

Implementation of a nationwide universal ultrasound screening programme for developmental dysplasia of the neonatal hip in Mongolia

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Abstract

Purpose Mongolia is the first Asian country to launch universal ultrasound screening for newborns with developmental dysplasia of the hip (DDH). The aims of this study were to determine the coverage and treatment rate of this programme.

Methods Data from birth statistics and ultrasound hip screening from 29 hospitals were retrospectively reviewed (2010 to 2016, pre-nationwide phase; and 2017 to 2019, nationwide programme). DDH was diagnosed using the Graf-technique and treated according to 'ABCD', a modified Graf classification (Group A: Graf Type 1, mature; B: 2a, physiologically immature; C: 2c to 3, early DDH; D: 4, dislocated). Group B children were followed with monthly ultrasound. Group C and D children were treated with a flexion and abduction orthosis (Tübingen). Screeners used a web-based platform to upload images for quality surveillance.

Results Between 2017 and 2019, 230 079 live births were registered and 176 388 newborns screened. The nationwide screening coverage rate in newborns increased from 73.6% in 2017 to 82.1% in 2019. Group A (148 510 children, 84.2%) was discharged, while Group B (25 820, 14.6%) was followed. Among children in Group B, 284 cases worsened to Group C and were, therefore, treated with a Tübingen orthosis. The remaining 2058 (1.2%) of newborns with DDH were treated with a Tübingen orthosis, including 1999 newborns in Group C and 59 in Group D. Since 2017, a total of 142 860 (81.0%) hip sonograms were uploaded to the platform.

Conclusion A simplified diagnostic and therapeutic framework for ultrasound DDH screening for newborns was successfully deployed in Mongolia, a developing country, providing high surveillance coverage and appropriate treatment.

Level of evidence: IV

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Introduction

Developmental dysplasia of the hip (DDH) is a major health problem that can lead to lifelong disability if diagnosis is missed in the first weeks of life.^{1,2} According to one study,³ 48.4% were found to have underlying hip dysplasia as the aetiology of total hip arthroplasty for osteoarthritis in patients under 50 years old. DDH has implications not only for the baby itself and its entire family, but is of enormous consequence for public health. The overall costs for orthopaedic DDH treatment, hospitalizations, surgery and rehabilitation are much higher than its prevention based on early detection by ultrasound with the Graf technique.^{4,5} The maturation of DDH by means of suitable, preventive treatment measures in the first 12 weeks of life leads to maturation of the hip joints,^{6,7} albeit residual acetabular dysplasia may develop.⁸ Treatment beyond this point is typically prolonged, and often results in an orthopaedic repair with poorer prognosis.⁹⁻¹²

Modern clinical screening for early detection of DDH, entailing a combination of risk factor identification and physical examination, is widely used in many countries. Approaches are either universal screening, in which all neonates are evaluated, or selective screening, in which only those at high risk are evaluated.¹³⁻¹⁵ Diagnosis of infants with risk factors or abnormal clinical findings are confirmed by hip ultrasonography, the benchmark diagnostic tool.^{16,17} An international consensus meeting of doctors of various disciplines, with expertise in the detection

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and treatment of DDH, strongly agreed in favour of universal ultrasound screening by Graf's method.¹⁸ Justification was given that the clinical examination screening is not sensitive enough to identify every child with DDH. The reported specificity is 90%; the sensitivity, however, is reported as being as low as 50%. Ultrasound by the Graf technique has been proved to be more sensitive and more specific for the diagnosis (> 90% for both measures).¹⁸

Although the simple and reproducible ultrasonographic diagnostic method for DDH known as Graf's method⁶ has been successfully used for over 40 years, it has not yet been adopted internationally as a standard. Studies have been indicating that ultrasonographic screening with the Graf technique is cost-effective,⁴ does not result in overtreatment and contributes to a reduction of long-term consequences.¹⁹ Studies from Germany²⁰ and Austria²¹ reported that a universal ultrasound screening programme by the Graf method resulted in a decrease in surgical procedures, hospitalizations and late presentation. Another study from Austria⁴ concluded that the small proportional increase in costs of the universal ultrasound screening by Graf is justifiable as it was associated with a reduction in the number of non-surgical and surgical interventions.

The Swiss Mongolian Pediatric Project (SMOPP), a humanitarian aid project, introduced Graf's method of hip ultrasound to Mongolia in 2007. In 2011, a prospective birth cohort study²² of 8356 newborns revealed that hip ultrasound is feasible in the tertiary paediatric hospital of Ulaanbaatar (National Center for Maternal and Child Health; NCMCH), that the incidence of DDH in neonates is comparable with that in European neonates (1% to 2%) and that early ultrasound-based assessment and flexion-abduction treatment of DDH leads to maturation of the hip joints in all cases. Based on the study results and NCMCH being a key player in nationwide policy and programme drafting in maternal and child health fields, the Mongolian Ministry of Health decided to initiate ultrasound for DDH in newborns throughout Ulaanbaatar.

In 2017, the Mongolian government approved a nationwide screening programme that required all maternity hospitals to offer ultrasound hip screening by Graf technique for all newborns. Trained neonatologists or paediatricians perform the hip ultrasound screening following standardized procedures and are responsible for flexion-abduction treatment, if necessary. Full documentation of all cases is mandatory. Screening, follow-up monitoring and flexion-abduction treatment are offered free of charge. The goal of the programme is to screen every newborn baby using hip ultrasound and to provide early preventive treatment to eliminate disability due to DDH. As Mongolia is the first Asian country to launch a programme of universal ultrasound hip screening for DDH, we aim to determine its coverage and treatment rate.

Materials and methods

Deliveries

Mongolia is divided into the capital city (Ulaanbaatar) and 21 aimags (provinces) in four regions: Western (five aimags), Khangain (six), Central (seven) and Eastern (three) regions. Each aimag has a general hospital that is responsible for deliveries of the geographic area of the aimag. Deliveries are centralized and occur mainly at three maternity hospitals, two district general hospitals and the NCMCH in Ulaanbaatar, and the aimag general hospitals. There are approximately 80 000 live births per year in the country and 99% of all deliveries occur in a hospital.²³ Half of deliveries occur in the capital city Ulaanbaatar. The average hospital stay following a normal delivery is one to two days.

Hip ultrasound screening was introduced stepwise until all before mentioned 29 hospitals across the country were equipped with necessary ultrasound machines, transducers and cradles, and doctors were trained by SMOPP. From 2010, screening was implemented at the NCMCH in Ulaanbaatar. In 2012, the screening was expanded to all three maternity hospitals and two remote district general hospitals of Ulaanbaatar, all five aimags of the Western region and one aimag of the Khangain region. Based on the financial and teaching resources available to SMOPP, one more aimag of the Khangain region in 2014, four aimags of the Central region in 2015, three aimags of the Khangain region and one more aimag of the Central region launched screening in 2016. The remaining six aimags (all Eastern aimags, one Khangain aimag and two Central aimags) started screening in 2017.

Diagnosis and treatment

The hip ultrasound screening guidelines used in the programme follow the diagnostic and therapeutic framework of Graf, which was modified by SMOPP.²⁴ SMOPP realized during implementation of the screening, that age-dependent Graf subtypes 2a-/+/b, 3b are irrelevant in the screened population, i.e. newborns in the first week of life. Moreover, it was not practical for the screeners at maternity hospitals to learn all of the different diagnostic (sub) types and subsequent treatments outlined by Graf⁶ because Graf Type 2c, D and 3 hips are treated in the same way (i.e. flexion-abduction orthosis). Therefore, the original Graf system required some adjustments into a simpler 'ABCD' system. This system is entirely based on the technique described by Graf and translates his initial differentiation into four groups. The ABCD system is focused on treatment recommendations for screeners at maternity hospitals. Therefore, depending on the management decision, the system uses four groups instead of 12 subtypes of Graf classification: Group A includes Graf Type 1; Group

Graf type	Graf angle α (°)	Graf angle β (°)	Graf therapy	ABCD group	ABCD angle α (°)	ABCD therapy		
1a	> 60	< 55	None	A	> 60	None		
1b		> 55	None					
2a	50–59	> 55	Control	B	> 50 ≤ 60*	Control		
2a+			Control					
2a-			Spreading device/Pavlik	C			< 50	Tübingen orthosis
2b								
2c stable	43–49	< 77	Plaster	D	-	Tübingen orthosis/Operation		
2c unstable								
D								
3a	< 43	> 77	Extension	D	-	Tübingen orthosis/Operation		
3b								
4			Operation					

* Group B hips were defined as angle alpha minus the age in weeks = between 50° and 60°

Fig. 1 Comparison of Graf types and ABCD groups for hip ultrasound (blinded for review)

B includes Graf Type 2a; Group C includes Graf Types 2c, D and 3; and Group D includes Graf Type 4 (Fig. 1).²⁴

The Mongolian national guidelines for the neonatal hip ultrasound screening are as follows:

- Ultrasound screening must be completed within one to two days after birth in healthy newborns before discharge from the hospital.
- All newborn children with ultrasonographically mature Group A hips at birth can be discharged without follow-up.
- Children with physiologically immature Group B hips require monitoring of maturation by ultrasound after four weeks. Traditional swaddling with legs adducted and extended must be avoided, because it might harm the immature socket and hinder maturation. A child can be discharged at follow-up if previously Group B hips have matured to Group A hips. In case of a deterioration to Group C, a treatment with a Tübingen orthosis (Otto Bock HealthCare Deutschland GmbH, Duderstadt, Germany) is started with monthly ultrasound follow-up until full maturation is achieved.
- For children with Group C hips, treatment with a Tübingen orthosis for 23 hours a day and an ultrasound follow-up after four weeks is indicated. Differentiation

between stable and unstable hips is not essential for ABCD, because all group C hips are treated in the same way.

- Children with Group D hips are also initially treated with a Tübingen orthosis. This primary procedure comprehends a chance of success, if the cartilage or soft tissue between bony socket and the femoral head do not obstruct reduction. Treatment is continued until full maturation to Group A. However, if treatment is unsuccessful, orthopaedic measures must be taken into account: (overhead) extension and closed or open reduction, depending on the reducibility of the hip. Postoperatively, these children need a plaster for six to eight weeks followed by continuation with the Tübingen orthosis until they reach Group A.
- Monitoring by radiograph at age four to six years can be considered as a long-term follow-up for treated children.

Data collection and quality control

SMOPP trained all screeners to apply standardized hip ultrasound methods. In all hospitals, ultrasound machines (GE Logiq series, GE Healthcare, Chicago, Illinois, USA) were equipped with a linear array transducer operating on

an ultrasound frequency of 7 MHz to 10 MHz. A Sonofirst holding cradle and transducer fixation unit (Orthopunkt, Solothurn, Switzerland) were used to prevent tilting errors and to standardize examination techniques.⁶

Since data were collected at multicentre sites, a quality control scheme was required. A web-based, password-protected platform (WebWaren, Bern, Switzerland)²⁴ was used for this purpose. The tool enabled screeners in all hospitals to upload Digital Imaging and Communications in Medicine files exported from the ultrasound machines. Four images (two per hip side; one of them with measured alpha and beta angles according to Graf), a unique identification number, age, sex and diagnosis were required for all examinations. We avoided double-counting by using unique identification numbers for all examinations. After the procedure was defined (discharge, control monitoring or Tübingen orthosis) and quality was controlled by a local expert (an on-site doctor with profound knowledge of the original Graf method and at least three years of experience of hip ultrasound and treatment), each examination could be completed and removed from the dashboard. This allowed continuous and reliable review of hip grading, diagnosis of DDH and treatment decisions. In addition, trained Mongolian and Swiss supervisors checked examinations from all hospitals and promptly sent comments to the experts and screeners. Discrepancies between Mongolian and Swiss assessments were resolved by discussion and consensus. The hip ultrasound screening coordinator (MU) performed weekly and monthly checks of screening and treatment rates, and the quality of the programme's application in each maternity hospital in Ulaanbaatar and the aimags. Regular visits or phone calls were made to each site, and screeners regularly received refresher training in screening methods. This quality control concept is especially important in remote and rural hospitals where human resources are lacking, i.e. only one doctor

performs the screening examinations and doctors often change. Most importantly, the quality control system allows the screeners to receive continuous education regarding hip grading, diagnosis of DDH and treatment decisions.

Birth data were collected from national registers.²⁵ Information on hip ultrasound screening, treatment and control data were collected retrospectively from paper-based hospital registers of hip ultrasound screening (for screening coverage and treatment proportion) and the telemedical platform (for online quality surveillance coverage). We cross-checked data with the mothers' and newborns' identification numbers and verified all information from hospital records and the platform.

Statistical analysis

Number of births, screening examinations (overall and by diagnostic group) and quality-checked examinations were imported into Stata 15 (Stata Corporation, Austin, Texas) for each hospital and region, respectively. We performed descriptive analyses to determine proportions. We used the child (not the hip) as the unit of analysis. If a child had hips with different morphologies, we evaluated that child based on the worst hip.

Results

Pre-nationwide hip ultrasound in Mongolia

After a first feasibility study in Mongolia in 2010,²² the coverage rate in newborns increased from 8.6% in 2010 to 67.4% in 2016 (Table 1). In the capital city, where half of the country's 3.4 million population reside, the rate reached 94.7% in 2016 but in rural provinces (aimags) the screening reached only 37.7% in 2016. During the pre-nationwide period (2010 to 2016), we could detect and treat 2429 cases of DDH (Table 1).

Table 1 Pre-nationwide implementation of neonatal hip ultrasound screening (2010 to 2016), percentage and ratio of screened and diagnosed newborns

Region	2010	2011	2012	2013	2014	2015	2016
Rate of hip ultrasound screening							
National	8.6	16.1	42.1	44.4	45.5	52.4	67.4
	5640/65889	11367/70576	31493/74778	35456/79780	37182/81715	42343/80875	52666/78194
Ulaanbaatar	18.6	33.5	69.0	68.9	69.7	76.8	94.7
	5640/30273	11367/33924	25680/37214	27709/40187	29120/41786	32037/41731	38518/40687
Aimag/rural provinces	na	na	15.5	19.6	20.2	26.3	37.7
			5813/37564	7747/39593	8062/39929	10306/39144	14148/37507
DDH incidence							
National	1.3	1.1	0.9	1.4	1.4	1.2	0.8
	72/5640	121/11367	292/31493	479/35456	506/37182	520/42343	439/52666
Ulaanbaatar	1.3	1.1	0.9	1.4	1.4	1.2	0.7
	72/5640	121/11367	219/25680	386/27709	401/29120	399/32037	286/38518
Aimag/rural provinces	na	na	1.3	1.2	1.3	1.2	1.1
			73/5813	93/7747	105/8062	121/10306	153/14148

na, not available (screening not yet introduced); DDH, developmental dysplasia of the hip

Table 2 Hip ultrasound screening coverage in Mongolia (2017 to 2019), percentage and ratio of screened and diagnosed newborns

Region	2017	2018	2019	Total
Rate of hip ultrasound screening				
National	73.6 54706/74328	74.1 57461/77528	82.1 64221/78223	76.7 176388/230079
Ulaanbaatar	86.0 33045/38404	80.2 32630/40703	88.2 36344/41206	84.8 102019/120313
Aimag/rural provinces	60.3 21661/35924	67.4 24831/36825	75.3 27877/37017	67.7 74369/109766
DDH incidence				
National	1.2 640/54706	1.2 661/57461	1.2 757/64221	1.2 2058/176388
Ulaanbaatar	1.2 397/33045	1.2 401/32630	1.2 444/36344	1.2 1242/102019
Aimag/rural provinces	1.1 243/21661	1.2 260/24831	1.1 313/27877	1.1 816/74369

DDH, developmental dysplasia of the hip

Table 3 Developmental dysplasia of the hip incidence in Mongolia (2017 to 2019), percentage and ratio of hip groups

Hip Group by ABCD system (Graf type)	2017	2018	2019	Total
A (Graf Type 1)	83.23 45532/54706	85.05 48870/57461	84.25 54108/64221	84.2 148510/176388
B (Graf Type 2a)*	15.6 8534/54706	13.8 7930/57461	14.57 9356/64221	14.64 25820/176388
C (Graf Type 2c, D, 3)	1.13 618/54706	1.12 645/57461	1.15 736/64221	1.13 1999/1760388
D (Graf Type 4)	0.04 22/54706	0.03 16/57461	0.03 21/64221	0.03 59/176388

*cases with Group B hips that worsened to DDH and were treated with a Tübingen orthosis (n = 284) are not included in this table

Table 4 Online surveillance coverage by year and places, percentage and ratio of quality-checked examinations

Region	2017	2018	2019	Total
National	84.3 46090/54706	79.5 45668/57461	79.6 51102/64221	81.0 142860/176388
Ulaanbaatar	80.1 26468/33045	67.7 22079/32630	71.4 25946/36344	73.0 74493/102019
Aimag/rural provinces	90.6 19622/21661	95.0 23589/24831	90.2 25156/27877	91.9 68367/74369

Nationwide hip ultrasound screening programme implementation

A total 230 079 live births were registered during the nationwide study period (1 January 2017 to 31 December 2019) and overall screening coverage in newborns was 76.7% (176 388/230 079). The hip ultrasound screening coverage rate in newborns increased from 73.6% in 2017 to 82.1% in 2019 (Table 2). The mean and median age at screening was 1 day (interquartile range: 0.1; range: 1.4; SD 0.34).

Of the 176 388 newborns screened during the study period, 148 510 (84.2%) were assigned to Group A (Graf Type I hips) and 25 820 (14.6%) to Group B (Graf Type 2a or physiologically immature hips). Children in Group B were monitored by ultrasound on a monthly basis until reclassified to Group A (Graf Type 1). The remaining 2058 (1.2%) newborns were diagnosed with DDH and treated with a Tübingen orthosis: 1999 (1.1%) newborns in Group C (Graf Type 2c, 3 or D) and 59 (0.03%)

newborns in Group D (Graf Type 4). Among the 25 820 children categorized as Group B, 284 (1.1%) worsened and were also treated with Tübingen orthosis. Among the 59 children diagnosed as Group D, 48 were referred to the orthopaedic surgeons. More detailed information by years of screening are shown in Table 3. There was no relevant difference in DDH incidences between Ulaanbaatar and the aimags.

The online surveillance coverage is shown in Table 4. Since 2017, a total of 142 860 (81.0%) ultrasound examinations of infants' hips were uploaded to the web-based platform from 29 hospitals.

Discussion

The implementation of a nationwide universal ultrasound screening of DDH allowed a rather high coverage rate even in otherwise low-resource rural areas in Mongolia. The study results reveal that the hip ultrasound screen-

ing coverage rate in newborns increased from 73.6% to 82.1% even though the screening was comparatively new to both the general population and to healthcare professionals. Nevertheless, considering the criteria of successful newborn screening programmes ($\geq 95\%$ of all newborns must be screened),²⁶ this screening programme is still in its infancy. Notably, the screening rate in Ulaanbaatar dropped in 2018, probably due to the increased numbers of births taking place in private maternity hospitals²³ that do not yet take part in the national screening programme.

Screening programmes for DDH are controversial; there is no current consensus on screening methods. Globally, there are different diagnostic and therapeutic algorithms and guidelines. Literature is difficult to interpret because different methods are used and often combined in reviews; furthermore, screening highly depends on the health systems and politics in the different countries.^{13-15,27,28} In addition, there are discursive assessments regarding the evaluation of screening programmes (methods, universal *versus* selective) and their economic viability.^{29,30} In Mongolia, initiating a universal screening programme was justifiable, since almost all births occur in just a few centralized maternity hospitals,²³ and most newborns are provided with a general checkup by neonatologists or paediatricians within first two days of life, before most families leave the hospital and return to the vast countryside. These exams offer an opportunity for a routine check of hips by ultrasound, and the earliest possible start for preventive flexion-abduction treatment if it is indicated. Furthermore, statistical reports describe serious concerns within the country in relation to DDH:³¹ there were no reliable examination and treatment methods in place, and significant variations in diagnosis of DDH were common prior to the advent of the standardized screening programme, leading to confusion for medical professionals and parents.

International data on frequency of hip dysplasia in newborns diagnosed by ultrasound varies widely.^{14,16,21,28,32} This broad range can be explained by the use of different methodologies and grading systems in DDH diagnosis. In the German-speaking world, the Czech Republic and Poland, the ultrasonic method developed by Graf⁶ has established its place as primary diagnostic tool in screening for DDH. It can quantify the degree of DDH, and is highly reproducible in its ability to visualize all important anatomical structures. Furthermore, it is painless for a child, safe and can be used quickly by trained and experienced examiners.²⁴

Studies using the Graf method show rather low incidences of DDH.²² In Mongolia, the original 12 Graf subtypes have been replaced by a simpler and more practical system, named the ABCD system.² It classifies the bony and cartilaginous roof in a manner correlated to age, comparing the spontaneous maturation during the first weeks of

life to the average 1° alpha per week by Graf.⁶ All children diagnosed with DDH in Mongolia are now treated with a Tübingen orthosis. The resulting incidence rate of DDH was 1.1% to 1.2% during our study period (Table 2). This rate and associated treatment can be considered reasonable, compared with other universal ultrasound screening (3.4%), selective ultrasound screening (2.0%) and clinical screening (1.8%) reported in previous studies.^{10,16,28,33,34}

Although the simplified ABCD system allowed narrowing of treatment strategies in the first days of life and helped to compensate for the huge turnover of screeners in governmental maternal hospitals in Mongolia, the method is only as good as its users. When the Graf technique was used correctly, the rates of open reductions, acetabuloplasty and head necrosis were reduced.²¹ To achieve such results, the quality of knowledge and skills of screeners is crucial; under- or over-diagnosis would lead to costly and unnecessary treatment or handicaps.³⁵ One of the main causes of misdiagnosis in infant hip sonography are errors due to poor-quality hip ultrasound.³⁵ Errors come from poor technique, tilting of transducers and subsequent measurement bias that leads to the wrong diagnosis and ultimately incorrect procedures that cause unnecessary harm for babies. Such errors may be more likely to occur in the absence of a quality control system.²⁴ Therefore, a quality control system should allow not only reliable review of diagnosis and treatment of DDH, but also continuous education of screening doctors. This Mongolian example shows that an internet-based quality platform can have good acceptance and support screeners, even in developing countries where knowledge and experience may be lacking.

The major strength of this study is its data that allows us to describe the implementation of the first universal ultrasound screening for DDH in neonates in Asia. This study also has some limitations. Firstly, retrospective reviews of screening reports may experience in a limitation to finding information on some newborns who might have been delivered in private hospitals and possibly screened without reporting to the screening programme, leading to an underestimation of the screening coverage. However, such cases are probably few because the ultrasound examination is expensive in private hospitals. Our study reviewed all 29 governmental maternity hospitals and should represent the Mongolia well. Secondly although recommended, consistent uploading of all hip sonograms into the telemedical platform has not yet been established which leads to incomplete online surveillance. Low upload rates are probably due to technical problems and limited human resources but might also indicate confident screeners after a concise and regularly refreshed teaching programme. Qualitative studies would be needed to better understand the surveillance process. Currently, the screening for DDH in newborns using a modified version

of Graf's method is implemented at a national scale in all major hospitals; however, the screening has not been implemented at the soum (subprovincial) level, a more resource-poor setting. In such contexts, we will have to look critically at operational issues that might affect implementation on a larger scale, and the ability to prevent hip dislocation and avoid complex surgical procedures. Lastly, the current study does not report on treatment compliance and outcomes of DHH, as the focus here is on diagnostic and quality surveillance coverage rates. We will report on follow-up results and treatment outcomes in separate publications.

After a successful pilot of ultrasound screening of newborns in the capital, we saw the potential to expand and include a quality control system. This study demonstrated that a population-based ultrasound screening programme for DDH in children is feasible. The Mongolian government has committed to continue the nationwide screening programme, indicating the sustainability of this approach.

Conclusion

In Mongolia, an ultrasonic method of DDH screening was introduced, piloted and expanded as a nationwide screening programme. The Mongolian experience may support further international discussion on universal ultrasound screening using the Graf technique. Our study demonstrated that a simplified diagnostic and therapeutic framework of hip ultrasound screening for DDH in newborns could be deployed in a developing country, providing high screening and quality surveillance coverage and an appropriate treatment rate in Mongolia with remarkable results.

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COMPLIANCE WITH ETHICAL STANDARDS

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ETHICAL STATEMENT

Ethical approval: The study received ethical approval (2012/04 & 2018/13) from the Ethical Review Committee of Ministry of Health Mongolia and institutional review board of the National Center for Maternal and Child Health, Mongolia.

Informed consent: Not required.

ICMJE CONFLICT OF INTEREST STATEMENT

None declared.

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AUTHOR CONTRIBUTIONS

MU: Conceived, designed and performed the study, Analyzed the data, Wrote the manuscript

BM: Conceived, designed and performed the study, Analyzed the data, Wrote the manuscript

RS: Conceived, designed and performed the study, Wrote the manuscript

TB: Conceived, designed and performed the study, Wrote the manuscript

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