Association of bioavailable inhibin B and oocyte yield in controlled ovarian stimulation

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Objective: To determine if the biologically active or bioavailable inhibin B (bio-inhB) correlated with the oocyte yield in controlled ovarian stimulation (COS).
Design: Cross-sectional study.
Setting: Academic center.
Patient(s): Women undergoing oocyte cryopreservation.
Intervention(s): None.
Main Outcome Measure(s): Serum of women were sampled to measure bio-inhB at three points: baseline ("start"); middle ("mid"); and end of COS. A validated, highly specific enzyme-linked immunosorbent assay (Ansh Labs, Webster, TX) measured bio-inhB. The Spearman tests analyzed correlations between bio-inhB and other ovarian reserve markers, including age, follicle-stimulating hormone (FSH), antral follicle count (AFC), and antimüllerian hormone (AMH), and correlations between these markers and oocyte yield.
Result(s): A total of 144 women were included. Bioavailable inhibin B at the mid and end of COS, plus its delta, were strongly correlated with other ovarian reserve markers. As the bio-inhB concentration increased, the AFC and AMH levels also increased, whereas the FSH concentration and age decreased. Bioavailable inhibin B values, except at the start of COS, were more strongly correlated with oocyte yield than the FSH concentration ($r = 0.72–0.82$ vs. $r = -0.44$) and correlated similarly to the AFC and AMH concentration ($r = 0.79$ and 0.81, respectively). These correlations strengthened in those with diminished ovarian reserve, specifically age ≥ 35 years or AMH concentration <2 ng/mL ($r = 0.71–0.86$ vs. $r = 0.49–0.67$).
Conclusion(s): Predicting COS outcome is imperfect. When using a highly specific enzyme-linked immunosorbent assay, bio-inhB correlated with the oocyte yield similar to or more strongly than traditionally used ovarian reserve markers. These correlations strengthened in cases of diminished ovarian reserve. Bioavailable inhibin B provides physicians with an additional clinical tool for estimating COS outcome. (Fertil Steril Rep® 2021;2:189–94. ©2021 by American Society for Reproductive Medicine.)
Key Words: Inhibin B, ovarian reserve, controlled ovarian stimulation, oocyte yield

Inhibin B (inhB) is a heterodimeric protein composed of an alpha (α) and a beta-B (β_B) subunit. In women, inhB is produced by granulosa cells in the ovary with the goal of suppressing follicle-stimulating hormone (FSH) production (1–3). As the FSH concentration increases, the inhB concentration subsequently increases. Inhibin B peaks in the midfollicular phase, with a second rise after the preovulatory luteinizing hormone (LH) surge, and then declines to a persistently low concentration in the luteal phase (1, 4, 5). Past research suggested that the relationship between inhB and folliculogenesis would enable inhB to be considered as a marker of ovarian reserve (1, 5, 6). Inhibin B concentrations were found to be significantly lower in both serum and follicular fluids of older-aged women than those in younger-aged women (2, 7). This was thought to be because of a decreasing FSH-sensitive follicular pool with increasing age, leading to a decline in the granulosa cell production of inhB (7, 8).

However, several studies have failed to show inhB to be an ovarian reserve marker (6, 7). This led investigators to then consider inhB as merely a marker of ovarian activity because of

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its production by rapidly proliferating granulosa cells of preantral follicles in response to FSH (6). This relationship has been demonstrated in cases of controlled ovarian stimulation (COS) (9–13). When comparing healthy volunteers in a natural menstrual cycle to when they underwent various regimens of gonadotropin stimulation, those with multifollicular development demonstrated significantly elevated concentrations of inhB compared to those with monofollicular development in natural cycles (10). When examining poor, normal, and hyper-responders undergoing COS, the concentration of inhB mid-stimulation (stimulation day 5) demonstrated significant direct relationships with the number of oocytes retrieved (9, 11, 13). In addition to measuring inhB at a particular point in time, the change in inhB concentration over the course of COS ("delta" inhB) has shown strong positive associations with the number oocytes retrieved (12, 14–17).

The inconsistent results regarding the clinical utility of inhB have been attributed to assay variability and intercycle variability of inhB. These limitations affect the specificity and reproducibility of inhB as a marker of ovarian reserve, ovarian activity, and predictor of oocyte yield (18, 19). Previous inhB assays exhibit cross-reactivity with other glycoproteins in the transforming growth factor beta family, causing detector antibodies to bind with not only β_B but also β_A and other similarly structured subunits (19). The poor specificity and poor precision of older assays had made inhB a poor marker of ovarian reserve/activity or oocyte yield in COS (20).

A newly designed highly specific enzyme-linked immunosorbent assay (ELISA) was recently developed (Ansh Labs, Webster, TX) to accurately measure bioavailable inhB (bio-inhB) (21, 22). The primary objective of this study was to determine if concentrations of bio-inhB, either at certain time points in a cycle or its change over the course of COS, correlate with the oocyte yield in women undergoing COS. The secondary objectives were to determine if bio-inhB was a stronger predictor of oocyte yield than traditionally used ovarian reserve markers.

MATERIALS AND METHODS

Study Design

This retrospective study, approved by the Institutional Review Board, Study ID HS-15-00859, was conducted at a single-site fertility clinic affiliated with the University of Southern California, Los Angeles, California, USA. Oocyte cryopreservation cases from January through December 2019 were reviewed. Women undergoing oocyte cryopreservation for social, medical, or situational reasons were eligible. Women with only one ovary or those with infertility who planned to undergo in vitro fertilization were excluded. Controlled ovarian stimulation protocols consisted of FSH preparations and recombinant LH (or human chorionic gonadotropin in place of LH) with either a gonadotropin-releasing hormone antagonist or a gonadotropin-releasing hormone analog.

Demographic data and baseline ovarian reserve testing were collected, including age, body mass index (kg/m^2), ethnicity, antimüllerian hormone (AMH) level, baseline FSH concentration, and baseline antral follicle count (AFC). Stimulation and retrieval data were collected. Retrieval data included both the total numbers of metaphase II and immature oocytes and the number of metaphase II oocytes only.

Serum was collected at three time points: at the start of stimulation, which was the morning before starting gonadotropins; at the middle ("mid-stimulation"), which was days 5–7, depending on the person's duration of COS; and at the end of stimulation, which was the day before retrieval (approximately 12 h after trigger). Samples were previously frozen at our center's biospecimen repository at −20 °C and obtained retrospectively. Frozen samples were shipped to and then thawed at Ansh Labs (Webster, TX), where the assay was developed. Bioavailable inhibin B was measured at each of these three time points, and its change in concentration between the end and start of stimulation was considered the delta.

Serum Hormone Assays

Serum AMH was quantified using the Gen II ELISA immunoassay (Beckman Coulter, Inc., Brea, CA). The assay sensitivity was 0.16 ng/mL, and the interassay coefficient of variation (CV) was <8%. Serum FSH was measured using the cobas 6000 analyzer series (Roche Diagnostics, Indianapolis, IN). The FSH assay sensitivity was 0.1 mIU/mL, and the CV was 4.9%, on average. Serum estradiol (E2) was measured by electrochemiluminescence immunoassay on the cobas analyzer. The limit of detection of the assay was 5 pg/mL; the cross-reaction of estrone was <1%; the interassay CV was 6.8% at 45 pg/mL and 2.5% at 1,308 pg/mL.

Bioavailable inhibin B was measured using the InhB ELISA (AL-107; Ansh Labs, Webster, TX). This assay uses two monoclonal antibodies directed toward the α and β_B subunits of inhB, which limit cross-reactivity with inhibin A, activin A, activin B, activin AB, and other structurally similar glycoproteins in the transforming growth factor beta family, thereby making the assay 100% specific to inhB (21–23). The assay was validated previously and shown to have a dynamic range of 2–1,400 pg/mL and has a limit of detection of 1.6 pg/mL (23). The interassay CV in our study was <7.5%.

Statistical Analysis

Descriptive statistics were used to analyze demographic variables. The Spearman tests evaluated correlations between bio-inhB values and other ovarian reserve markers, as well as correlations between bio-inhB or ovarian reserve markers and oocyte yield. These markers included age, day 2/3 FSH, day 2/3 AFC, and AMH. Following this analysis, data were stratified by diminished ovarian reserve (DOR) parameters: age <35 or ≥35 years, FSH concentration <10 or ≥10 mIU/mL, AFC <7 or ≥7 follicles, and AMH level <2 or ≥2 ng/mL. These parameters were decided upon after the investigators reviewed the consensus on the definition of "poor response" to ovarian stimulation in conjunction with incorporating how our fertility center counseled patients on ovarian reserve status (24).

Since AFC and concentrations of serum E2 24h before retrieval ("peak E2") have typically been used during COS to

prospectively estimate oocyte yield, receiver operating characteristic (ROC) curves were created to determine whether bio-inhB values were predictive of oocyte yield when AFC was <7 versus ≥7 and when peak E2 was <1,000 pg/mL versus ≥1,000 pg/mL.

RESULTS

Patient Demographics

A total of 144 women undergoing oocyte cryopreservation met the inclusion criteria for this study. Most patients were young, with a median age of 35 years (interquartile range, 31–37 years). Most women were Caucasian (n = 81, 56.3%), and most had a normal body mass index, with a median of 22.3 kg/m^2 (interquartile range, 20.3–24.5 kg/m^2). Only a minority of women met the DOR criteria (Table 1).

Bio-inhB Throughout COS

The bio-inhB concentration progressively increased throughout the cycle (Fig. 1). Bioavailable inhibin B at the middle and end of stimulation, as well as the delta bio-inhB, was correlated with the ovarian reserve markers (Supplemental Table 1, available online). As the AFC and AMH levels increased, the bio-inhB concentration also increased. On the other hand, as the age and FSH concentration increased, the bio-inhB concentration decreased. Similarly, larger increases in bio-inhB over the course of COS (generating a larger delta) coincided with younger age, lower FSH concentration, increasing AFC, and increasing AMH level. Bioavailable inhibin B at the start of the cycle, before ovarian stimulation, did not correlate with the ovarian reserve markers.

Predictors of Oocyte Yield

As indicated in Table 2, age and baseline FSH concentration were negatively associated with oocyte yield (r = −0.40 to −0.44; $P<.05$), yet the AMH level and AFC were positively associated with oocyte yield (r = 0.79–0.81; $P<.05$). Among all four ovarian reserve markers, the AFC and AMH levels were the strongest predictors.

As for bio-inhB and oocyte yield, bio-inhB at the middle and end of stimulation, as well as the delta bio-inhB, all directly correlated with oocyte yield. These correlations were stronger than those between oocyte yield and age or baseline FSH concentration (r = 0.71–0.82 vs. r = −0.4 to −0.44, respectively; $P<.05$; Table 2). Concentration of bio-inhB at the end of stimulation and its delta over the course of stimulation were similarly strong predictors of oocyte yield as compared with the AFC and AMH level (r = 0.78–0.82 vs. r = 0.79–0.81; $P<.05$; Table 2). Delta bio-inhB exhibited a slightly stronger correlation to oocyte yield than bio-inhB levels at any specific time point during COS, but this correlation was too small for clinical significance. The difference between the predictability of the total number of oocytes and that of only mature oocytes was minimal (Table 2).

Inhibin B was historically used as a potential marker of ovarian aging in COS. Therefore, we stratified correlations between bio-inhB values and oocyte yield by DOR parameters (Table 3). Correlations between oocyte yield and bio-inhB, including mid-stimulation, end of stimulation, and its delta, all strengthened in women ≥35 years of age versus those in women <35 years of age. Similarly, bio-inhB values and its delta had stronger correlations with oocyte yield in women with lower AMH values. Bioavailable inhibin B was generally a stronger predictor of oocyte yield in women with an AMH level <2 ng/mL (n = 63) compared with that in women with an AMH level ≥2 ng/mL (n = 77). Correlations were similar when stratified by FSH concentrations <10 (n = 107) and ≥10 (n = 18) mIU/mL and not significant when stratified by AFC <7 (n = 8) and ≥7 (n = 132) (Table 3).

Since delta bio-inhB and bio-inhB at the end of stimulation generated the strongest correlation to the total number of oocytes retrieved, ROC analyses investigated their sensitivity and specificity with respect to AFC and peak E2. The areas under the curves (AUCs) for assessing the value of delta bio-inhB in women with an AFC of <7 or peak E2 of <1,000 pg/mL were 0.96 and 0.79, respectively. The AUCs with regard to

TABLE 1

Patient demographics and stimulation outcomes.

	Median (IQR)	Cutoff	N per cutoff (%)
Age (years)	35 (31–37)	<35	63 (43.8%)
		≥35	81 (56.3%)
FSH[a] (mIU/mL)	6.8 (5.3–8.3)	<10	107 (85.6%)
		≥10	18 (14.4%)
AFC[b] (follicles)	17 (12–24)	<7	8 (5.7%)
		≥7	132 (94.3%)
AMH[b] (ng/mL)	2.2 (1.1–3.7)	<2	63 (45.0%)
		≥2	77 (55.0%)
Peak E2 (pg/mL)	3,009 (1,854–4,272)		
Number of MII oocytes	9 (5–15)		

Note: AFC = antral follicle count; AMH = antimüllerian hormone; FSH = follicle-stimulating hormone; IQR = interquartile range; MII = metaphase II.
[a] Data for only 125 participants.
[b] Data for only 140 participants.

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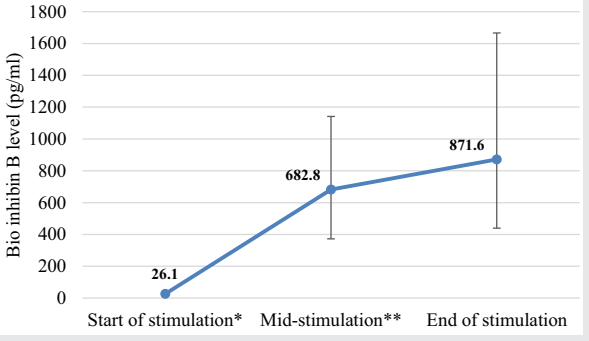
FIGURE 1



Trend of bioavailable inhibin B (bio-inhB) during controlled ovarian stimulation. Median concentration of bio-inhB at the start, middle, and end of stimulation; error bars indicate interquartile range. *n = 141; **n = 143.

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TABLE 2

Correlations between bioavailable inhibin B and oocyte yield.

	Age*	FSH*	AFC*	AMH*	Bio-inhB, start*	Bio-inhB, mid*	Bio-inhB, end*	Bio-inhB, delta*
Total number of oocytes	−0.44	−0.44	0.79	0.81	0.19	0.72	0.82	0.82
MII, only	−0.40	−0.41	0.80	0.81	0.20	0.71	0.79	0.78

Note: AFC = antral follicle count; AMH = antimüllerian hormone; Bio-inhB = bioavailable inhibin B; FSH = follicle-stimulating hormone; MII = metaphase II.
* $P<.05$.

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TABLE 3

Correlations between bioavailable inhibin B and the total number of oocytes retrieved when stratified by age and antimüllerian hormone level.

	Bio-inhB, mid	Bio-inhB, end	Bio-inhB, delta
Normal and hyper-responders			
Age <35 years*	0.60	0.75	0.73
FSH <10 mIU/mL*	0.67	0.82	0.82
AFC ≥7*	0.70	0.82	0.82
AMH ≥2 ng/mL*	0.49	0.67	0.66
Poor responders			
Age ≥35 years*	0.78	0.86	0.86
FSH ≥10 mIU/mL*	0.73	0.78	0.81
AFC <7#	0.31	0.59	0.89
AMH <2 ng/mL*	0.74	0.71	0.73

Note: Correlation coefficients calculated using the Spearman tests, r. AFC = antral follicle count; AMH = antimüllerian hormone; Bio-inhB = bioavailable inhibin B; FSH = follicle-stimulating hormone.
* $P<.05$.
# When AFC was <7, only the correlation between the delta bio-inhB and total number of oocytes was statistically significant.

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the predictability of bio-inhB at the end of stimulation with an AFC of <7 or peak E2 of <1,000 pg/mL were 0.97 and 0.79, respectively.

DISCUSSION

Theories behind the clinical significance of inhB and its utility for being a marker of ovarian reserve and/or activity in COS have been nebulous at best. Knauff et al. (6) conducted a nationwide prospective cohort study to assess whether certain ovarian reserve markers changed with respect to age, baseline FSH, and menstrual history. While the AFC and AMH levels decreased with increasing age, the inhB concentration did not (6). Conversely, other studies have shown direct associations between inhB and ovarian activity in infertile women undergoing COS (9–13). Given these mixed findings and wanting a broader understanding of the clinical utility of bio-inhB, we included all women undergoing oocyte cryopreservation versus those with infertility and/or significant DOR (Table 1).

When using this highly specific ELISA to accurately measure the active, or bioavailable, inhB, in the serum of women undergoing COS, bio-inhB was strongly correlated with oocyte yield. This was evident when predicting both the mature and total number of oocytes retrieved. The bio-inhB levels at the end of stimulation, or 12 h after the trigger, and the delta bio-inhB performed more strongly or similarly to age, baseline FSH concentration, AFC, or AMH levels. The bio-inhB concentration at the start of stimulation did not significantly correspond to oocyte yield. This is no surprise since folliculogenesis had not been initiated at this point, so there was no need for inhB to suppress FSH. However, it is important to note that measuring bio-inhB at baseline still has clinical value. The baseline concentration is required for the calculation of the delta bio-inhB, which did demonstrate a strong positive correlation with oocyte yield.

Correlations between oocyte yield and bio-inhB values strengthened even more when stratified by age <35 or ≥35 years and AMH levels <2 or ≥2 ng/mL. In older women as well as in women with lower AMH values, there was a stronger correlation between bio-inhB and oocyte yield. The higher the bio-inhB value at mid-stimulation, and the more robust of a change in bio-inhB over the course of COS (the higher the delta), the more oocytes retrieved. However, correlations did not improve when stratified by FSH concentrations <10 or ≥10 mIU/mL, nor when stratified by AFC <7 or ≥7. Reasons for why correlations between bio-inhB and oocyte yield did not strengthen under these circumstances could be because of both the cutoff values used to classify DOR, as well as the patient population at hand. This study had a small sample size of only 144 women. These women were not inherently infertile or with DOR, as they all underwent oocyte cryopreservation and had not attempted conception previously. To see the true effects of DOR on correlations between bio-inhB and oocyte yield, one would ideally have a substantial number of participants in incremental classes of ovarian reserve status, such as women with FSH concentrations <10, 10–20, and >20 mIU/mL or AMH levels <1, 1–2, and >2 ng/mL. Our study did not focus on a DOR population and was, therefore, underpowered to study this subgroup of women.

The fact that delta bio-inhB over the course of COS was the strongest predictor of oocyte yield, more so than at a given time point, deserves special attention. The biphasic pattern of inhB has been well described: inhB peaks in the midfollicular phase, corresponding to granulosa cell activity in preantral follicles, and peaks for a second time in response to the LH surge just before ovulation (4, 5).In addition to assay variability, a reason for the previous inconsistent findings regarding inhB as a marker of ovarian reserve or activity, in both natural and COS cycles, could be that the focus of prior studies had been on inhB as an isolated value. Studies were not focusing on inhB as a fluid hormone measurement. Inhibin B is dynamic in vivo and should be measured

throughout different time points in a COS cycle to understand its relationship with outcome variables. A key strength of this study was that with the use of a more precise and specific assay, we were able to accurately characterize the changes in bio-inhB over the course of COS and find meaningful results regarding its predictive value for oocyte yield.

Given that bio-inhB at the end of stimulation and the delta bio-inhB were the strongest predictors of oocyte yield out of all bio-inhB values, we attempted to investigate their sensitivity and specificity with respect to AFC and peak E2. While the ROC analyses showed promising AUCs (0.97 and 0.79, respectively), these need to be interpreted with caution, as only 6.4% (n = 9) women in this study had a baseline AFC of <7 and only 6.9% (n = 10) had a peak E2 of <1,000 pg/mL. We cannot assume sensitivity and specificity for oocyte yield prediction change with respect to changing AFC or peak E2. Future studies with uniformly large populations of women exhibiting various levels of ovarian reserve and response to exogenous gonadotropins should be performed to investigate the validity of bio-inhB values as predictors of oocyte yield with respect to incremental AFC and peak E2 cut points. Taken one step further, future studies should compare the predictability of bio-inhB values with each other. It is possible that the delta bio-inhB or a bio-inhB value at a specific time point during COS has a stronger predictive value with respect to oocyte yield. Given that this was a pilot study, we were initially uncertain which, if any, of the bio-inhB values would correlate with oocyte yield, which was why we chose to focus our investigation on the relationships between bio-inhB values and oocyte yield and other ovarian reserve markers, versus investigating the clinical significance of each bio-inhB value.

An important question to ask is: What is the clinical utility of measuring bio-inhB to only generate a predictive value that is similar to the currently used ovarian reserve markers? The goal of ovarian reserve testing at the initial consultation is to serve as a prognostic tool for the clinician, who then uses this information to counsel the patient (20, 25). The predictive value of ovarian reserve markers depends on the population being tested, as screening tests are generally dependable on the prevalence of disease (i.e., DOR) (20, 26). Additionally, the predictability and accuracy of these markers for assessing ovarian response may be a result of the cutoff values used, like in the case of AMH. Published data have used a variety of AMH cutoff values for predicting poor response to COS, and even the best cutoff value for an ovarian reserve marker can be associated with a false-positive rate of 10%–20% (14, 20, 24, 27–29). While this study does not demonstrate the superiority of bio-inhB to other currently used markers of ovarian reserve when predicting oocyte yield, bio-inhB can be a valuable additional tool for the clinician when estimating oocyte yield preretrieval.

CONCLUSION

The ability to accurately predict the number of oocytes is of great clinical interest to both reproductive endocrinologists and patients. Prior studies have used older assays with high cross-reactivity and have not found inhB to be a clinically useful marker for assessing ovarian reserve or response to COS. This may be because of inaccuracies in measuring the true biologically active concentration of inhB. To our knowledge, our study was one of the first to use this highly specific assay to measure bio-inhB. We were able to demonstrate a strong relationship between the delta bio-inhB and bio-inhB at the end of stimulation with oocyte yield during COS. These relationships may have a similar or more predictive value of oocyte yield than other ovarian reserve markers, particularly in older women and those with low AMH values. Future studies should explore the utility of bio-inhB prospectively as women undergo COS. Nevertheless, findings from this retrospective study are encouraging. Reproductive endocrinologists may use bio-inhB as an additional tool when counseling patients on their estimated oocyte yield.

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