

## PEER REVIEW HISTORY

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## ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Prevalence and Outcomes of Twin Pregnancies in Botswana: a National Birth Outcomes Surveillance Study
<b>AUTHORS</b>	Isaacson, Arielle; Diseko, Modiegi; Mayondi, Gloria; Mabuta, Judith; Davey, Sonya; Mmalane, Mompoti; Makhema, Joseph; Jacobson, DL; Lockett, Rebecca; Shapiro, Roger; Zash, Rebecca

## VERSION 1 – REVIEW

<b>REVIEWER</b>	Meyer, Raanan Sheba Medical Center
<b>REVIEW RETURNED</b>	13-Feb-2021

<b>GENERAL COMMENTS</b>	<p>I would like to thank the Editors for the opportunity to review this manuscript. This study aimed to evaluate the outcome of twin pregnancies in Botswana, and compared these pregnancies outcomes to singleton pregnancies' outcomes. The study is well written and clear. However, although trying to provide information on twin pregnancies' outcomes in this country, it is severely limited by a lack of detailed data on these pregnancies and deliveries. Following are my comments on specific points in the manuscript.</p> <p><b>Abstract</b> Participants- as the number of deliveries is provided in the Results section, this information is redundant in the Participants section.</p> <p><b>Background</b> Page 6 lines 6-8- the authors state that "prior studies of twins in sub-Saharan Africa pre-date the implementation of the SDG and do not evaluate birth outcomes". Please provide references to this claim.</p> <p><b>Methods</b> I would like to highlight some issues regarding the primary outcomes chosen by the authors: Stillbirths- there is no information as to whether predelivery intrauterine fetal demise occurred, or if it occurred intrapartum. These are two different entities with different etiologies. Furthermore, in page 7, line 40, the authors refer to "neonatal death", after stating "stillbirths" as the selected outcome several lines above. Please correct. Birthweight- the authors chose 2500 and 1500 grams as cutoffs. While these cutoffs are indeed usually used in the literature, I suggest that the fetuses' birthweight percentiles would be more appropriate as primary outcomes, as they represent intrauterine fetal growth more accurately and associated adverse outcomes in cases of growth restriction.</p> <p><b>Results</b></p>
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	<p>The proportion of induction of labor in each group is not provided and should be added. In addition, indications for cesarean deliveries are not provided.</p> <p>Gestational age during ultrasound- was this the only US during pregnancy? Were there additional visits at later stages of the pregnancy? This information is important as it is associated with pregnancy outcomes.</p> <p>What is the proportion of pregnancies with no information on gestational age at delivery? The primary outcome of this manuscript is preterm deliveries, yet this essential information is missing. Is there a correlation with the proportion of women that had US examinations?</p> <p>Table 1- why are p-values not provided in this Table?</p> <p>Tables 2,3- data presentation is confusing</p> <p>Discussion</p> <p>The authors appropriately discuss the limitations of this study. I agree with those stated and suggest adding the lack of information on out-of-hospital/home deliveries. An additional major limitation is the lack of accurate gestational age of the study cohort. As this is the primary outcome of this study, it is vital that the authors provide information on the proportion of pregnancies without this data.</p>
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<b>REVIEWER</b>	Senat, Marie Victorie Hôpital Bicêtre, Departement de Gynécologie-Obstétrique
<b>REVIEW RETURNED</b>	24-Apr-2021

<b>GENERAL COMMENTS</b>	<p>Prevalence and Outcomes of Twin Pregnancies in Botswana: a National Birth Outcomes Surveillance Study</p> <p>This study report the prevalence and outcome of twin pregnancies in Botswana in a country with low socio-economic resources. This study is very interesting and provides an inventory of the outcome of twin pregnancies in a country with low socio-economic resources in order to make an assessment. In this way, this study is very informative and includes a large number of women with a comparison over the same period of time of pregnancy with singleton. It reports a very high rate of IUGR and stillbirth reflecting the absence of adequate monitoring during pregnancy, particularly regular ultrasound. The major limitation of this study is that without knowing the % of spontaneous and induced prematurity, nor the fetal or maternal causes leading to delivery, it will be difficult to improve the quality of care. And as pointed out by the authors another limitation is the lack of diagnosis of chorionicity which does not allow for planning an appropriate gestational age of birth which given the median age of birth in the study is far too late for monochorionic pregnancies and surely a source of stillbirth...although I do understand that changing the culture of pregnant women to go for early antenatal care and getting ultrasound scans and sonographers in Botswana is a challenge. It may then be just a first study to say that it is necessary to reinforce the monitoring of pregnancy and particularly ultrasound and to carry out a second study which will specifically analyse the different categories and causes of prematurity</p> <p>Do we know how many ultrasound scans (mean and range) twin and singleton patient had in their pregnancy ?</p> <p>I am not sure that it is relevant to have added the year 2018-2019</p>
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	<p>even if it increases the number of patients. Indeed, as there are new centers added, the care provided to patients may not be the same as those included between 2014 and 2018, the characteristics of the patients may be different and the efficiency of the teams too. For a better homogeneity the authors should consider removing the data of the 9 news centers added between 2018-2019</p> <p>I think it would be better to give the median birth weight per twin, distinguishing between the weight of the first and second twin Idem for mean head circumference and mean length at birth In MM the authors said that “Neonatal death status was only collected on the first twin and therefore neonatal deaths in twins could not be analyzed”. Why? I don't understand the difficulty of collecting neonatal deaths of the 2nd twin. If it is possible to have this data for the first twin it should be possible to have it for the second. Please comment on this. Anyway we have no data on neonatal death although this is stated in MM even for the first twin...</p> <p>The lack of information on the cause of preterm birth, especially spontaneous and induced, is a major limitation of the study in the perspective of a potential improvement of care in Bostwana. This seems particulary true as the caesarean section rate for prematurity before 32 weeks is the same as for singletons, suggesting a possible induced prematurity of maternal origin or IUGR after 32 weeks , particularly for emmergency C section due to an absence of care or prevention before. It is only by knowing the causes of prematurity that authors will be able to correct the rate of caesarean section and neonatal mortality rates in the next few years.</p> <p>Why statistical tests, especially the p, do not appear in the tables. It is said in the text for example that patients pregnant with twins are significantly older than those pregnant with singletons... Please add this p in the tables when appropriate</p> <p>In the table 1 and 2 there is a formatting problem with the rows not matching each other in the different columns which makes it impossible to read correctly. Please make a proper formatting. Also the columns are too small with truncated headings...</p>
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### VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Raanan Meyer, Sheba Medical Center

Comments to the Author:

I would like to thank the Editors for the opportunity to review this manuscript. This study aimed to evaluate the outcome of twin pregnancies in Botswana, and compared these pregnancies outcomes to singleton pregnancies' outcomes. The study is well written and clear. However, although trying to provide information on twin pregnancies' outcomes in this country, it is severely limited by a lack of detailed data on these pregnancies and deliveries.

Following are my comments on specific points in the manuscript.

Abstract

Participants- as the number of deliveries is provided in the Results section, this information is redundant in the Participants section.

--We have taken out number of deliveries as suggested.

## Background

Page 6 lines 6-8- the authors state that “prior studies of twins in sub-Saharan Africa pre-date the implementation of the SDG and do not evaluate birth outcomes”. Please provide references to this claim.

--We have provided references as recommended.

## Methods

I would like to highlight some issues regarding the primary outcomes chosen by the authors:

Stillbirths- there is no information as to whether predelivery intrauterine fetal demise occurred, or if it occurred intrapartum. These are two different entities with different etiologies.

--Unfortunately, we do not have accurate information on whether fetal demise occurred predelivery or intrapartum. We recognize that this is a major limitation of our study and have added text emphasizing this in the discussion (p. 18, final paragraph) “Also, our study did not collect information necessary to determine the etiology of preterm delivery and stillbirth among twins, such as induction status, spontaneity of preterm delivery, whether c-sections were planned or emergent, indication for c-section (including presentation of infants), or fetal heartbeat on admission.”

Furthermore, in page 7, line 40, the authors refer to “neonatal death”, after stating “stillbirths” as the selected outcome several lines above. Please correct.

--The reviewer is correct that neonatal death is not an outcome that we were able to assess—we have tried to clarify further and now state “While APGAR scores and birthweights were recorded for each individual twin, neonatal death status was only collected on the first twin per the original protocol of our study. Therefore, neonatal deaths in twins could not be analyzed and were not included as a primary outcome assessed. We chose not to provide data on neonatal deaths in twin 1 without data on twin 2 because it would only provide an incomplete comparison to singletons, which could over or underestimate the total NND among twins.”

Birthweight- the authors chose 2500 and 1500 grams as cutoffs. While these cutoffs are indeed usually used in the literature, I suggest that the fetuses’ birthweight percentiles would be more appropriate as primary outcomes, as they represent intrauterine fetal growth more accurately and associated adverse outcomes in cases of growth restriction.

--We chose to use birthweight because we were unable to find published norms for weight-for-GA applicable to twin gestation in Botswana. Typically we use INTERGROWTH-21, but this applies only to singletons as twins are expected to have lower birthweights than singletons at every gestational age.

## Results

The proportion of induction of labor in each group is not provided and should be added. In addition, indications for cesarean deliveries are not provided.

--Unfortunately, we do not have this information because it is not routinely captured in our dataset. We agree that this is a limitation of our study and that it would be very helpful for understanding the etiology of adverse birth outcomes among twins and have added these specific details into the limitations section of our discussion (final paragraph), "Also, our study did not collect information necessary to determine the etiology of preterm delivery and stillbirth among twins, such as induction status, spontaneity of preterm delivery, whether c-sections were planned or emergent, indication for c-section (including presentation of infants), or fetal heartbeat on admission. Understanding the causes of adverse outcomes is clearly necessary before effective interventions can be designed and implemented. However, we hope our findings will be the catalyst for further research to elucidate these causes and ultimately lead to fewer adverse birth outcomes among twins."

Gestational age during ultrasound- was this the only US during pregnancy? Were there additional visits at later stages of the pregnancy? This information is important as it is associated with pregnancy outcomes.

--The study captures information on the first ultrasound during pregnancy. In table 1 we include the median number of antenatal care visits in pregnancy (9 for twins, 10 for singletons), but ultrasound is not routinely performed during these visits (recommended once per Botswana guidelines). Some women may have additional ultrasounds after the first one, but our study did not collect this information.

What is the proportion of pregnancies with no information on gestational age at delivery? The primary outcome of this manuscript is preterm deliveries, yet this essential information is missing. Is there a correlation with the proportion of women that had US examinations?

--We have added the number of pregnancies with missing gestational age to the first paragraph of results ("Gestational age was known in 98.8% of singletons and 98.7% of twins"). Nearly all births had a gestational age and this was not correlated with the proportion of women who had ultrasound.

Table 1- why are p-values not provided in this Table?

Tables 2,3- data presentation is confusing

--It is our practice, from the recommendation of our statisticians, not to provide p-values for Table 1 description of baseline statistics [per STROBE guidelines, Vandembroucke et al Annals of Internal Med 2007]. Additionally, in this case, due to the extremely large number in the singletons, a significant p-value does not necessarily indicate a meaningful difference and could be misleading to readers. We have noted that the formatting of table 2 and 3 appears different in the PDF created by the website than it appears in the word document of our manuscript. We have provided images of the tables below for reference.

**Table 1.** Maternal Characteristics and Obstetric Care

	<b>Twin Pregnancies (N=1859)</b>	<b>Singleton Pregnancies (N=117,593)</b>
<b>Maternal Characteristics</b>		
Maternal Age (median, IQR)	29 [24,34]	26 [22,32]
Missing	0	71
Primigravid	405 (21.8%)	42385 (36.1%)
Grand Multip (>4 prior pregnancies)	280 (15.1%)	12369 (10.5%)
Missing	3	391
Low Maternal Education (completed none or primary only)	167 (9.2%)	8624 (7.5%)
Missing	55	2934
Botswana Citizen	1761 (94.7%)	113386 (96.4%)
Non-Citizen	89 (4.79%)	3674 (3.12%)
Missing	9	400
HIV-Infected	562 (30.5%)	28264 (24.2%)
Missing	17	934
<b>Obstetric Care</b>		
Antenatal Care Visits (median, IQR)	9 [6,12]	10 [7,12]
Missing (%)	21 (1.1%)	1112 (1.0%)
Ultrasound		
Total with Ultrasound during pregnancy*	980/1248 (78.5%)	52,012/77786 (66.9%)
Median Gestational Age at U/S	25 [19, 31]	27 [20, 33]
Ultrasound Scan <20wks gestation	243/1199 (20.3%)	10706/73522 (14.6%)
Missing Ultrasound Date	49	4264
Delivered in Tertiary Hospital	1019 (54.8%)	53040 (45.1%)
Missing	0	1
C-Section	1015 (54.6%)	25887 (22.0%)
Missing method of delivery	0	2

\*Captured in Tsepamo beginning 3/31/2016

**Table 2.** Preterm Birth among twin and singleton pregnancies

	<b>Twin Pregnancies (N=1859)</b>	<b>Singleton Pregnancies (N=117,593)</b>	<b>Relative Risk (RR)</b>	<b>Adjusted Relative Risk* (ARR)</b>
<b>Preterm birth (&lt;37 wks)</b>	873 (47.6%)	19462 (16.7%)	2.8, 95% CI	2.8, 95%
Missing (%)	25 (1.3%)	1391 (1.2%)	2.7, 3.0	CI 2.7,2.9
<b>Very Preterm birth (&lt;32 wks)</b>	217 (11.8%)	4664 (4.0%)	2.9 95% CI	3.0, 95%
Missing (%)	25 (1.3%)	1391 (1.2%)	2.6, 3.4	CI 2.6, 3.4

**Table 3.** Birth Outcomes among twins by birth order

	<b>Twin Pregnancies (N=1859, resulting in 3718 Infants)</b>				<b>Singleton Pregnancies (N=117,594)</b>	<b>RR (singleton vs. any twin)</b>	<b>aRR* (singleton vs. any twin)</b>
	<b>Twin 1 N=1859 (Infants)</b>	<b>Twin 2 N=1859 (Infants)</b>	<b>Any Twin N=1859 (Pregnancies)</b>	<b>Both Twins N=1859 (Pregnancies)</b>			
<b>Low birthweight (&lt;2500g)</b>	1158 (62.4%)	1239 (67.0%)	1430 (77.1%)	967 (52.0%)	16019 (13.6%)	5.7 (95% CI 5.5, 5.8)	4.5 (95% CI 4.3, 4.6)
Missing (N,%)	4	9	4	4	138		
<b>Very Low birthweight (&lt;1500g)</b>	237 (12.8%)	271 (14.6%)	311 (16.8%)	197 (10.6%)	3757 (3.2%)	5.2 (95% CI 4.7, 5.8)	5.2 (95% CI 4.7, 5.8)
Missing (N,%)	4	9	4	4	138		
<b>Stillbirth</b>	70/1859 (3.8%)	96/1855 (5.2%)	128 (6.9%)	38 (2.0%)	2845 (2.4%)	2.8, (95% CI 2.4, 3.4)	2.8 (95% CI 2.3, 3.3)
Missing (N,%)	0	4	4	0	18		

\*adjusted for maternal age, gravida and educational attainment and maternal HIV status

## Discussion

The authors appropriately discuss the limitations of this study. I agree with those stated and suggest adding the lack of information on out-of-hospital/home deliveries.

--We have added this to our limitations section in the discussion: "Finally, we do not have data from deliveries occurring outside the hospital, though this is rare (<5%) in Botswana [15, 16]."

An additional major limitation is the lack of accurate gestational age of the study cohort. As this is the primary outcome of this study, it is vital that the authors provide information on the proportion of pregnancies without this data.

--As noted above, we have added the proportion of pregnancies with missing gestational age. We agree that potential for inaccurate gestational age is a limitation of our study and have highlighted this in our discussion (final paragraph).

Reviewer: 2

Dr. Marie Victorie Senat, Hôpital Bicêtre

Comments to the Author:

Prevalence and Outcomes of Twin Pregnancies in Botswana: a National Birth Outcomes Surveillance Study

This study report the prevalence and outcome of twin pregnancies in Botswana in a country with low socio-economic resources.

This study is very interesting and provides an inventory of the outcome of twin pregnancies in a country with low socio-economic resources in order to make an assessment. In this way, this study is very informative and includes a large number of women with a comparison over the same period of time of pregnancy with singleton. It reports a very high rate of IUGR and stillbirth reflecting the absence of adequate monitoring during pregnancy, particularly regular ultrasound. The major limitation of this study is that without knowing the % of spontaneous and induced prematurity, nor the fetal or maternal causes leading to delivery, it will be difficult to improve the quality of care. And as pointed out by the authors another limitation is the lack of diagnosis of chorionicity which does not allow for planning an appropriate gestational age of birth which given the median age of birth in the study is far too late for monochorionic pregnancies and surely a source of stillbirth...although I do understand that changing the culture of pregnant women to go for early antenatal care and getting ultrasound scans and sonographers in Botswana is a challenge. It may then be just a first study to say that it is necessary to reinforce the monitoring of pregnancy and particularly ultrasound and to carry out a second study which will specifically analyse the different categories and causes of prematurity.

Do we know how many ultrasound scans (mean and range) twin and singleton patient had in their pregnancy ?

--This information is not collected in our study. There are no clinical guidelines for monitoring (twins or singletons) by ultrasound in Botswana, so we unfortunately do not expect to be able to find this information. As noted in comments to reviewer 1, we believe that this is an important limitation of our research and hope that this information can be collected in future studies.

I am not sure that it is relevant to have added the year 2018-2019 even if it increases the number of patients. Indeed, as there are new centers added, the care provided to patients may not be the same as those included between 2014 and 2018, the characteristics of the patients may be different and the efficiency of the teams too. For a better homogeneity the authors should consider removing the data of the 9 new centers added between 2018-2019

--We chose to include all sites in the study because our primary aim was to describe the outcomes of twins in Botswana and these added sites help to give a better overview of birth outcomes on a national level. We agree with the reviewer that the addition of new sites does decrease the homogeneity over time, but it should not be differential since both twins and singletons from all the sites are included.

I think it would be better to give the median birth weight per twin, distinguishing between the weight of the first and second twin. Idem for mean head circumference and mean length at birth

--We have added information on the median birth weight (Results/Birth Outcomes, First Paragraph), head circumference and length (Results/Birth Outcomes, First Paragraph) distinguishing between the first and second twin.

In MM the authors said that "Neonatal death status was only collected on the first twin and therefore neonatal deaths in twins could not be analyzed". Why? I don't understand the difficulty of collecting neonatal deaths of the 2nd twin. If it is possible to have this data for the first twin it should be possible to have it for the second. Please comment on this. Anyway we have no data on neonatal death although this is stated in MM even for the first twin...

--In the original protocol for our study we only collected data on the first twin's death (occurring in hospital within 28 days of delivery among infants who never left the hospital) and we are unable to go back and retrospectively look at deaths for twin 2 (data was abstracted anonymously). We realize that this is a major limitation of our study. We have clarified this in the methods section as mentioned in response to the first reviewer, " We chose not to provide data on neonatal deaths in twin 1 without data on twin 2 because it would only provide an incomplete comparison to singletons, which could over or underestimate the total NND among twins." We were concerned this could be misleading.

The lack of information on the cause of preterm birth, especially spontaneous and induced, is a major limitation of the study in the perspective of a potential improvement of care in Bostwana. This seems particularly true as the caesarean section rate for prematurity before 32 weeks is the same as for singletons, suggesting a possible induced prematurity of maternal origin or IUGR after 32 weeks , particularly for emergency C section due to an absence of care or prevention before. It is only by knowing the causes of prematurity that authors will be able to correct the rate of caesarean section and neonatal mortality rates in the next few years.

--We agree with this reviewer's comments and appreciate the thoughtful feedback. We have tried to emphasize this point by clearly laying out the next steps and the need for subsequent research to understand the causes of prematurity and have added additional text to the limitations section of our discussion (final paragraph) "our study did not collect information necessary to determine the etiology of preterm delivery and stillbirth among twins, such as induction status, spontaneity of preterm delivery, whether c-sections were planned or emergent, indication for c-section (including presentation of infants), or fetal heartbeat on admission. Understanding the causes of adverse outcomes is clearly necessary before effective interventions can be designed and implemented. However, we hope our findings will be the catalyst for further research to elucidate these causes and ultimately lead to fewer adverse birth outcomes among twins."

Why statistical tests, especially the p, do not appear in the tables. It is said in the text for example that patients pregnant with twins are significantly older than those pregnant with singletons... Please add this p in the tables when appropriate

--Please see response to reviewer 1.

In the table 1 and 2 there is a formatting problem with the rows not matching each other in the different columns which makes it impossible to read correctly. Please make a proper formatting. Also the columns are too small with truncated headings...

--Please see comments to reviewer 1

## VERSION 2 – REVIEW

<b>REVIEWER</b>	Meyer, Raanan Sheba Medical Center
<b>REVIEW RETURNED</b>	29-Jul-2021

<b>GENERAL COMMENTS</b>	While this study has several major limitations, the authors appropriately addressed them in the revised manuscript.
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<b>REVIEWER</b>	Senat, Marie Victorie Hôpital Bicêtre, Departement de Gynécologie-Obstétrique
<b>REVIEW RETURNED</b>	24-Aug-2021

<b>GENERAL COMMENTS</b>	No comment for this revised version
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