also cause AMH variations. Contraceptive use causes an approximately 30% decrease in AMH levels [25]. One of the main reasons for AMH level fluctuations is the laboratory [26]. Bungum et al. reported differentiating AMH results of three most well-known kits [26]. The study identified significant physiological, intra-individual biodiversity that questions the clinical validity of a single AMH measurement in specific clinical settings. They also noted that commercial assays may not measure ovarian reserve in some individuals due to the presence of different forms of AMH or interaction with other proteins that alter epitope exposure [26]. AMH levels can also decrease as a result of smoking, acute sickness, and autoimmune diseases [22].

AMH is widely acknowledged as a reliable indicator of ovarian reserve that is not influenced by the menstrual cycle. However, is it essential to measure AMH when AFC also indicates the reserve? It is feasible to manage the cycle without relying solely on AMH. Furthermore, the exact application of AMH is still being explored. Measurement of AMH can cause misclassification of approximately 20% of normal responders as poor responders and vice versa due to inter-cycle variability. This misclassification can lead to confusion and undue distress for patients. Therefore, while we are capable of monitoring ART cycles with AFC, it is crucial to consider the inter-cycle variability of AMH to fully comprehend its significance and proper utilization. Since the AMH value measured at the beginning of the COS cycle predicts the number of MII oocytes more successfully, it seems more appropriate to measure AMH in the early follicular phase of that cycle.

The main strengths of our study include the consistency in the timing of measurements (early follicular phase), the use of the same test kit, and the performance of all tests in the same laboratory. While there is a mid-follicular peak in AMH, a periovulatory decline, followed by a slope during the luteal phase, conducting measurements in the same period minimized the effect of intra-cycle fluctuations [27]. The AMH assay we used has demonstrated strong performance and reliability, as supported by robust data [28]. To maintain the integrity of the study results, we excluded women with polycystic ovarian syndrome. This helped to prevent the impact of high AMH levels, which are associated with higher variance, from affecting the study outcomes. The main limitations of our study were its retrospective nature and relatively small sample size. However, including prospectively

recorded data may add credence to our observations. In addition, the study population consists of ovulatory infertile women. Although the variation in AMH within this cohort is clinically significant, it may not adequately represent the general population.

Conclusion

In conclusion, although it's a highly stable test, there may be significant differences in AMH measurements between consecutive cycles and this may lead to misclassification of patients. Measuring AMH in the early follicular phase of the COS cycle rather than measuring it in an earlier cycle will show a higher correlation with the numbers of total and MII oocytes collected.

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Author contributions

YEŞ proposed and designed the study, and drafted the first version of the manuscript. BA conducted the statistical analyses and drafted the first version of the manuscript. NBK and MD completed the literature search and data extraction. BÖ, MS, BB, CA, and RA participated in the critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript.

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There is no funding for the project.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

The study protocol was approved by the Institutional Review Board of Ankara University School of Medicine (approval no: E-12405952-050.01.04-1373265).

Competing interests

The authors declare no competing interests.

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