Neoadjuvant Treatment With Müllerian-Inhibiting Substance Synchronizes Follicles and Enhances Superovulation Yield

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Müllerian-inhibiting substance (MIS), also known as anti-Müllerian hormone, is thought to be a negative regulator of primordial follicle activation. We have previously reported that treatment with exogenous MIS can induce complete ovarian suppression within 5 weeks of treatment in mice. To investigate the kinetics of the return of folliculogenesis following the reversal of suppression, we treated animals with recombinant human MIS (rhMIS) protein for 40 days in adult female Nu/Nu mice and monitored the recovery of each follicle type over time. Following cessation of MIS therapy, secondary, and antral follicles returned within 30 days, along with the normalization of reproductive hormones, including LH, FSH, MIS, and Inhibin B. Furthermore, 30 days following MIS pretreatment, the number of antral follicles were significantly higher than controls, and superovulation with timed pregnant mare serum gonadotropin and human chorionic gonadotropin stimulation at this time point resulted in an approximately threefold increased yield of eggs. Use of the combined rhMIS-gonadotropin superovulation regimen in a diminished ovarian reserve (DOR) mouse model, created by 4-vinylcyclohexene dioxide treatment, also resulted in a twofold improvement in the yield of eggs. In conclusion, treatment with rhMIS can induce a reversible ovarian suppression, following which a rapid and synchronized large initial wave of growing follicles can be harnessed to enhance the response to superovulation. Therapies modulating MIS signaling may therefore augment the response to current ovarian stimulation protocols and could be particularly useful to women with DOR or poor responders to controlled ovarian hyperstimulation during *in vitro* fertilization.

Freeform/Key Words: AMH, MIS, superovulation, diminished ovarian reserve, folliculogenesis, primordial follicles

Müllerian-inhibiting substance (MIS; also known as anti-Müllerian hormone) is a ligand of the TGF-β superfamily. In the female, MIS is secreted by the granulosa cells of growing preantral and early antral follicles of the ovary [1–5]. The development of an ELISA [6] to measure circulating MIS concentration in the blood permitted monitoring the hormone in

Abbreviations: DOR, diminished ovarian reserve; H&E, hematoxylin and eosin; InhB, Inhibin B; MIS, Müllerian-inhibiting substance; MISR2, Müllerian-inhibiting substance type II receptor; mMIS, mouse Müllerian-inhibiting substance; MWCO, molecular weight cutoff; PMSG, pregnant mare serum gonadotropin; rhMIS, recombinant human Müllerian-inhibiting substance; VCD, 4-vinylcyclohexene dioxide.

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many clinical applications, including its use as a marker of ovarian age. This property stems from the fact that the size of the growing pool of follicles that produces MIS is proportional to the quiescent pool of primordial follicles (*i.e.*, the ovarian reserve), which proportionately declines with age [7]. MIS remains one of the most reliable markers for ovarian reserve [8], and the increasing clinical use of competing commercial ELISA platforms has raised the need for a universal standard with a defined mass [9, 10].

The MIS type II receptor (MISR2; also known as anti-Müllerian hormone receptor 2) is likewise expressed by granulosa cells, but persists from the primordial to the late antral stage [5, 11–13]. MIS is thought to act as a negative-feedback paracrine signal that inhibits both primordial follicle activation [13–17] and the sensitivity of growing follicles to FSH [18, 19]. The MIS-MISR2 signaling axis is therefore thought to fine tune follicular homeostasis by balancing growing and quiescent follicle pools to regulate oocyte quality and reproductive longevity [20].

Superovulation, or controlled ovarian hyperstimulation therapy, is a treatment strategy for infertility in which gonadotropins are administered to induce the release of multiple eggs at once. Effectiveness of this treatment largely depends on the size of the ovarian reserve, which is often estimated using the MIS ELISA. MIS levels have therefore been used to predict the response to superovulation, allowing personalized stimulation protocols to identify patients with likely poor ovarian response or conversely at risk for ovarian hyperstimulation syndrome [21, 22].

In cases of diminished ovarian reserve (DOR), which are associated with low circulating MIS, the success rate of controlled ovarian hyperstimulation is generally low, because of both poor oocyte yield and low oocyte quality. DOR may be caused by aging, genetic factors, and iatrogenic factors such as chemotherapy, and few adjuvant treatments are available to enhance *in vitro* fertilization success in these patients [23].

We recently reported that prolonged treatment with recombinant human MIS (rhMIS) protein completely and reversibly stops the folliculogenesis in the ovary [13]. Following reversal of the ovarian suppression, we observed a large initial synchronized wave of follicle growth, with accelerated developmental kinetics in an environment of high gonadotropins and low endogenous serum MIS. Based on these findings, we hypothesized that timed hyperstimulation to the ovary coinciding with the maturation of this initial wave should enhance the response to superovulation. In this study, we report that neoadjuvant rhMIS therapy can increase the yield of superovulation and that this treatment could enhance stimulation in a mouse model of DOR.

1. Materials and Methods

A. Research Animals

This study was performed in accordance with a Massachusetts General Hospital Institutional Animal Care and Use Committee–approved experimental protocol (2009N000033). Mice were housed in the room maintained at 25°C, 30% to 60% humidity, regular light cycle (7 AM to 7 PM), and were given food and water *ad libitum*. Nu/Nu mice (Gnotobiotic Mouse Cox 7 Core, Rakesh Jain Laboratory, Massachusetts General Hospital, Boston, MA) were used for the experiment to eliminate any possibility of immunoreaction to the recombinant human protein given to the mice for more than a month.

B. Ovarian Suppression and Superovulation Protocol

Adult 7-week-old female mice were pretreated by subcutaneous injections with 750 μg/kg of rhMIS or vehicle control (saline) every 12 hours for 40 days (day −40 to day 0) to establish complete ovarian suppression as previously described [13]. Following cessation of rhMIS pretreatment and release from ovarian suppression (day 0), mice (N = 3) were euthanized on days 0, 5, 10, 15, 30, or 50 for rhMIS treatment or days 0 and 30 for vehicle control to collect ovarian tissue and blood samples for follicle counts and hormonal analysis, respectively.

To induce superovulation, adult 7-week-old female mice were pretreated by subcutaneous injections of 750 μg/kg of rhMIS or vehicle control (saline) (N > 5/group) every 12 hours for 40 days (day −40 to day 0) and subsequently injected IP with 7.5 IU of pregnant mare serum gonadotropin (PMSG) (Calbiochem, San Diego, CA) on days 15, 30, or 50 for rhMIS treatment or on day 30 for vehicle control, followed 49 hours later by 7.5 IU of human chorionic gonadotropin (hCG; Millipore Sigma, St. Louis, MO). The mice were euthanized 16 hours after the hCG injection to collect and count the ovulated eggs from the oviduct.

C. DOR Model

To reduce the number of primordial follicles, mice (N = 6) were given daily injections of 4-vinylcyclohexene dioxide (VCD; Millipore Sigma, St. Louis, MO) at 160 mg/kg or vehicle control (corn oil) IP for 5 days [24], and 9 days later, mice were euthanized, ovaries recovered, and follicle counts were performed. In a parallel cohort (N = 8), mice received an identical 5-day course of VCD, followed, 2 days later, by a 40-day rhMIS (or vehicle control) pretreatment and, 30 days later, finally underwent the superovulation protocol (PMSG plus hCG) as detailed previously (N = 4/group).

D. Histology and Follicle Counts

For histological analysis, harvested tissues were immersed in 4% paraformaldehyde overnight at 4°C and then processed through a series of graded alcohols and xylenes for 24 hours prior to paraffin embedding. The entire ovary was sectioned at an 8-μm interval and mounted on glass slides for staining. Slides were deparaffinized, rehydrated, and stained with hematoxylin (Mayer's; Dako, Carpinteria, CA) and eosin (EosinY; Sigma-Aldrich, St. Louis, MO) (H&E).

H&E-stained ovary sections were examined under the light microscope, and follicles were counted on every fifth section of the whole ovary. The follicles were categorized into five stages; primordial, primary, secondary, antral, or atretic follicles, based on their morphological character as previously described [25]. The total number of follicles was estimated by applying fivefold correction factor to the sum of follicles.

E. Measurements of Blood Hormones

Blood sample were procured via tail vein puncture, and 10 μL of blood was collected and mixed with 90 μL of 1% BSA/PBS with Tween 20. Terminal blood samples were collected immediately after euthanasia via cardiac puncture; serum was retrieved following room-temperature coagulation and centrifugation at 1000*g* for 10 minutes and stored at −20°C until use.

Endogenous mouse MIS (mMIS) was measured by ELISA [26] (Beckman Coulter, Brea, CA) following the manufacturer's protocol, whereas Inhibin B [27] (InhB; Ansh Labs, Webster, TX) and LH/FSH [28] (EMD Millipore, Burlington, MA) were measured by the Ligand Assay and Analysis Core of the Center for Research in Reproduction at University of Virginia School of Medicine under a cooperative agreement. The core is supported by the Eunice Kennedy Shriver NICHD/NIH (NCTRI), Grant P50-HD28934.

F. Recombinant MIS Protein Production

rhMIS was produced as previously described [29]. Briefly, a CHOK1 clone (LR11) stably transfected with a human MIS transgene (albumin leader-Q425R "LRMIS") was cultured in hyperflasks (Corning, Corning, NY) maintained in DMEM with 5% fetal bovine serum (Gibco, Gaithersburg, MD) and cycled weekly for 3 days in serum-free DMEM with 1% insulin-transferin-selenium (Gibco, Gaithersburg, MD) to produce conditioned media. Conditioned media was concentrated eight times through a crossflow cassette filter [Vivaflow 200, 10,000 molecular weight cutoff (MWCO) PES; Sartorius, Goettingen, Germany]. The concentrated

media was incubated with activated immuno-support beads (Bio-Rad, Hercules, CA) coupled to anti-human MIS monoclonal antibody (6E11 [30], produced in our laboratory [31]) overnight. rhMIS protein was eluted with 0.1 M glycine pH 2.9, concentration was measured by a Bradford assay, and the eluate was concentrated to 1400 μg/mL using a spin column (10,000 MWCO; Millipore Sigma). The concentrate was then dialyzed in 1 L of PBS (Gibco, Gaithersburg, MD) in a floating cassette (Slide-A-Lyzer, 3500 MWCO; Thermo Fisher Scientific, Waltham, MA) overnight with gentle rotation. Finally, the protein concentration was readjusted to 1200 μg/mL in PBS (Gibco, Gaithersburg, MD). The purified rhMIS solutions were stored in −80°C until use and diluted with a saline solution to the desired working concentration.

G. Statistical Analysis

Statistical analysis was performed using the Prism 7 software (GraphPad Software, La Jolla, CA). The Student *t* test was used to analyze the results from two experimental groups, whereas one-way ANOVA followed by Tukey or Holm-Sidak *post hoc* test was used to analyze the results from more than two experimental groups.

2. Results

A. Reversible Ovarian Suppression With rhMIS

To determine the kinetics of the return of folliculogenesis following complete ovarian suppression, we pretreated 7-week-old Nu/Nu female mice with rhMIS protein (or vehicle control) from day −40 to day 0 (750 μg/kg of rhMIS every 12 hours), terminated treatment, and then euthanized animals on days 0, 5, 10, 15, 30, and 50 as previously described (Fig. 1A) [13]. We collected ovaries and performed follicle counts on each time point using H&E-stained sections; a subset of primary data (day 0 to day 15) was previously reported [13]. As expected, MIS-treated ovaries contained equivalent numbers of primordial and primary follicles, but significantly fewer growing secondary, antral, and atretic follicles compared with the vehicle-treated mice at the end of the pretreatment (day 0) (Fig. 1B'–1F'). The kinetics of the return of growing follicles following MIS treatment was examined in detail with a time series at day 0 to day 50 (Fig. 1B–1F).

There was a time-dependent trend for reduction in primordial follicle numbers, which were significantly depleted by days 30 to 50 compared with earlier time points (day 0 to day 10) (Fig. 1B). Furthermore, the number of primordial follicles in the MIS pretreated group, although not significantly different than saline-pretreated control at day 0, was significantly reduced at day 30 compared with the respective day 30 saline control (49% decrease; $P = 0.01$) (Fig. 1B). In contrast, primary follicle abundance did not significantly change during the day 0 to day 50 posttreatment release period, with a trend suggesting a rapid normalization of abundance within the first 5 days following release (Fig. 1C).

Conversely, both the populations of secondary and antral follicles were significantly higher by days 30 to 50 compared with earlier timepoints (day 0 to day 10 and day 0 to day 15, respectively) (Fig. 1D' and 1E'). Although this translated into a trend for higher numbers of secondary follicles in MIS vs saline pretreated groups at day 30, this difference was not statistically significant (51% increase; $P = 0.07$) (Fig. 1D'). However, antral follicle counts of MIS pretreated mice at day 30 were significantly higher than saline control–treated mice at day 30 (71% increase; $P = 0.04$) (Fig. 1E').

Finally, there were significantly fewer atretic follicles in MIS-pretreated mice at days 0 and 30 than saline control mice at those same respective time points (Fig. 1F'). Furthermore, atretic follicles remain undetectable from days 0 to 10 and only significantly return on day 50 (Fig. 1F).

To track the functional return of folliculogenesis and confirm our follicle counts, we examined a panel of reproductive hormone in the blood by ELISA, including endogenous

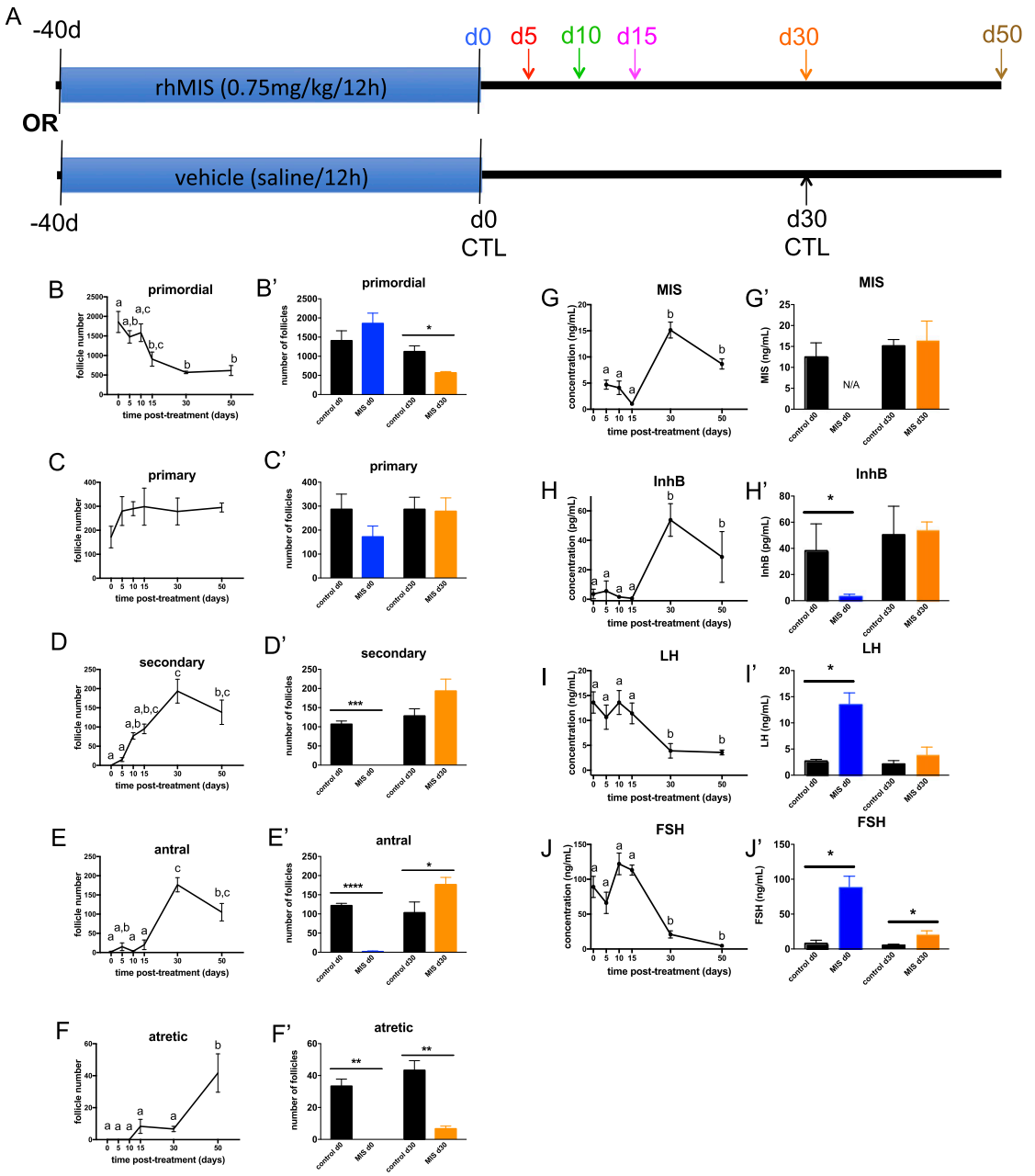

Figure 1. Total ovarian follicle counts and serum hormone levels following MIS pretreatment and release. (A) Six-week-old Nu/Nu mice were pretreated with 750 μg/kg of rhMIS (or vehicle control) twice a day for 40 d (days −40 to 0). Treated animals were euthanized on days 0, 5, 10, 15, 30, and 50 (N = 3), whereas control mice were treated with saline (vehicle of rhMIS) with the same dosing schedule and age and killed on days 0 (d0) and 30 (d30). (B–F) Total follicle counts were performed on ovaries retrieved from the mice on. (B'–F') Statistical comparisons of the d0 and d30 time points in MIS and vehicle-treated animals. (G–J) Serum samples were collected, and MIS, InhB, LH, and FSH were measured by ELISA in all samples from days 0–50. (G'–J') Statistical comparisons of the d0 and d30 time points in MIS and vehicle-treated animals. Different letters above each data bar indicate significance (a, b, and c; $P < 0.05$) within each category of group by one-way ANOVA followed by Holm-Sidak multiple-comparison test. Asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$) indicate statistical significance and P value by pairwise t test with respective controls. Values are presented as mean ± SEM.

mMIS, InhB, LH, and FSH (Fig. 1G–1J). Measurements of endogenous mMIS were not performed at day 0 due to interference from the rhMIS, which is eliminated by day 5 considering its 4-hour half-life in mice [13]. Both mMIS and InhB levels remained low until day 30, at which point they rose and remained significantly higher on days 30 to 50 compared with days 0 to 15 (Fig. 1G and 1H). Furthermore, both mMIS and InhB levels are significantly lower at day 0 in MIS-pretreated animals compared with vehicle controls at the same time point, but were not different from controls at day 30. The timing of return of MIS coincides with the appearance of antral follicles (Fig. 1E), confirming a previous report indicating they contribute the majority of the MIS in circulation [32]. Both gonadotropins LH and FSH displayed similar kinetics of reduction over time following release from MIS pretreatment and remained high from days 0 to 15 followed by a considerable reduction at days 30 to 50 (Fig. 1I, J). Interestingly, whereas both LH and FSH levels were significantly higher in MIS-treated animals than saline-treated controls at day 0 and were decreased by day 30, FSH still remained significantly higher than controls at day 30 (Fig. 1I' and 1J'), suggesting its return to homeostasis may be slower than LH.

B. Pretreatment With rhMIS Increases Superovulation Yield

Based on the timing of resumption of folliculogenesis following MIS pretreatment as determined by follicle counts, we hypothesized that stimulating antral follicles on day 30 could induce a large synchronized cohort of follicles to mature. To test this hypothesis, we induced superovulation with gonadotropins (PMSG and, 48 hours later, hCG) on days 15 (N = 8), 30 (N = 7), and 50 (N = 4) following MIS pretreatment release in mice or in age-matched saline control–pretreated mice at day 30 (N = 5) (Fig. 2A). The average number of eggs ovulated in MIS-pretreated animals stimulated on day 15 postrelease was only one per animal, indicating that the growing follicle pool was not yet responsive to gonadotropin stimulation by day 15 (Fig. 2B). In contrast, day 30 pretreated mice ovulated on average 55.4 eggs, which was 3.4 times more than age-matched saline-treated day 30 control animals (Fig. 2B; $P < 0.001$). Consistent with the hypothesis that the large wave of synchronized follicles reaches an apex by day 30, which is gradually followed by a return of normal follicular homeostasis, day 50 MIS-pretreated mice ovulated 23.8 eggs on average, which is significantly fewer than the MIS-pretreated animals stimulated at day 30 (Fig. 2B).

C. Neoadjuvant rhMIS Pretreatment Augments the Response to Superovulation in a Mouse Model of DOR

To validate the enhancement of gonadotropin stimulation in a clinically relevant model, we evaluated neoadjuvant rhMIS treatment in a mouse model of DOR. To induce DOR, we treated mice (N = 3/group) with once-a-day IP VCD (160 mg/kg) for 5 days (Fig. 3A), a well-characterized alkylating agent used for sterilization and modeling of menopause in mice [33]. This treatment resulted in a 73% reduction in primordial follicles compared with vehicle control treatment as observed 14 days after treatment (Fig. 3A). Following the treatment of mice with VCD, mice (N = 8) received a 40-day course of rhMIS neoadjuvant or vehicle control, followed by a 30-day release and a gonadotropin-stimulation protocol (PMSG plus hCG) to induce superovulation (Fig. 3B). VCD-treated DOR mice receiving an rhMIS neoadjuvant treatment had twofold more eggs ovulated in response to stimulation, compared with those treated receiving vehicle control (Fig. 3C; $P < 0.05$), with no remarkable differences in the proportion of viable eggs recovered (Fig. 3C), although these DOR mice treatments were not directly compared with control mice treated with saline instead of VCD, as seen in Fig. 2A.

3. Discussion

The inhibitory effect of MIS on primordial follicle activation has long been suspected since the observation from Durlinger *et al.* [14] that MIS knockout mice deplete their ovarian reserve

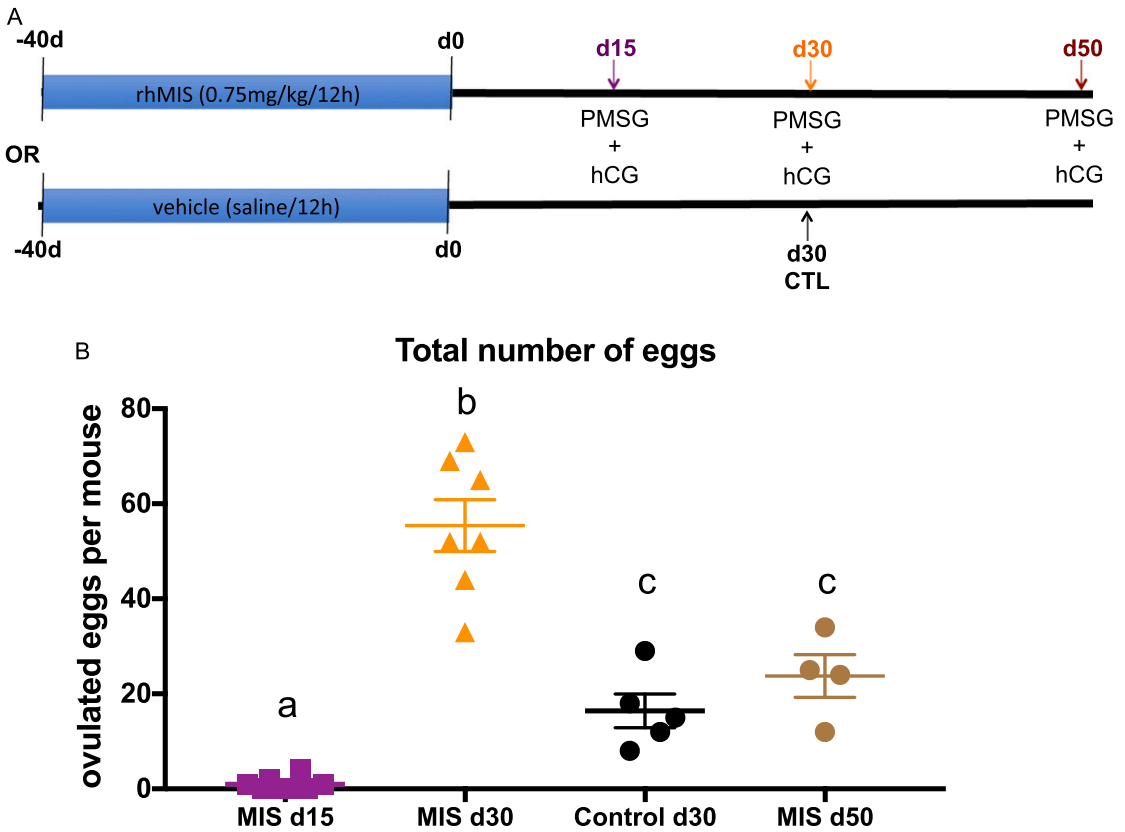

Figure 2. Superovulation yield following MIS adjuvant therapy and gonadotropin stimulation. (A) Six-week-old female Nu/Nu mice were treated with rhMIS twice daily for 40 d at 750 μg/kg/dose or saline as vehicle control (days −40 to 0). Following this pretreatment, the mice were induced to ovulate with a PMSG and hCG protocol starting on days 15 (MIS d15), 30 (MIS d30 or Control d30), or 50 (MIS d50) postrelease. (B) The number of eggs recovered in the oviduct 18 h after hCG stimulation were counted for each mouse. Statistical differences were analyzed by one-way ANOVA followed by Tukey multiple-comparison test. Different characters on the groups indicate significant difference between each groups (a, b, and c; $P < 0.05$).

faster than wild-type littermates and the albeit modest inhibitory properties of recombinant MIS in ovarian *ex vivo* cultures [15–17]. We recently reported that AAV9-rhMIS treatment *in vivo* completely halted the development of follicles, resulting in a quiescent ovary consisting almost entirely of primordial follicles [13]. This ovarian suppression could alternatively be induced using daily injections of rhMIS protein for 40 days and was reversible upon cessation of treatment. In the current study, we expand upon those findings and demonstrate that the initial wave of folliculogenesis immediately following release from MIS suppression displays rapid growth, reduced atresia, and synchronized maturation culminating at 30 days post-release, characteristics that may be exploited to enhance ovarian hyperstimulation protocols.

Our model of complete arrest and resumption of folliculogenesis allows for a unique experimental dissection of the kinetics of growths of follicles. Upon release from MIS suppression, the hormonal milieu of pretreated mice is akin to that of gonadectomized animals, with high gonadotropins and almost absent ovarian hormones, including endogenous MIS and inhibins. However, given that the ovary at that stage is composed almost entirely of primordial follicles, some parallels can be made to the first wave of folliculogenesis in the neonatal ovaries [34]. Similar to the neonate setting, the kinetics of maturation of the first wave of follicles goes from primordial to fully competent antral follicle within 30 days.

We hypothesize that the combination of absent or low endogenous MIS, high FSH, and low rates of atresia give rise to an outsized wave of coordinated follicles maturing in synchrony.

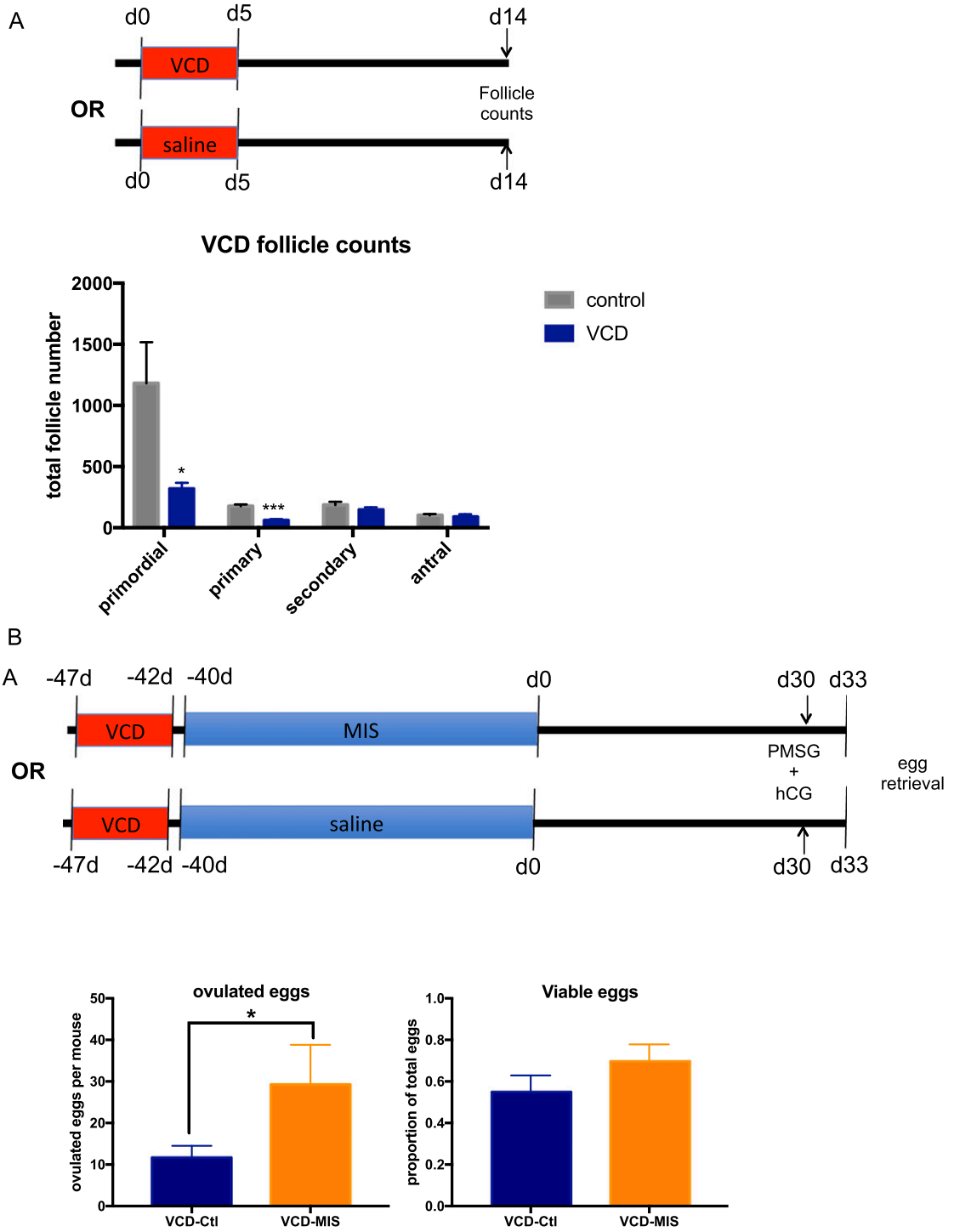

Figure 3. Superovulation yield and egg viability following MIS adjuvant therapy and gonadotropin stimulation in a mouse model of DOR. (A) Six-week-old female Nu/Nu mice (N = 3/group) were treated with VCD 160 mg/kg or vehicle control (corn oil) for 5 d, and 9 d later, ovaries were examined for follicle counts to assess the damage to the ovarian reserve. (B) Six-week-old female Nu/Nu mice (N = 4/group) were treated with VCD 160 mg/kg or vehicle control (corn oil) for 5 d, 2 d later treated with rhMIS twice daily (750 μg/kg/dose) for 40 d (or vehicle control), and induced to ovulate by treatment with PMSG and hCG on day 30 postrelease. The number of eggs in the oviduct 18 h after hCG stimulation were compared for each group. The number of viable vs degenerated eggs were also counted to produce the ratio of viable eggs as defined by normal cytology. Statistical differences were analyzed by Student *t* test. $^{*}P < 0.05$; $^{***}P < 0.001$.

Once this wave of maturing follicles reaches the antral stage, they start to reset the normal hormonal feedback that maintains follicular steady-state dynamics. Consistent with this hypothesis, pituitary hormones such as LH and FSH and ovarian hormones such as endogenous mMIS and Inhibin A normalize with the return of antral follicles at day 30 and return to equilibrium by day 50.

Only when the gonadotropin stimulation was made to coincide with the arrival of follicles to the antral stage at day 30, but not before (day 15) or after (day 50), did the superovulation of this synchronized pool result in a remarkable threefold increase in ovulated eggs. This outsized recruitment was accompanied by a corresponding decrease in ovarian reserve, consistent with the hypothesis that lack of negative feedback by endogenous MIS and other hormones accelerates activation and follicular development. It should also be noted that the decline in primordial follicles following MIS pretreatment is likely to cause a higher expenditure of ovarian reserve than gonadotropin stimulation alone. This depletion occurred acutely during the first 30 days of initial release, and no further loss was observed on days 30 to 50, during which time antral follicle counts and endogenous mMIS levels returned to normal. Alternatively, some of the ovarian reserve loss may be the direct consequence of prolonged and elevated exposure to superphysiological levels of MIS, which in transgenic models appears to be harmful to both follicles and egg quality [35]. In our model, however, egg viability appears to be unaffected, suggesting the adverse effects of MIS exposure on follicle health observed in these studies may be the sequela of fetal or neonatal exposure during follicular assembly [35–37]. Further studies examining the consequence of MIS pretreatment on early embryonic development of the zygote will be needed to confirm the lack of adverse effects on egg quality using a nonimmune compromised mouse model more suited for *in vitro* embryo maturation.

Although the neoadjuvant MIS treatment explored in this study allowed for complete control over folliculogenesis, the subsequent enhancement of activation and growth of follicles does translate into substantial ovarian reserve expenditure. Others have successfully used rhMIS pretreatment as a way to enhance superovulation albeit with a distinctly different methodology [38]; a short course of comparatively low doses of C-terminal mature rhMIS was used, which, although insufficient to arrest folliculogenesis, resulted in a 1.3-fold enhancement in ovulation yield in adult mice when immediately followed by gonadotropin stimulation. Surprisingly, however, the C-terminal mature MIS (R&D Systems, Minneapolis, MN) used in the aforementioned study does not cause regression of the Müllerian duct [13], and its pharmacological properties remain unknown. These data raise the tantalizing possibility that low doses of MIS C-terminal mature domain, unassociated with MIS N-terminal prodomain, could act on the pituitary, given that this same recombinant protein preparation was shown to modulate gonadotropin secretions in other studies [39–41].

The increased yield of eggs following neoadjuvant MIS therapy and gonadotropin stimulation on day 30 postrelease suggests a potential application of MIS to enhance current *in vitro* fertilization protocols in patients with DOR or poor ovarian response. To model this clinical setting, we evaluated the effectiveness of MIS neoadjuvant therapy in a mouse model in which VCD treatment was used to diminish the ovarian reserve. In this DOR model, MIS therapy augmented the recovery of eggs by twofold, indicating that increased activation can partially rescue the response to gonadotropins and overcome the deficits in ovarian reserve. Many adjuvant treatments to enhance subsequent ovarian stimulation have been investigated previously, such as dehydroepiandrosterone, although with mixed success [42–44], highlighting the need for more clinical options in patients with DOR. Experimentally, blocking other negative-feedback inhibitors, such has inhibins, has been proposed as a way to enhance gonadotropin stimulation using anti-inhibin serum [45]. Our data suggest the same concept could be applied to MIS, by either using neutralizing antibodies to MIS or blocking MISR2 signaling with antagonists to relieve temporarily the negative feedback exerted on primordial follicle activation.

Given the complete control of follicle activation and synchronization of folliculogenesis, afforded by treatment with MIS, we anticipate many applications for this hormone in the

management of fertility and ovarian pathologies. A careful dissection of the mechanisms of action of MIS on each different follicle type, as well as on the pituitary, will be required to understand the full extent of its clinical utility. In this study, we reported experimental evidence that one such use of MIS could be as a neoadjuvant therapy to enhance response to gonadotropin stimulation in refractory patient populations, such as those with DOR.

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Disclosure Summary: The authors have nothing to disclose.

Data Availability: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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