AMH as an ovarian function predictor in premenopausal female breast cancer patients receiving chemotherapy is effective only for those over 35 years old

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Abstract
Background The predicting value of AMH for ovarian dysfunction after chemotherapy is controversial. This study is designed to evaluate the value of serum AMH clinically and theoretically. Methods We detected the serum estradiol, FSH and AMH in 144 pre-menopausal women with breast cancer receiving cyclophosphamide-based chemotherapy. The differences of hormones before and after chemotherapy were compared; the correlations among the hormones and amenorrhea, menstrual recovery were analyzed. In addition, serum AMH was detected randomly in 177 normal healthy women and 36 normal female C57BL/6J mice of different ages, meanwhile the status of ovary follicles was observed. Furthermore, 72 Balb/c nude mice with breast cancer were randomly assigned to three groups with different dosage of CTX (control, 100mg/kg, 200mg/kg), the alterations of serum AMH and ovary follicles were recorded and analyzed. Results Chemotherapy induced amenorrhea was associated with pre-chemo AMH level, E2 level and FSH level (P <0.0001). Recovery of menstruation was associated with pre-chemo AMH level (P <0.0001) but not E2 and FSH level (P >0.05). In breast cancer patients received chemotherapy, the serum AMH did not differ significantly between pre- and post- chemotherapy in patients younger than 35 years old (P>0.05), while dramatic decline was detected in patients over 35 years old (P<0.0001). In healthy women, the AMH level sharply declined after 35 years old ( P <0.0001) and remained relatively stable in early age. Similar results were obtained in normal mice experiments. The cancer-bearing mice exposed to 200mg/kg CTX endured significant decline of AMH levels and remarkable decrease of primordial and growing follicles (P <0.0001). Conclusion Our results indicate that AMH is effective for predicting post-chemo ovarian function only in premenopausal female breast cancer patients over 35 years old.

1. Background
Anti-Müllerian hormone (AMH), a dimeric glycoprotein member of the transforming growth factor-β superfamily, is composed of two identical subunits and is also called as Müllerian inhibiting substance (MIS) [1–3]. In females, AMH is produced predominantly by granulosa cell and its expression begins in the primary follicles and peaks in the secondary and small antral follicles, but is not detected in primordial follicles and atretic follicles [4, 5]. It has an inhibitory role in recruiting and developing the
primordial follicles into growing follicles [6]. Previous investigations have validated serum AMH levels as a quantitative marker for ovarian reserve and ovarian dysfunction in in vitro fertilization (IVF) and polycystic ovary syndrome (PCOS) [7, 8]. But the role of AMH on predicting ovarian function recovery in post-chemotherapy patients is still in controversy.

Chemotherapeutic regimen including gonadotoxic molecules are usually used to treat those younger patients with advanced malignancies [9, 10], while cyclophosphamide (CTX) is one of the most used cell-cycle non-specific cytotoxic drugs with frequent ovarian function damage [11]. Ovarian toxicity due to CTX usually manifests as amenorrhea or even premature ovarian failure (POF) [12]. Many serum markers, such as follicular stimulating hormone (FSH), luteinizing hormone (LH), inhibin B and AMH, have been employed to evaluate the ovarian reserve and the ability to conceive [13–17]. For AMH is relative stable for not been influenced by the menstrual cycle, many studies have adopted AMH as a prominent biomarker to assess ovarian function during the post-chemotherapy follow-up period [18, 19]. However, the results of those studies were contradictory, and there is no consensus on the predictive value of AMH for ovarian function under chemotherapeutic damage till now [18–21].

Considering the controversies mentioned above, here we conduct this study to clarify whether AMH is an effective biomarker for predicting the ovarian function in premenopausal female breast cancer patients receiving chemotherapy, and to determine in which patients the serum AMH is the most valuable predictor of ovarian function.

2. Methods

Study population

During Jan 2016 to Dec 2017, we randomly recruited 144 pre-menopause women with breast cancer (27–49 years old, median 43y) who need post-operative chemotherapy in our breast center. The regimens used were TEC (docetaxol 75 mg/m² + epirubicin 60 mg/m² + CTX 500 mg/m², D1) or FEC (5-fluorouracil 500 mg/m² + epirubicin 75 mg/m² + CTX 500 mg/m², D1), every 3 weeks for 6 cycles. Patients with previous chemotherapy for malignancy or other disease, or with comorbidities related to fertility, and those breast cancer patients treated without chemotherapy were excluded. The serum estradiol (E2), FSH and AMH levels were detected twice in the breast cancer patients, once before
chemotherapy and once six months after the last injection of chemotherapy. Amenorrhea occurred within six months after chemotherapy was recorded, and subsequent menstruation recovery was recorded one year after chemotherapy.

At the same time 177 normal healthy women (17–75 years old, median 44y, without history of gynecological disease or any cancer) were recruited and serum AMH of each person was detected randomly. Written informed consent was obtained from all participants and ethical approval was granted by the Shanghai Jiao Tong University affiliated Shanghai Sixth People’s Hospital Medical Ethics Committee.

Animals

The project was approved by the Animal Ethics and Welfare Committee of Shanghai Jiao Tong University affiliated Shanghai Sixth People’s Hospital. Thirty-six young female wild-type C57BL/6J mice (obtained from Shanghai SLAC Laboratory Animal Co., Shanghai, China), aged 1, 4, 6, 8, 12, 16 months (6 mice per age group) were anesthetized and euthanasia by terminal cardiac puncture. Blood was collected from the heart and the ovaries were removed for further tests.

In addition, 72 young female inbred Balb/c nude mice (obtained from Shanghai SLAC Laboratory Animal Co., Shanghai, China), aged 6 weeks and weighed 15–20 g, were randomly divided into three groups. \(1 \times 10^7\) MCF7 cells were injected subcutaneously into every mice of each group. We observed and recorded the tumor growth every two days. At 8 weeks old, mice in group 1 (n = 24), group 2 (n = 24) and group 3 (n = 24) were treated with a single intraperitoneal dose of 0.1 ml saline, 100 mg/kg of CTX and 200 mg/kg of CTX, respectively. The dosages of CTX were based on a previous study that demonstrated a significant dose-dependent ovarian toxicity but not sterilization [15, 22]. Six stochastic mice of each group underwent heart blood collection at the time of euthanasia (anesthetization and euthanasia by terminal cardiac puncture) and ovaries excision before chemotherapy. Then 1, 3, 5 weeks after chemotherapy, six stochastic mice of each group underwent blood collection before euthanasia and ovaries excision each time (Fig. 1). Ovaries were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin (H&E), while the serums were
isolated from blood samples for hormonal measurements.

Serum E2, FSH assay

Serum E2 and FSH in all 144 breast cancer patients were test by ADVIA Centaur XP Immunoassay System (SIEMENS) respectively.

Serum AMH assay

Human and mouse serum AMH levels were measured using the Human AMH ELISA kit (Cusabio Biotech, CATALOG #:11351) and Mouse AMH ELISA kit (XLPM0168) respectively. Assays were performed according to the manufacturer’s protocol and concentrations of AMH was determined from the standard curve. All serum samples were assayed in duplicate.

Follicles counting

We performed the follicles counting as previously described in reference[23]. Briefly, the total number of follicles in each ovary was estimated by counting the numbers of follicles in every five sections of H&E-stained whole ovaries and applying a fivefold correction factor. Five µm-thick sections were serially cut and every 5th section analyzed for follicles counting. There are five stages in all follicles: primordial, primary, secondary, antral, and corpus luteum follicles. Only follicles that had an oocyte nucleus were scored. Primary follicles, secondary follicles and antral follicles were collectively referred to growing follicles.

Statistical analysis

The data were expressed as the mean ± standard error of the mean. Statistical analysis was performed using GraphPad Prism 7 and SAS 8.02 software and differences among the chemotherapy groups and the control were determined using one-way analysis of variance followed by multiple comparison test. Correlations between menstruation status and hormones were performed using logistic regression analysis. P < 0.05 was considered to indicate a statistically significant difference.

3. Results

Patient Characteristics

The clinicopathological characteristics of the breast cancer patients were displayed on Table 1. Most of the patients were diagnosed over 35 years old (86.1%) and primarily diagnosed with a T1 (39.6%)
or T2 (44.4%) tumor. Histological diagnoses comprised invasive duct carcinomas (89.6%), lobular carcinomas (4.9%), and others (5.5%); In this cohort, 67 (46.5%), 41 (28.5%), 12 (8.3%), and 24 (16.7%) patients’ molecular subtype were HR+/HER2-, HR+/HER2+, HR-/HER2 + and TNBC, respectively. A total of 12 (8.3%), 92 (63.9%), and 40 (27.8%) women received total mastectomy, modified radical mastectomy, breast conserving surgery, respectively.

| Characteristics                  | Number of Patients (N = 144) |
|----------------------------------|-----------------------------|
| Age (years)                      |                             |
| < 35                             | 20 (13.9%)                  |
| ≥ 35                             | 124 (86.1%)                 |
| T stage                          |                             |
| 1                                | 57 (39.6%)                  |
| 2                                | 64 (44.4%)                  |
| 3                                | 19 (13.2%)                  |
| 4                                | 4 (2.8%)                    |
| Axillary lymph node              |                             |
| Negative                         | 94 (65.3%)                  |
| Positive                         | 50 (34.7%)                  |
| Histology                        |                             |
| Invasive ductal carcinoma        | 129 (89.6%)                 |
| Invasive lobular carcinoma       | 7 (4.9%)                    |
| Others                           | 8 (5.5%)                    |
| Grade                            |                             |
| I                                | 35 (24.3%)                  |
| II                               | 54 (37.5%)                  |
| III                              | 40 (27.8%)                  |
| undifferentiated                 | 15 (10.4%)                  |
| Molecular subtype                |                             |
| HR+/HER2-                        | 67 (46.5%)                  |
| HR+/HER2+                        | 41 (28.5%)                  |
| HR-/HER2+                        | 12 (8.3%)                   |
| TNBC                             | 24 (16.7%)                  |
| Operation                        |                             |
| Total mastectomy                 | 12 (8.3%)                   |
| Modified radical mastectomy      | 92 (63.9%)                  |
| Breast conserving surgery        | 40 (27.8%)                  |

Table 1 displayed the clinicopathological characteristics (age, axillary lymph node, histology, and so on) of the breast cancer patients in this research.

The menstruation status and productive hormone levels in breast cancer patients with chemotherapy.

The relationship between menstruation status and hormones was analyzed by Logistic regression. As shown on Tables 2 & 3, Chemotherapy induced amenorrhea was significantly correlated with pre-chemo levels of AMH, E2, FSH and post-chemo levels of AMH, E2. Recovery of menstruation was significantly correlated with pre-chemo AMH level but not E2 and FSH levels. This indicates that amenorrhea may be predicted through the pre-chemo levels of AMH, E2 and FSH, but menstruation
recovery can only be predicted through pre-chemo AMH level.

Table 2
Menstruation status in breast cancer patients with chemotherapy

| Total          | < 35y  | ≥ 35   |
|----------------|--------|--------|
| Amenorrhea     | 112/144(77.8%) | 8/20(40.0%) | 104/124(83.9%) |
| Menstruation recovery | 28/112(25.0%) | 6/8(75.0%) | 22/104(21.2%) |

Table 3
The relationship between menstruation and reproductive hormone (Logistic regression analysis)

|                | Amenorrhea | Menstruation recovery |
|----------------|------------|-----------------------|
|                | OR (95%)   | P value               | OR (95%)   | P value               |
| AMH            |            |                       |            |                       |
| Pre-chemo      | 0.31(0.157-0.612) | < 0.001               | 0.224(0.079-0.631) | < 0.005               |
| Post-chemo     | 0.319(0.167-0.611) | < 0.001               | 0.161(0.053-0.484) | < 0.001               |
| Oestradiol     |            |                       |            |                       |
| Pre-chemo      | 0.983(0.873-0.994) | 0.002                 | 1.003(0.991-1.015) | 0.637                 |
| Post-chemo     | 0.980(0.969-0.991) | 0.001                 | 0.985(0.973-0.988) | 0.023                 |
| FSH            |            |                       |            |                       |
| Pre-chemo      | 1.022(1.004-1.041) | 0.016                 | 1.027(0.959-1.101) | 0.444                 |
| Post-chemo     | 1.002(0.963-1.943) | 0.913                 | 1.041(1.016-1.068) | 0.002                 |

Moreover, in breast cancer patients treated with chemotherapy, the serum AMH and E2 did not differ significantly between pre- and post- chemotherapy in patients younger than 35 years old (P > 0.05), while dramatic decline was detected in patients over 35 years old (P < 0.0001) (Table 4). It suggests that the AMH level will be more effective on assessing the chemo-induced ovarian injury in premenopausal women older than 35 years old.

Table 4
Reproductive hormone levels in breast cancer patients before and after chemotherapy (Wilcoxon Signed Ranks Test)

|                | All          | < 35         | ≥ 35          |
|----------------|--------------|--------------|--------------|
| AMH (ng/ml)    |              |              |              |
| Pre-chemo      | 1.22 (0.01, 8.68) | 3.29 (0.81, 8.68) | 1.13 (0.01, 5.32) |
| Post-chemo     | 1.08 (0.01, 8.52) | 3.24 (0.06, 8.52) | 0.97 (0.01, 4.02) |
| P-value        | < 0.001      |              |              |
| Oestradiol (pg/ml) | 122.43 (18, 335) | 173.2 (18, 335) | 119.44 (27, 242) |
| Pre-chemo      | 63.88 (5, 287)  | 134.7 (15, 287) | 64.56 (5, 234)  |
| Post-chemo     |              |              |              |
| P-value        | < 0.0001     |              |              |
| FSH (mIU/ml)   | 10.51 (1.32, 83.22) | 8.30 (1.55, 38.82) | 10.54 (1.32, 83.22) |
| Pre-chemo      | 51.22 (3.45, 121.06) | 30.82 (3.56, 119.83) | 51.82 (3.45, 121.06) |
| Post-chemo     |              |              |              |
| P-value        | < 0.0001     | 0.014        | < 0.0001     |

*The data was described by mean, minimum, maximum.

The trends of AMH level in healthy women and female mice and the correlation between follicle numbers and AMH level in mice.

Table 5 depicts the age distribution of all healthy women and the median AMH levels by age group.

The AMH level sharply declined in women over 35 years old (P < 0.0001, Fig. 2A) and remained relatively stable in early age.
Table 5 depicts the age distribution of all healthy women and the median AMH levels by age group.

Serum AMH levels were determined in C57BL/6J wild-type female mice of various ages. It was increasing at the early time and declined significantly with increasing age (Fig. 2B). We found three phases in the changes of AMH level. At the first phase the serum AMH levels were increasing obviously before 4 months of age ($r = 0.97, P < 0.0001$). At the second phase mice of 4–8 months of age had relatively stable AMH levels ($r = 0.97$, 4 months vs. 6 months $P = 0.28$, 4 months vs. 8 months $P = 0.98$). A significant decline of the AMH level was observed in mice elder than 8 months ($r = 0.97$, $P < 0.0001$), which represents mice being with an irregular cycle and getting into anestrus.

Analysis of the follicles counting revealed that the number of primordial follicles declined with increasing age ($r = 0.99$, $P < 0.0001$, Fig. 2C). While the number of growing follicles is increasing before 6 months old ($r = 0.91$, $P < 0.0001$, Fig. 2D), remaining stable in 6–8 months old ($r = 0.91$, 6 months vs. 8 months, $P = 0.21$), and declined significantly after 8 months old ($r = 0.91$, $P < 0.001$).

Histological appearance was revealed in the Fig. 2F. It’s correlated strongly between the serum AMH levels and the number of growing follicles ($r = 0.887$, $P < 0.0001$, Table 6 and Fig. 2E).

Table 6 showed the strongly correlation between the serum AMH levels and the number of growing follicles ($r = 0.887$, $P < 0.0001$).

The effect of cyclophosphamide on AMH level and follicles in Balb/c nude mice.
There was no significant difference of the tumors size among the three groups before chemotherapy (Control vs. CTX100 vs. CTX200, P > 0.05). The growth of tumors in CTX 100 mg/kg group was similar to that in controlled group, while the tumor growth in CTX 200 mg/kg was slowed down more effectively (Control vs. CTX200, P < 0.0001). The trends of serum AMH, primordial follicles and growing follicles counting at 8 weeks old (pre-chemotherapy), 9 weeks old (1 week after CTX or saline), 11 weeks old (3 weeks after CTX or saline), 13 weeks old (5 weeks after CTX or saline) in Balb/c nude mice with breast cancer were shown in Fig. 3(A-C). The relation between AMH levels and follicle numbers in control and CTX-treated group was shown on Table 7. No significant difference of serum AMH levels was observed in control group among mice aged from 8 to 13 weeks, as well as among mice at 8 weeks old (pre-chemo) in each group. In 200 mg/kg CTX group, the AMH levels were going down significantly over time (P < 0.0001). Interestingly, in 100 mg/kg CTX group, the AMH levels were rising slightly at 9 weeks, declining sharply at 11 weeks, and then increasing at 13 weeks eventually. What’s more, the primordial follicles consistently declined by time lapse in each group (P < 0.0001), while the trend was dramatic in 200 mg/kg CTX group. The number of growing follicles was always in accordance with the changes of serum AMH levels. Changes of the number of growing follicles were revealed by H&E sections (Fig. 3D).

### Table 7

The comparison of AMH levels and follicle numbers in control and CTX-treated groups (ANOVA test)

| Comparison | Time point | P value | P value |
|------------|------------|---------|---------|
|            |            | G1 vs. G2 | G1 vs. G3 |
|            |            | AMH | PDF | GF | AMH | PDF | GF |
| Pre-CTX    | ns         | 0.03 | ns  | ns  | ns  | ns  | ns  |
| 1 week after CTX | 0.037 | ns  | < 0.001 | < 0.0001 | 0.001 | ns  | ns  |
| 3 week after CTX | < 0.001 | ns  | < 0.001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| 5 week after CTX | ns         | 0.028 | ns  | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

Note: G1, Control group; G2, CTX100 group; G3, CTX200 group; PDF, primordial follicles; GF, growing follicles; ns, not significant.

### 4. Discussion

In both species the decline in fertility is closely related to the reduction of the primordial follicle pool. Moreover, the relationships between ages, numbers of oocytes and fertility in virgin and multiparous mice have been confirmed years before. Furthermore, cytotoxic drugs, especially CTX, will cause various follicles destruction, which was more significant in primordial follicles [20, 22]. The injury
caused by CTX will eventually lead to ovarian dysfunction, although the specific mechanism is not very clear yet. It was reported that CTX injures the follicles in a direct way by DNA alkylation and subsequent disruption of normal cellular processes and depletes the primordial follicles by destruction of granulosa cells [11, 12, 22, 24, 25]. In the absence of AMH, ovarian follicles depletion was accelerated for more primordial follicles recruited into growing follicles [20, 26].

Although it was ever reported that CTX-induced acute ovarian follicular destruction couldn’t be predicted by serum AMH levels [20]. According to our study, the happening of amenorrhea six months after chemotherapy was correlated with pre-chemo levels of AMH and E2 negatively, with pre-chemo FSH level positively. While the recovery of menstruation one year after chemotherapy was correlated with pre- and post-chemo AMH level positively, but not correlated with pre-chemo levels of E2 and FSH. That means patients with higher AMH levels are less likely to have amenorrhea and more likely to return to normal even after chemotherapy, whereas patients with lower AMH levels are more likely to have amenorrhea and less likely to recover after amenorrhea [27]. Although pre-chemo E2 and FSH levels were correlated with amenorrhea, we did not find a correlation between them and menstrual recovery after chemotherapy, which was in line with expectations: the levels of E2 and FSH fluctuated greatly with the physiological cycle, while the levels of AMH were relatively stable [5]. Further stratified analysis revealed interesting findings: in our clinical study there were no significant changes of AMH level in young patients accepted chemotherapy, while AMH levels declined dramatically in patients over 35 years old with chemotherapy [28, 29]. It implied that in younger patients the sufficient primordial follicles reserve and strong compensation ability resulted in a stable AMH levels. Therefore, the serum AMH as a biomarker for ovarian function predicting will be more accurate in women receiving chemotherapy older than 35 years old, but not in women younger than 35 years old. There is no consensus on whether AMH can be used as a predictor of ovarian function in women undergoing chemotherapy, especially in younger patients [28–30]. Although studies have shown that AMH is of little value in predicting ovarian function in underage chemotherapy patients, no reasonable explanation has been found [20, 21, 28, 30]. In our study on healthy women, the AMH level has a persistent trend of decline with ageing in women over 35 years old but remains relatively stable in
women younger than 35 years old, which means AMH may not be an accurate and suitable biomarker of ovarian reserve for younger women [31, 32]. We got a similar result in mice study, the AMH level decreased with ageing in mice after an increasing during the first 6 months. Following study revealed that the number of growing follicles has a close correlation with primordial follicles, as well as the serum level of AMH. And in mice toxicity experiment, the primordial follicles consistently declined by time lapse in each group (P < 0.0001), while the trend was dramatic in 200 mg/kg CTX group. The number of growing follicles was always in accordance with the changes of serum AMH levels. In mice older than 8 months, both the serum AMH level and the number of growing follicles underwent a steady decline, which is in accordance with the opinion of Scheffer et al [32]. It was suggested that in immature female mice and women, the level of AMH increases due to the increasing of growing follicles. After then, the number of growing follicles remained constant during the early reproductive period in female, accompanied with the constant levels of AMH. With the increasing of age, serum AMH level will gradually decrease accompanied with the decrease of growing follicles in female.

It’s well understood that AMH regulates the rate of primordial follicles recruited for further growth, and the recruitment of primordial follicles is inhibited with the presence of AMH in mature ovaries [4, 5, 26]. In the juvenile, because the follicles were mostly primordial follicles, AMH could not accurately evaluate the ovarian functional impairment. However, in young adults, even if the growing follicles were largely killed by chemotherapy drugs, the remaining growing follicles and the timely replenishment of the primordial follicular pool could maintain the stability of AMH, so the serum AMH level could not accurately reflect the damage degree of ovarian function. On the contrary, AMH can accurately reflect the injury of ovarian function in middle-aged and elderly female due to the limited ovarian reserve [31, 33].

5. Conclusions
According to our research, AMH is an effective biomarker for predicting the ovarian function in premenopausal female breast cancer patients receiving chemotherapy, but only in those older than 35 years old. AMH as a predictor of ovarian function and an ovarian protectant is worthy of further study [23, 34]. Therefore, AMH may not only be used as a predictive and evaluation indicator for
ovarian function in premenopausal female with multiple tumors, but also would be a potential therapeutic target.

List Of Abbreviations
AMH: Anti-Müllerian hormone; MIS: Müllerian inhibiting substance; IVF: in vitro fertilization; PCOS: Polycystic Ovary Syndrome; CTX: Cyclophosphamide; POF: Premature ovarian failure; FSH: Follicular stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; TNBC: Triple Negative Breast Cancer.

Declarations
Ethics approval and consent to participate
The study was approved by the Institutional Research Committee, Animal Ethics and Welfare Committee of Shanghai Jiao Tong University affiliated Shanghai Sixth People’s Hospital. All patients had given consent before enrollment.

Consent for publication
Not applicable.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests
The authors declare that they have no competing interests.

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Authors’ contributions
XZ, XL, and SL participated in the study design. XL, YY, SL and RH conducted experiments. XL, YY, LM, and CX did the statistical analysis. XL and HW drew the figures using software. XL, RH, and XZ wrote the manuscript. All authors reviewed the report and gave final approval to submit for publication.

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Figures
10^7 MCF7 cells were injected subcutaneously into Balb/c nude mice 6 weeks, 15-20g, N=72

Figure 1

Flowchart of cancer bearing mice experiments 72 Balb/c nude mice, aged 6 weeks and weighed 15–20 g, were randomly divided into three groups. 10^7 MCF7 cells were injected subcutaneously into every mice of each group. At 8 weeks old, mice in group 1 (n=24),
group 2 (n=24) and group 3 (n=24) were treated with a single intraperitoneal dose of 0.1 ml saline, 100 mg/kg of CTX and 200 mg/kg of CTX, respectively. Six stochastic mice of each group underwent heart blood collection at the time of euthanasia and ovaries excision before chemotherapy. Then 9, 11, 13 weeks, six stochastic mice of each group underwent blood collection before euthanasia and ovaries excision each time.

The trends of AMH level in healthy women and female mice and the correlation between follicle numbers and AMH level in mice. A, The AMH levels obviously declined in women over 35 years old. B, Serum AMH levels remained stable in 4-8 months old and declined after 8 months old. C, The number of primordial follicles decreased with increasing age. D, The number of growing follicles was significantly declined in mice older than 8 months. E, The correlation between AMH levels and growing follicles was strongly significant (r= 0.887, P <0.0001, Pearson test). F, H&E stained ovaries from mice of different age. (B-D, each bar represents a minimum of 6 mice, ANOVA test. #, no statistical significance; **, P <0.001; ***, P <0.0001.)
Serum AMH levels and ovarian follicles of Balb/c nude mice. A, The levels of serum AMH remained stable in controlled group and declined significantly in CTX 200 mg/kg group (P <0.0001). B, The number of primordial follicles was not changed obviously in controlled group (P >0.05) and 100 mg/kg CTX group at early time, while the decrease in CTX 200 mg/kg group was apparent (P <0.0001). C, The number of growing follicles remained stable in controlled group and 100 mg/kg CTX group (P >0.05). However, it was decreased significantly in 200 mg/kg CTX group at 5 weeks after chemotherapy (P <0.0001). D, H&E stains of representative sections from all mice in different treatment groups. (A-C, each bar represents a minimum of 6 mice, ANOVA test)
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