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Minimally Invasive Method of Measuring Anti-Müllerian Hormone Level in Dried Blood Spots of Indonesian Infertile Population

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ABSTRACT

This study aims to evaluate the certainty of anti-Müllerian hormone (AMH) levels in dried blood spots (DBS) compared to serum AMH. A total of 109 infertile women who planned to undergo their controlled ovarian stimulation cycle were recruited. Serum and DBS samples were collected from each patient for the quantification of AMH levels. Serum AMH was measured with an automated clinical chemistry assay (Roche) while DBS AMH was measured using the Ansh Labs DBS AMH ELISA reagent kit. Mean AMH level in DBS was 4.02 ± 3.89 ng/mL, which ranged from 0.01 to 17.3 ng/mL, while the mean AMH in serum samples was 2.44 ± 2.29 ng/mL (0.01–10.8 ng/mL). Comparable accuracy for low AMH quantification was observed between the two methods over basal antral follicle counts (AFC) as a clinical comparator (AUC 86%, cutoff ≤1.95 ng/mL, $R = 0.67$, $P < 0.001$ vs. 85%, cutoff ≤1.15 ng/mL, $R = 0.66$, $P < 0.001$, respectively for DBS and serum sample). Likewise, DBS AMH achieved high concordance for low AMH discrimination in comparison to serum AMH method (AUC 99% vs. 93%, respectively). A strong linear Spearman regression ($R = 0.92$, $P < 0.001$) and sufficient agreement (94.5%, mean difference of −1.6, 2.7 to −5.9) was demonstrated among studied groups with mathematical Passing and Bablok regression equation of $y = -0.12+1.80\times$. The confidence interval of intercept (−0.12, 95% CI [−0.29 to −0.01]) and slope (1.80, 95% CI [1.66–1.94]) indicated a constant and proportional difference between the two methods but it was not significant ($P = 0.893$). AMH measurement in DBS could be useful as an alternative approach to screening ovarian reserve through a more convenient remote sample collection.

Keywords: Anti-Müllerian Hormone; Dried Blood Spot; In-vitro Fertilization.

ABSTRAK

[ABSTRACT IN BAHASA INDONESIA]

Penelitian ini bertujuan untuk mengevaluasi kadar hormon anti-Müllerian (AMH) menggunakan sampel darah kering (dried blood spots, DBS) dibandingkan dengan serum darah. Sampel DBS dan serum dikoleksi dari 109 wanita infertil yang akan menjalani program bayi tabung. Kadar AMH sampel DBS dianalisis menggunakan reagen kit ELISA DBS AMH (Ansh Lab) sedangkan kadar AMH sampel serum dianalisis menggunakan Elecsys® AMH plush Assay (Roche). Rerata kadar AMH pada sampel DBS adalah 4,02 ± 3,89 ng/mL (0,01 – 17,3 ng/mL), sedangkan rerata kadar AMH pada sampel serum adalah 2,44 ± 2,29 ng/mL (0,01–10,8 ng/mL). Diperoleh akurasi sebanding antara kedua sampel dalam mengukur AMH kadar rendah dibandingkan terhadap jumlah folikel basal sebagai pembanding klinis (AUC 86%, titik potong ≤1.95 ng/mL, R = 0.67, P < 0.001 vs. 85%, titik potong ≤1.15 ng/mL, R = 0.66, P < 0.001, masing-masing untuk sampel DBS dan serum). Sampel DBS juga mencapai kesesuaian yang sangat baik dengan sampel serum untuk menentukan AMH kadar rendah (AUC 99% vs. 93%). Regresi linear Spearman yang kuat (R = 0.92, p < 0.001) dan tingkat kesesuaian yang cukup (94.5%, perbedaan rerata −1.6 (2.7 sampai −5.9)) diamati pada kedua group sampel

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dengan rumus matematika regresi Passing dan Bablok y = −0.12+1.80×. Selang kepercayaan untuk nilai intercept ((−0.12, 95% CI [−0.29 to −0.01]) dan slope (1.80, 95% CI [1.66–1.94]) menunjukan adanya perbedaan konstan dan proporsional antara kedua sampel, namun tidak bermakna (P = 0.893). Dapat disimpulkan bahwa pengukuran kadar AMH dengan sampel DBS dapat menjadi pilihan untuk evaluasi cadangan folikel ovarium melalui koleksi sampel jarak jauh yang lebih nyaman.

Kata kunci: Hormon Anti-Mullerian, Sampel darah kering, Fertilisasi in-vitro

INTRODUCTION

Anti-Müllerian Hormone (AMH) is a prominent biomarker to measure ovarian reserve (Nelson et al., 2015a) and a reliable prognostic factor to predict ovarian response to gonadotropin stimulation (Visser et al., 2006). The clinical application of AMH has also been proposed to be more accurate in predicting ovarian response to gonadotropin in IVF stimulation than other evaluations, such as age, basal follicle-stimulating hormone, estradiol, and inhibin B (La Marca et al., 2009). Moreover, AMH levels have been utilized to assess the effect of cancer treatment on ovarian reserve in a specific group of female cancer survivors (Gracia et al., 2012).

AMH is produced by granulosa cells of small pre-antral and antral follicles within the ovary in the form of glycoprotein dimer with minimal level fluctuations during menstrual cycles (Peluso et al., 2014). Measurement of AMH level is commonly performed in serum or plasma from venous blood collected through phlebotomy, which is quite invasive. In addition, the stability of the AMH is easily compromised during the handling of the serum samples. This has been a concern, discussed by experts due to the possible bias produced because of the varying handling process (Li et al., 2021).

Dried blood spots (DBS) have been proposed as an alternative tool for measuring the AMH level (McDade et al., 2012). This method quickly gained popularity among other micro-sample-based methods (<50 µL) and continues to be developed to measure other analyte types, including genomic, epigenomic, transcriptomic, proteomic, and metabolic markers. The current practice of DBS is established in the pediatric clinical setting for the screening of metabolic disorders (Freeman et al., 2018). DBS is a well-known approach to studying public health surveillance and epidemiological research (Sakhi et al., 2015) or to evaluating fecundability in a specific population (Hall et al., 2020) that could be processed through assays with high sensitivity.

Utilizing a sterile disposable lancet, approximately five drops of whole blood are applied to a specific filter paper for analysis (Hall et al., 2020). User-friendliness is one benefit of measuring AMH levels using DBS that a patient can self-collect the blood sample either at home or in a nonclinical setting. Samples are allowed to dry and are stored in a bag to be transported to the laboratory for measurement (Sakhi et al., 2015). Moreover, collecting DBS samples is less painful and invasive in comparison to phlebotomy. Nowadays, with increased productivity in the daily activities of the population, the availability of easy-to-use, minimally invasive, and reliable diagnostic tools is mandatory in the healthcare industry. This study aims to validate the effectiveness of DBS AMH assay over a conventional serum AMH measurement in infertile populations for ovarian reserve measurement.

MATERIALS AND METHODS

Study subjects

This prospective study was conducted in Morula IVF Jakarta clinic, Indonesia, between August 2021 and June 2022. The eligibility criteria were infertile women who underwent a controlled ovarian stimulation cycle. Infertile women with specific underlying diseases, such as systemic lupus erythematosus and Crohn's disease, were excluded. Women undergoing chemotherapy and women aged above 45 years old were also excluded. In regard to sampling time, samples were collected either on day 2–5 or random days of the menstrual cycle considering that small fluctuation in AMH levels due to intracycle variation is regarded as not relevant clinically (Li et al., 2021). A total of 109 women agreed to participate in this study. The study protocol was approved by the local ethics committee of the Faculty of Medicine, Universitas Indonesia (KET-358/UN2.F1/ETIK/PPM.00.02/2021), and all infertile women involved in this study had given their signed informed consent to participate.

Samples collection

Matched samples of DBS and serum were collected from each woman. Approximately 3 mL of blood was drawn into a clot-activator tube through phlebotomy. Collected samples were processed immediately by allowing samples to clot for 2 hours at room temperature. Samples were then centrifuged at 3,500 RPM for 15 minutes. Serum samples were then processed using the fully automated Roche Elecsys AMH assays. Following the venipuncture procedure, each participant also received a finger-prick utilizing a DBS 5/16″ lancet. A large blood droplet from a pricked finger was applied to the DBS paper and allowed to blot on the paper. At least two blots of whole blood from the finger were collected on the DBS paper (Whatman 903) for the quantification of AMH. Blood on the DBS papers was dried at room temperature for at least 1 hour before storage at −20°C. Each sample was sealed in an individual ziplock bag with desiccant.

Dried blood spots anti-Müllerian hormone assay protocol

Kits of DBS AMH ELISA (AL-129, Ansh Labs, USA) supplied 10 materials as follows: (a) one vial of AMH calibrator A (containing 0 ng/mL AMH); (b) five vials of AMH ELISA calibrators labeled B–F with known AMH concentration (ranging from 0.2 to 22 ng/mL); (c) two vials of AMH control labeled levels 1 and 2 (containing low and high known AMH concentration); (d) one strip holder constituting 12 strips and 96 microtitration wells coated with immobilized AMH antibody; (e) one bottle (45 mL) DBS AMH extraction buffer; (f) one bottle (12 mL) ready-to-use AMH biotin conjugate (RTU); (g) one bottle (12 mL) RTU streptavidin-enzyme conjugate; (h) one bottle (12 mL) TMB chromogen solution; (i) one bottle (12 mL) stopping solution; and (j) one bottle (60 mL) wash concentrate "A" containing nonionic detergent buffered saline.

Dried blood spots extraction

DBS extraction procedures were conducted according to the manufacturer's instructions (Ansh Labs, 2019). Briefly, all working solutions were placed at room temperature (23–25 °C) for 30 minutes on the day of measurement. After preparing two labeled tubes for each participant, DBS extraction was started by punching two disc spots (±7.9 mm) on DBS paper and directly plugging the two discs into the first tube. Subsequently, 450 µL DBS AMH extraction buffer was added to the corresponding tube followed by incubation with shaking (500–600 RPM) for 60 minutes. The resultant supernatant was then transferred into the second labeled tube to be analyzed. During the incubation period, several reagents for the AMH quantification were prepared, including AMH calibrators B–F, AMH control levels 1 and 2, and a washing solution. Each calibrator and

control vial were diluted with 1 mL of deionized water, while the washing solution was diluted 25-fold with deionized water.

Assay procedure

Quantification of DBS AMH levels were carried out immediately after a successful DBS extraction as follows: (a) adding 100 µL of calibration (A–F) and control (levels 1 and 2) reagent into the appropriate wells and adding 50 µL DBS AMH extraction buffer; (b) adding 150 µL of extracted DBS samples into appropriate wells; (c) incubation of the assay plate in shaker machine at slow speed (600–800 RPM) for 3 hours followed by five times of washing using diluted washing solution; (c) 100 µL antibody-biotin conjugate RTU was then added into each well followed by incubation for 1 hour with shaking (600–800 RPM) and five times of washing; (d) 100 µL streptavidin-enzyme conjugate RTU was added into each well and incubated for 30 minutes with shaking (600–800 RPM); (e) after five times of washing, TMB chromogen solution was added into each well and incubated with shaking for 8–12 minutes at low speed (600 RPM); and (f) 100 µL stopping solution was added into the wells and AMH concentration was subsequently measured at 450 nm absorbance. Duplicate measurements were performed for each sample, calibrator, and control.

Serum anti-Müllerian hormone measurement protocol

AMH levels in serum were performed using Elecsys® AMH plush Assay (Roche Diagnostics International) in an automated standardized instrument (Cobas e601). The detection limit of measurement was 0.01–23 ng/mL. After incubating the tube sample for 2 hours at room temperature, blood samples were centrifugated at 3,500 RPM for 15 minutes to retrieve serum for further analysis. And 50 µL of serum were incubated with a biotinylated monoclonal AMH-specific antibody, and a monoclonal AMH-specific antibody labeled with a ruthenium complex. Streptavidin-coated microparticles were added to the mixture reaction. Streptavidin will then form a strong bond with biotin to facilitate targeted complex binding to a solid phase. A mixture reaction was run into measuring cells and ProCell solution was introduced to wash away any untargeted molecules. Applied voltage to the electrode allowed a chemical reaction that produces chemiluminescent emission in which the emitted light was captured by a photomultiplier. AMH concentration was calculated via a calibration curve.

Sample size, statistical analysis, and performance evaluations

The sample size was calculated using the comparison group formula. At least 107 participants were required to detect differences between the two methods. Data distribution normality was evaluated utilizing Kolmogorov–Smirnov test. Numerical variables were analyzed through a *t*-test or Mann–Whitney test depending on data distribution. The data were presented as mean ± SD or median (Q1–Q3), accordingly. Categorical variables were presented as a proportion (*n* [%]). The contingency table of each method against basal antral follicle count (AFC) as a clinical comparator was constructed. AFC cutoff used in the present study followed the previous studies, which classify AFC into three categories (low: <7; normal: 8–15; high: >15; Anderson et al., 2015; van Tilborg et al., 2012). Twelve expert fertility clinicians were involved in AFC scanning. The contingency table was also developed using the standard reference of each sample (the Ansh Labs reference for DBS AMH and Roches for serum-based samples; Supplementary Tables 1 and 2) to generate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy (receiver operating characteristic area under the curve, ROC AUC) for DBS samples. Bland–Altman and Passing and Bablok regression analysis using MedCalc statistics Software version 16.0 was conducted to measure agreement and potential systematic bias between the two samples. Interpretation of agreement was performed as follows: <90% were considered poor; 90%–95% as sufficient; and >95% as a strong concordance.

Table 1. Characteristics of subjects.

Women characteristics	*n* = 109
Female age (years)	35 ± 5
Body mass index (kg/m²)	23.68 (21.48–26.19)
Infertility duration (years)	6 (3–8)
Type of infertility	
Primary infertility	84 (77.1%)
Secondary infertility	25 (22.9%)
Etiology of infertility	
Tubal factor	11 (10.09%)
Endometrial factor	24 (22.02%)
Sperm factor	49 (44.95%)
Unexplained infertility	13 (11.9%)
Recurrent IUI Failure	16 (14.7%)
Other factors	7 (6.49%)
Basal antral follicle counts	10 (5–15)

Data were presented as mean ± SD; median (Q1–Q3), *n* (%).
IUI: intrauterine insemination.

Table 2. Agreement between AFC and each AMH measurement method.

	Basal AFC		
DBS AMH	Low (0–7)	Normal (8–15)	High (>15)
Low (≤1.95 ng/mL)	31 (79.5%)	6 (15.4%)	2 (5.1%)
Normal (1.95–4.22 ng/mL)	2 (6.7%)	24 (80.0%)	4 (13.3%)
High (>4.22 ng/mL)	3 (10.3%)	11 (37.9%)	15 (51.7%)
	Basal AFC		
Serum AMH	Low (0–7)	Normal (8–15)	High (>15)
Low (≤1.15 ng/mL)	29 (80.6%)	5 (13.9%)	2 (5.6%)
Normal (1.15–1.70 ng/mL)	2 (15.4%)	9 (69.2%)	2 (15.4%)
High (> 1.70 ng/mL)	5 (10.2%)	27 (55.1%)	17 (34.7%)

Data were presented as *n* (%).
DBS AMH: dry blood spot anti-Müllerian hormone; AFC: antral follicle count.

RESULTS

Women who participated in the present study were relatively young with a mean age of 35 years (SD 5) and had a normal body mass index (<25 kg/m²). Most women had primary infertility (77.1%) with a median infertility duration of 6 years. Sperm quality was the most prominent cause of infertility among any other etiologies followed by endometrial factors and recurrent intrauterine insemination

Table 3. Performance of each AMH measurement method against defining AFC.

DBS AMH	AUC	Sensitivity	Specificity	PPV	NPV	*P*-value
Low AMH	86% (78–94)	86% (71–94)	86% (76–92)	76% (61–86)	93% (84–97)	<0.001
High AMH	75% (63–87)	71% (50–86)	78% (68–86)	44% (29–61)	92% (83–96)	<0.001
Serum AMH	**AUC**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	***P*-value**
Low AMH	85% (76–93)	81% (65–90)	89% (80–94)	78% (63–89)	90% (81–95)	<0.001
High AMH	69% (57–81)	81% (60–92)	56% (46–66)	31% (20–44)	93% (82–97)	0.008

Data were presented as *n* (%).
DBS AMH: dry blood spot anti-Müllerian hormone; AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value.

Table 4. Contingency table between DBS and serum sample.

	Serum AMH		
DBS AMH	**Low**	**Normal**	**High**
Low	4 (100.0%)	0 (0.0%)	0 (0.0%)
Normal	9 (9.5%)	85 (89.5%)	1 (1.1%)
High	0 (0.0%)	5 (55.6%)	4 (44.4%)

Data were presented as *n* (%).
DBS AMH: dry blood spot anti-Müllerian hormone.

failure. The median basal AFC measured on day 2 or 3 of menstrual cycle was 10 (Table 1). The mean levels of AMH in DBS samples were relatively higher than that of the serum samples (4.02 ± 3.89 ng/mL vs. 2.44 ± 2.29 ng/mL, respectively). AMH levels measured in all DBS and serum samples were within the normal reference range (0.01–17.3 ng/mL and 0.01–10.8 ng/mL, respectively).

In an attempt to evaluate the clinical value of both methods for ovarian reserve screening, basal AFC was used as a clinical comparator for each method. Spearman correlation described a strong positive correlation of AFC with both AMH measurement methods ($R = 0.67$ and 0.66, $P < 0.001$, respectively for DBS and serum samples). Utilizing reference value of AFC in each group (low, normal, and high), a new cutoff of AMH levels has been constructed by using Youden Index for defining low, normal, and high ovarian reserve in each method. Youden index calculation ([sensitivity + specificity] − 1) included overall participants (21–45 years old) without female age stratification. The optimum cutoff value that offered a good balance between sensitivity and specificity was as follows: ≤1.95 ng/mL, 1.95–4.22 ng/mL, and >4.22 ng/mL, respectively for low, normal, and high DBS AMH group. On the other hand, the favorable cutoff value of ≤1.15 ng/mL for low, 1.15–1.70 ng/mL for normal, and >1.70 ng/mL for high were chosen for serum AMH.

Table 2 displayed a contingency table of both methods against basal AFC. In DBS, the concordance rate between AMH categories and AFC groups was 79.5%, 80%, and 51.7% for low, normal, and high, while serum AMH was shown to have an agreement in 80.6%, 69.2%, and 34.7% for low, normal, and high group.

A comparison of the results revealed that both methods attained to achieve almost similar performance for discriminating low AFC (AUC 86%, cutoff ≤1.95 ng/mL, $P < 0.001$ vs. 85%, cutoff ≤1.15 ng/mL, $P < 0.001$, respectively for DBS and serum sample) as well as comparable PPV (76% vs. 78%, respectively for DBS and serum samples). In addition, accuracy and PPV performance for detecting high AFC were higher in DBS when compared to that of the serum method (AUC 75% vs. 69%, $P < 0.001$ and 0.008, respectively, PPV 44% vs. 31%, respectively; Table 3).

In comparison to the serum sample, Spearman correlation demonstrated a very strong and linear correlation between DBS and serum samples ($R = 0.92$, $P < 0.001$). The agreement between DBS AMH against serum AMH methods was highlighted in Table 4. The contingency table was constructed by using cutoff references from each method (Supplementary Tables 1 and 2). The concordance rate between the two methods was 100%, 89.5%, and 44.4% for low, normal, and high AMH levels.

The performance of DBS for defining low AMH was higher in comparison to that of the serum method (99% vs. 93%, $P < 0.001$, respectively). DBS AMH also achieved high sensitivity over serum samples (75% vs. 62%, respectively). Both methods attained comparable specificity for low AMH discrimination. On the other hand, the PPV of DBS method was low than that of serum method (60% vs. 80%, respectively). Further analysis of the data revealed comparable ability of the two AMH measurement methods in quantifying high AMH levels in terms of ROC AUC, sensitivity, specificity, and PPV, as well as NPV (Table 5).

Further statistical analysis by utilizing Bland–Altman tests revealed that agreement between the two methods was sufficient (94.5%) with a mean difference of −1.6 (limits of agreement: upper 2.7, lower −5.9). Six measurement values were outliers (5.5%; Fig. 1a). Passing and Bablok regression was $y = -0.12 + 1.8\times$. The intercept of Passing and Bablok regression (−0.12 [95% CI {−0.29 to −0.01}]), as well as the slope (1.80, 95% CI [1.66–1.94]), demonstrated that there was a constant and proportional difference between the two methods. However, the linearity difference was not significant ($P = 0.893$).

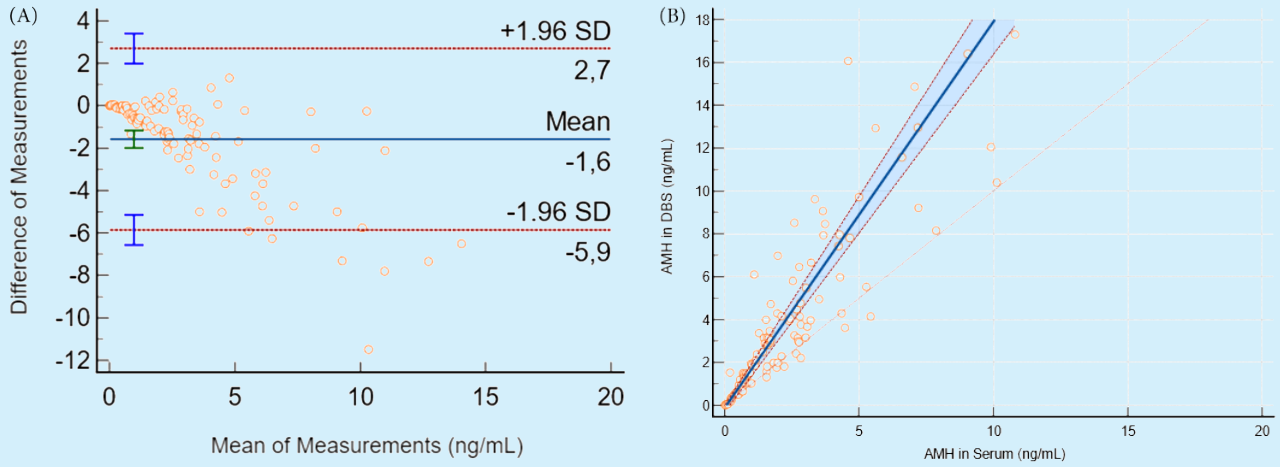
DISCUSSION

The present study demonstrated that DBS sample was acceptable as an alternative approach to screening AMH levels in the infertile Indonesian population. As far as is known, this was the first study to report the clinical value of DBS assay by fully recruiting infertile women in the clinical setting of IVF clinic. Since there is no standardization on which method is preferred for AMH quantification, we compared our standard procedure of serum AMH

Table 5. DBS AMH performance and result interpretation concordance in comparison to serum samples.

DBS AMH	AUC	Sensitivity	Specificity	PPV	NPV	*P*-value
Low AMH	99% (97–100)	75% (30–95)	98% (93–99)	60% (23–88)	99% (95–100)	<0.001
High AMH	100% (100)	100% (70–100)	100% (96–100)	100% (70–100)	100% (96–100)	<0.001
Serum AMH	**AUC**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	***P*-value**
Low AMH	93% (88–99)	62% (36–82)	98% (93–99)	80% (49–94)	95% (87–98)	<0.001
High AMH	100% (100)	100% (57–100)	100% (96–100)	100% (57–100)	100% (96–100)	<0.001

Fig. 1. Comparison of DBS and serum samples: (A) outlier measurement between DBS and serum samples through Bland–Altman test; (B) Passing and Bablok regression.



measurement (Elecsys serum AMH plus, Roche) over DBS AMH ELISA Ansh Labs to measure the feasibility use of DBS. What stands out in the current analysis of the present study was the good concordance of DBS over clinical comparator (AFC) as well as the serum method for quantifying AMH levels for low ovarian reserve group. Here we proposed DBS AMH cutoff value of ≤1.95 ng/mL as a new reliable reference for predicting low ovarian reserve in Indonesian population. Moreover, when compared to serum samples, DBS performance was also higher when it was used for low AMH discrimination (Table 5). Therefore, although AMH levels were likely to be higher in DBS samples than that of matched serum samples, clinical interpretation of the results corresponding to ovarian reserve (low, normal, or high) in the current clinical IVF practice is quite similar.

Previous studies highlighted that AMH serum levels measured by Elecsys AMH plus (Roche) are relatively lower than that of picoAMH (Ansh Labs, 2019) and Gen II AMH (Beckman Coulter) due to different calibration and/or dilution step processes (Moolhuijsen et al., 2022; Nelson et al., 2015b). Our result added new information that AMH levels in the DBS approach are likely to be higher than that of serum-based AMH levels evaluated with Elecsys plus (Roche). On the other hand, a study has demonstrated a similar mean of AMH value in DBS and serum when the Beckman Coulter kit was utilized (McDade et al., 2012). Currently, 21 platforms of AMH assay are available commercially and variability of measurement is reported reflecting the urgent need for standardization of the AMH assay platform (Punchoo and Bhoora, 2021). The lack of accurate AMH preparations to be utilized as a proper international reference for calibrating each serum AMH assessment platform is the root cause of differing results (Li et al., 2021). The specificity of antibodies being used in each platform varied and could contribute to result discrepancy (Punchoo and Bhoora, 2021). In addition, the nature of different sample sources between DBS and serum could also be a potential factor for elucidating the aforementioned results (Freeman et al., 2018).

A 2020 study has reported the utility of DBS AMH to study fecundability in adolescent and young adult American population with the mean age of women 20 years (AMH mean of 5.66 ng/mL, 1.02–22.23 ng/mL; Hall et al., 2020). It was found that AMH levels were in the normal reference range. In our study, AMH concentrations of infertile women group in the DBS samples were also within the acceptable range according to its manufacturer's reference (mean of 4.02 ± 3.89 ng/mL, ranging from 0.01 to 17.3 ng/mL).

AMH is a prominent marker for evaluating ovarian reserve and has consistently been included as a predictive factor for ovarian response and personalized ovarian stimulation dosage (La Marca

et al., 2012; Nelson et al., 2019). Following proteolytic activities within the ovaries, the precursor protein of AMH is circulated as an active 140 kDa complex of homodimer glycoprotein consisting of pro-region (55 kDa N-terminal) and mature peptide (12.5 kDa C-terminal) linked via a non-covalent bond (Dewailly et al., 2014). Given the biological structure of AMH, the hormone is categorized as large molecules (>70 kDa), which are ideally captured using a specific cotton filter paper. AMH protein that is dried on a matrix could be subsequently quantified. A supporting study investigating the effect of varying compound physicochemical characteristics and paper lots variability in DBS performance analysis has demonstrated the sufficient sensitivity of DBS in detecting the targeted compounds, advocating its utilization to capture a wide variety of molecules. Moreover, different lots of DBS paper did not impact the assay performance indicating a stable manufacturing process (Pham et al., 2011).

In IVF, DBS AMH offers several advantages as it is less invasive, less painful, and easy to obtain a specimen by patient self-collection. The DBS approach allows infertile patients to independently collect the samples at their convenience (Roberts et al., 2016; Sakhi et al., 2015). The availability of DBS AMH would benefit a specific group of reproductive-age women who prioritize their careers over marriage and having children. These women would be able to screen their AMH levels from time to time without the need for an appointment with the doctors; hence saving their valuable time visiting the clinics. The samples would then easily be dried at room temperature and transported to a referral clinic. DBS AMH could also be useful for women undergoing cancer therapy (Roberts et al., 2016). Since most therapeutic agents for cancer are gonadotoxic, DBS AMH could be used to track the side effects of cancer treatment on ovarian reserve as demonstrated by the previous study (Su et al., 2020). As a micro-sample method, DBS also warrants an attractive approach for collecting samples from IVF patients with HIV or Hepatitis by reducing biohazard risks as smaller blood volumes are required for assessment (Freeman et al., 2018).

Notably, the heterogeneity of analytes in whole-blood DBS samples is a challenging issue that needs to be addressed; for instance, the presence of lysed blood cells or cellular fracture, which could potentially interfere with the analysis of the target molecule (Hall et al., 2020). Therefore, the DBS specimen type should have its own reference range or specific cutoff for its clinical application as we offered in this study. Another limitation of DBS AMH compared to serum samples is the workflow in which DBS must be performed. The extraction procedure and long incubation for sensitive detection consume more time and labor than an automatic instrument for which the assay is not yet available. This may limit the application of DBS as a routine method in some laboratories. However, for high-workload laboratories, there are commercially available solutions to automate the DBS preparation as well as robotic immunoanalyzers for automated performance of 96 well microplate ELISA-based assays. In conclusion, DBS could be the method of choice for the quantification of AMH in infertile populations particularly for screening low ovarian reserve utilizing cutoff value of ≤1.95 ng/mL. Potential application in IVF practice is very promising, as the method offers a convenient self-collection feature, easy handling, and shipment to a clinical laboratory for assessment.

CONFLICT OF INTEREST

The authors declare that they have no competing interest to disclose.

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