# Ovarian Reserve Assessment among Healthy Women Using Hormonal Contraceptives Baghdad, Iraq

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**Abstract:**

**Background:** A woman's ovarian reserve and fertility may be affected by the long-term use of hormonal contraception. Anti-Mullerian hormone levels, the best available measure of ovarian reserve, were reduced by hormonal contraceptives methods, and were much lower in the majority of hormonal contraceptive methods, although this suppression effect is reversible.

**Objective :** The aim is to investigate the association between different types of contraceptives and ovarian reserve, probably affect fertility by measuring ovarian reserve parameters (Anti-Mullerian hormone, follicle-stimulating hormone).

**Methods:** A total of 60 women were eligible for inclusion and were divided into two groups. Group 1 included 40 married women who had regularly used combined oral contraceptives (ethinyl estradiol 30 µg and 150 µg levonorgestrel) or medroxyprogesterone acetate injection for at least 6 months. 21 women used combined oral contraceptives, and 19 women used injectable form. Group 2 included 20 married women who didn't take any hormonal contraceptives. Both groups were investigated for serum anti-Mullerian and follicle-stimulating hormone levels on the third day of the menstrual cycle.

**Results:** Among women aged 26-30, the mean age was 30.8±3.3 years. There were statistically significant differences between cases and non-users regarding anti-mullerian hormone level (*P*-value=0.001), significantly lower level among women who were taking combined oral contraceptives compared to those who were receiving birth control injections (*P*-value =0.001) regarding the anti-mullerian hormone level. There were no significant differences between cases and non-users regarding follicle-stimulating hormone levels (*P*-value = 0.772).

**Conclusion:** Hormonal contraceptive methods, both oral and injectable, seem to reduce anti-mullerian hormone levels, but not follicle-stimulating hormone and anti-mullerian hormone levels were suppressed more by oral compared to injectable contraceptives. Further studies using ultrasound and other tests for example luteinizing hormone, sex hormones, and inhibin to check ovarian reserve changes during contraception.

**Keywords:** ovarian reserve, hormonal contraceptives, Anti-Mullerian hormone, follicle-stimulating hormone, contraceptive use.

# تقييم احتياطي المبيض بين النساء الأصحاء اللائي يستخدمن موانع الحمل الهرمونية بغداد، العراق

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**الخلاصة:**

**الخلفية :**قد يتأثر احتياطي المبيض لدى المرأة وخصوبتها بالاستخدام طويل الأمد لوسائل منع الحمل الهرمونية .تم تخفيض مستويات هرمون مكافحة مولر، وهو أفضل مقياس متاح لاحتياطي المبيض، عن طريق وسائل منع الحمل الهرمونية، وكانت أقل بكثير في غالبية وسائل منع الحمل الهرمونية، على الرغم من أن تأثير القمع هذا قابل للعكس.

**الهدف:** والهدف من ذلك هو التحقيق في العلاقة بين أنواع مختلفة من وسائل منع الحمل واحتياطي المبيض، وربما تؤثر على الخصوبة عن طريق قياس المعلمات احتياطي المبيض (هرمون مكافحة مولر، هرمون تحفيز الجريب).

**الطريقة :**وكان ما مجموعه 60 امرأة مؤهلات للإدماج وتم تقسيمهن إلى مجموعتين .تضمنت المجموعة الاولى 40امرأة متزوجة استخدمن بانتظام وسائل منع الحمل المركبة عن طريق الفم ( إيثينيل استراديول 30 جم و150 جم ليفونورجيستريل) أو حقن أسيتات ميدروكسي بروجستيرون لمدة 6 أشهر على الأقل. استخدمت 21امرأة موانع الحمل الفموية المركبة واستخدمت 19امرأة شكل الحقن .تضمنت المجموعة الثانية 20امرأة متزوجة لم تتناولن أي موانع حمل هرمونية .تم التحقيق في كلا المجموعتين لمستويات هرمون المصل المضاد للموليريان والهرمون المنبه للجريب في اليوم الثالث من الدورة الشهرية.

**النتائج:** بين النساء اللواتي تتراوح أعمارهن بين 26 و30 عاما، كان متوسط العمر 30.8-3.3 سنوات. كانت هناك فروق ذات دلالة إحصائية بين الحالات وغير المستخدمين فيما يتعلق بمستوى الهرمون المضاد لموليريان (ف-القيمة=0.001)، وهو مستوى أقل بكثير بين النساء اللواتي تناولن موانع الحمل الفموية المركبة مقارنة بأولئك الذين كانوا يتلقون حقن تحديد النسل (ف-القيمة =0.001) فيما يتعلق بمستوى الهرمون المضاد لموليريان .لم تكن هناك فروق ذات دلالة إحصائية بين الحالات وغير المستخدمين فيما يتعلق بمستويات الهرمون المنبه للجريب (ف-القيمة = 0.772).

**الاستنتاج:** يبدو أن وسائل منع الحمل الهرمونية، سواء عن طريق الفم أو عن طريق الحقن، تقلل من مستويات الهرمون المضاد لموليريان، ولكن لم يتم قمع مستويات الهرمون المنبه للجريب والهرمون المضاد لموليريان عن طريق الفم مقارنة بموانع الحمل القابلة للحقن .مزيد من الدراسات باستخدام الموجات فوق الصوتية واختبارات أخرى على سبيل المثال الهرمون اللوتيني والهرمونات الجنسية والإنهيبين للتحقق من تغيرات احتياطي المبيض أثناء منع الحمل.

**الكلمات المفتاحية:** احتياطي المبيض، وسائل منع الحمل الهرمونية، هرمون مضاد مولر، هرمون تحفيز الجريب، استخدام وسائل منع الحمل.

## Introduction

The term "ovarian reserve" (OR) describes the pool of follicles in the ovaries that may produce egg cells during a woman's prime years and establish reproductive longevity (1). Anti-Mullerian hormone (AMH), follicle-stimulating hormone (FSH), antral follicle count (AFC), and ovarian volume are some of the indicators that have been utilized to evaluate OR (2). Assessment of AMH often called Mullerian inhibitory substance, has several potential advantages over other conventional markers of ovarian reserve. Firstly, it is the earliest marker to exhibit changes with age. Secondly, it has the lowest variability between menstrual cycles. Thirdly, it has the lowest variability within a single menstrual cycle. Serum AMH level analysis aids in evaluating ovarian aging, assessing the risk of ovarian hyperstimulation syndrome, and diagnosing and monitoring treatment for women with polycystic ovarian disease (3)._Lastly, it can provide useful information even if obtained randomly during the cycle (4). In women with polycystic ovarian syndrome, FSH levels are

often low or within the normal range, accompanied by an altered LH/FSH ratio that impairs follicular formation and leads to infertility (5). A higher day 3 FSH level serves as an indirect sign of lower ovarian reserve, reflecting the ovary's reduced capacity to deliver negative feedback to the pituitary gland (6). Conversely, AMH and antral follicle count (AFC) offers a more direct evaluation of ovarian reserve by quantifying the actual number of accessible follicles (7).The anti-Mullerian hormone is synthesized by granulosa cells in preantral and small antral follicles inside the ovary (8). The AMH has traditionally been thought to be unaffected by hormonal contraception use. Unfortunately, there is a lack of conclusive evidence on whether alternating methods of administering combined contraceptives affect follicle growth and serum AMH secretion dynamics (9). Thus, it is assumed that the decrease in blood AMH levels seen in women on combination contraceptives is due to a decrease in the number of small antral follicles, which are already vulnerable to cyclical changes in FSH (10). Combined contraceptives work by lowering FSH levels, which in turn prevents follicle formation, especially during the antral stage. Follicle granulosa cell (GC) mass reduction significantly reduces AMH release, mostly in antral follicles but presumably also in follicles at earlier stages to a lesser degree (11). According to certain research, using hormonal contraceptives reduces AMH levels (12). According to a comprehensive study, long-term users of hormonal contraceptives tended to have lower AMH levels, whereas individuals taking them for less than six months did not appear to have any changes in these levels (13). The mechanisms proposed for the reduction in ovarian reserve associated with hormonal contraceptive use encompass the suppression of follicular development and the selection of follicles transitioning from preantral to antral stages, resulting in decreased ovarian volume, antral follicle count, and anti-Mullerian hormone levels (14). When hormonal contraceptives are stopped, the long-term users' decrease in AMH levels seems to be reversible (15). Infertility and related issues may be mostly prevented by measuring OR markers, which are a useful tool for predicting possible reproductive ability (1). The aim is to study the association between different types of contraceptives and ovarian reserve which probably affect fertility by measuring hormonal profiles (AMH, FSH).

## Subjects and Methods

A convenient sample of married women receiving hormonal contraceptives was collected over the study period during the visit to the family planning division at Al-Yarmouk Teaching Hospital and others from a private clinic for gynecology. Apparently, healthy married women not receiving any hormonal contraceptives were collected from the community as non-users healthy by taking medical and gynecological history. Women with polycystic ovarian syndrome, pelvic inflammatory disease, and ovarian surgery were excluded. Users included women between the ages of 18 and 49. Who were regularly using hormonal contraceptives for at least 6 months and had regular menstruation before and during hormonal contraceptives (16). A sterile syringe was used to draw five ml of venous blood, which was then separated into a single-use gel tube. The serum was then centrifuged at 3000 rpm for ten minutes to measure AMH and FSH levels on the third day of the menstrual cycle (early follicular phase) (17). The time of sample collection was between 9 am to 2 pm.

### Study Design and Study Group

The current study was a cross-sectional community-based study, from October 2023 to March 2024. Eligible 60 women were

divided into two groups: Group 1: included 40 married women on regular use of hormonal contraceptive pills ethinyl estradiol 30 µg and 150 µg levonorgestrel or injections of medroxyprogesterone acetate 150 mg for at least 6 months. The mean duration was 2.5±0.7 years. Group 2: included 20 married women who didn't take any hormonal contraceptives.

### Statistical Analysis

The data were analyzed using the following software: Microsoft Excel 2019, and IBM SPSS Version 26. The results reported in this study were expressed as frequencies, percentages, and rank values. The Mann-Whitney test was used to compare between the study groups. Probability values less than 0.01 were highly significant, and those less than 0.05 were statistically significant.

## Results

### Socio-Demographic Characteristics and Description of Hormonal Contraceptive

Regarding the participants' sociodemographic characteristics, the mean age of cases was 30.8±3.3 years, with 50.0% of the women aged between 26 – 30 years, and 65.0% of them were urban. The mean age of non-users was 29.9±3.4 years. Around sixty percent of the women who received hormonal contraceptives were housewives. Most of the currently used hormonal contraceptives were combined oral contraceptives (52.5%), and 47.5% of injectable contraceptives. Regarding the ethinyl estradiol dose in the COC, 85.7% (n = 18) used 30 – 35 µg, and 14.3% (n = 3) used 20 µg. Regarding the generation of progestins, 89.5%used the second generation, and 10.5% used the third generation. Regarding the duration of contraceptive use, the mean was 2.5±0.7 years, and 12.5% used contraceptives for 6 – 12 months, 37.5% used contraceptives for 1 – 2 years, and 50.0% used contraceptives for more than two years.

**Table (1) Socio-demographic characteristics and description of hormonal contraceptives among study participants**

| Socio-demographic Characteristics | | Group 1 N= 40 | | Group 2 N= 20 | |
|---|---|---|---|---|---|
| **Age groups (in years)** | 18-25 years | 4 | 10.0% | 2 | 10.0% |
| | 26-30 years | 20 | 50.0% | 9 | 45.0% |
| | > 30 years | 16 | 40.0% | 9 | 45.0% |
| **Residence** | Urban | 26 | 65.0% | 11 | 55.0% |
| | Rural | 14 | 35.0% | 9 | 45.0% |
| **Occupation of the wife** | Housewife | 24 | 60.0% | 14 | 70.0% |
| | Worker | 16 | 40.0% | 6 | 30.0% |

| | | | | | |
|---|---|---|---|---|---|
| **Type of currently used hormonal contraceptive** | Combined oral contraceptives (ethinyl estradiol 30 µg and 150 µg levonorgestrel) | 21 | 52.5% | | |
| | Medroxyprogesterone acetate (Depo _Provera) 150mg injection | 19 | 47.5% | | |
| **Ethinyl Estradiol dose in the COC (µg)** | 20 | 3 | 14.3% | | |
| | 30 - 35 | 18 | 85.7% | | |
| **Generation of injection Progestogens** | Second | 17 | 89.5% | | |
| | Third | 2 | 10.5% | | |
| **Duration of contraceptive use retrospectively** | >6 months-12 months | 5 | 12.5% | | |
| | 1-2 years | 15 | 37.5% | | |
| | >2 years | 20 | 50.0% | | |

**Data presented as Number of patients (N), Percentage (%)**

**Assessment of Hormone Level and its Correlation to Type of Contraceptives**

The forty women who received hormonal contraceptives and twenty non-user women were investigated for AMH and FSH as presented in Figures (1) and (2), there were statistically significant differences between users and non-users regarding anti-Mullerian hormone level (*P*-value = 0.001), but there were no statistically significant differences between both study groups regarding FSH (*P* -value = 0.772), according to the Mann-Whitney test. The reference range of AMH is (4-6.8ng/ml). The reference range of FSH is (at Follicular phase 1.4-9.9 mIU/ml, at luteal phase 1.1-9.2 mIU/ml).

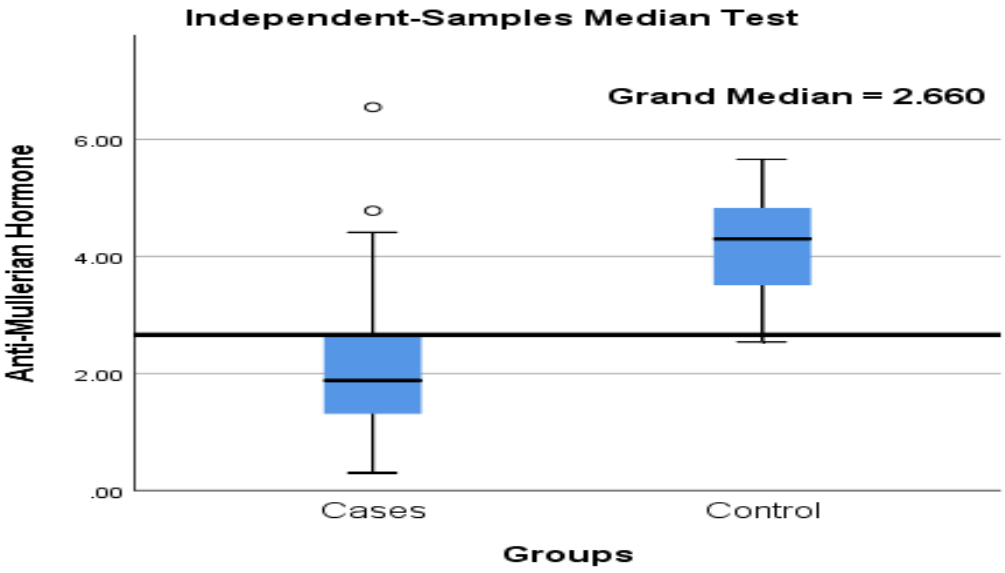


**Figure (1) Box-Plot showed the differences in the median AMH between cases and control women**

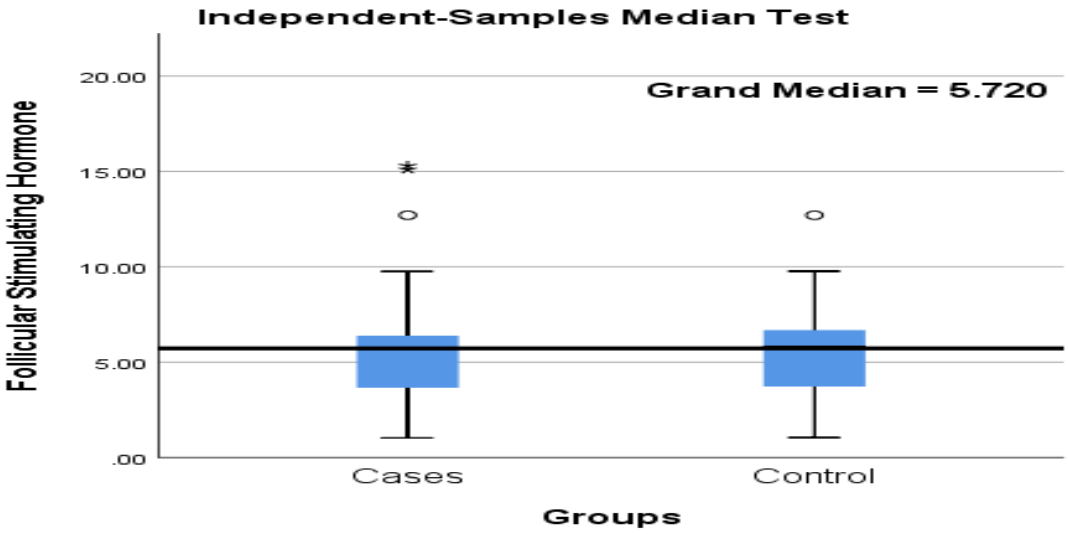


**Figure (2) Box-Plot showed the differences in the median FSH between cases and control women**

The results of Table (2) indicate that there was a highly significant difference between women who were taking Combined oral contraceptives and those who were receiving birth control injections (*P*-value =0.001) regarding the AMH level, the lowest level produced by Combined oral contraceptives.

**Table (2) Level of AMH according to Type of Contraceptives**

| Variable | Median Rank | | *P*-value |
|---|---|---|---|
| **Anti-Mullerian hormone** | Combined oral contraceptive | Birth control injection | |
| | 22.5 | 37.5 | **0.001** |

**Mann-Whitney test was used, and HS was highly significant at (*P* -value ≤ 0.01)**

## Discussion

In the current study, most of the women using hormonal contraceptives were middle-aged between 26-30 years, which was in agreement with the previous study (18). This age range is generally associated with higher fertility and a greater chance of pregnancy. Most of the women in the present study live in urban areas. By 2023, a recent previous study estimates that 32.6% of urban women used contraceptives, while only 7.41% of rural women used contraceptives. Urban women were more likely to have access to, know about, and use contraceptives than their rural counterparts (19). In the current study, most women use combined oral contraceptives for their easy use, reversibility of fertility, wide access, safety, and high effectiveness, as stated in a previous study for the preference of combined oral contraceptives (20). In contrast, other recent findings in 2023 showed that withdrawal was the primary contraception choice among a large portion of women, suggesting possible cultural or educational influences favoring non-hormonal and non-medical methods (21). Most women in this study use hormonal contraceptives for more than two years. They are highly effective in preventing pregnancy when used correctly, and they provide a convenient and reversible method of contraception, most women had a small failure percent in the last year as reported in a previous similar study where the failure rate of contraceptives has decreased in recent years (22). The AMH levels were significantly decreased in the 40 women who received hormonal contraceptives most of them for more than 2 years compared to control women, this is probably due to the suppressive effects of exogenous hormones on ovarian follicle development. The finding is in line with a recent previous study in 2022, which reported that AMH levels were lower in women with varying menstrual cycles, which could be attributed to the use of hormonal contraceptives, which in turn decreased the ovarian reserve marker (23). The suppressive effect of hormonal contraceptives on AMH is reversible, and AMH levels typically return to baseline levels after discontinuation of the contraceptive method as mentioned in another previous study (24). Similar previous findings analyzed the reproductive hormone levels of AMH and antral follicle count among 235 women who were using combined oral contraceptives from different clinics compared to a control group of 983 women, the AMH levels were significantly lower at 33.5% among COC users (25), and up to 23.68% lower AMH levels than those not using contraceptives (26). In the current findings, the FSH level was within normal levels among women in both study groups. This result was not in line with a previous study, since women taking hormonal contraceptives had the FSH typically lower compared to women not taking contraceptives (27). The difference in these

findings is probably due to the small sample size in the current study compared to the previous one, nevertheless, extensive research is warranted to ascertain whether this alteration in FSH levels holds significance. The FSH levels fluctuate throughout the cycle, potentially introducing variability during testing. In women on current hormonal contraceptive use, measuring AMH and FSH is crucial to ovarian reserve assessment. AMH, generated by follicular granulosa cells, is considered a more accurate indicator of ovarian reserve than FSH (23). AMH levels are generally constant during the menstrual cycle, whereas FSH levels might vary (28). The results of the current study indicate that there was a highly significant difference between women who were taking COC and those who were receiving birth control injections, where COC produced the lowest level of AMH. A previous study reported that oral contraceptive use can lead to a 19% lower AMH level compared to non-users (2). In contrast, injectable contraceptives like medroxyprogesterone acetate do not appear to have the same degree of impact on AMH levels (29), the findings in line with the current study. Oral and injectable contraceptives differ in their modes of action, which might explain the variation in AMH suppression. A more marked reduction in AMH may result from the suppression of ovarian function and inhibition of ovulation caused by oral contraceptives. However, injectable contraceptives like medroxyprogesterone acetate mainly work by thickening cervical mucus and weakening the endometrium (25).

## Conclusion

The study stated that women using hormonal contraceptives had decreased AMH levels, but not FSH compared to non-users. Combined oral contraceptives (ethinyl estradiol 30 µg and 150 µg levonorgestrel) reduced AMH levels more than medroxyprogesterone acetate injection. Further studies using ultrasound and other tests for example luteinizing hormone, sex hormones, and inhibin to check ovarian reserve changes during contraception.

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