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

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

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ABSTRACT

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Objectives: This study aims to evaluate the prevalence and outcome of twin pregnancies in Botswana.

Setting: The Tsepamo Study conducted birth outcomes surveillance at 8 government-run hospitals (~45% of all births in Botswana) from August 2014-June 2018 and expanded to 18 hospitals (~70% of all births in Botswana) from July 2018-March 2019.

Participants: Data were collected for all live-born and stillborn in-hospital deliveries with a gestational age (GA) greater than 24 weeks. This analysis included 117,593 singleton and 3,718 twin infants born to 119,477 women between August 2014-March 2019 and excluded 73 higher order multiples (23 sets of triplets and 1 set of quadruplets).

Outcomes Measured: Our primary outcomes were preterm delivery (<37 weeks GA), very preterm delivery (<32 weeks GA) and stillbirth (APGAR score of 0,0,0).

Results: Between August 2014 and March 2019, 119,477 deliveries were recorded, including 1859 (1.6%) sets of twins. Women with twin pregnancies had a similar median number of antenatal care visits (9 vs. 10), but were more likely to deliver in a tertiary center (54.8% vs. 45.1%, p<0.001) and more likely to have a C-section (54.6% vs. 22.0%, p<0.001) than women with singletons. Compared with singletons, twin pregnancies had a higher risk of preterm delivery (<37 weeks GA) (47.6% vs. 16.7%, aRR 2.8, 95% CI 2.7, 2.9) and very preterm delivery (<32 weeks) (11.8% vs. 4.0%, aRR 3.0 95% CI 2.6, 3.4). Among all twin pregnancies, 128 (6.9%) had at least one stillborn infant compared with 2845 (2.4%) stillbirths among singletons (aRR 2.8, 95% CI 2.3, 3.3).

Conclusion:
Adverse birth outcomes are common among twins in Botswana, and are often severe. Interventions that allow for earlier identification of twin gestation and improved antenatal management of twin pregnancies may improve infant and child survival.

Keywords: Twins, multiple gestation, Botswana, sub-Saharan Africa, stillbirth, preterm, low-birthweight

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Strengths and limitations of this study

- Our study uses a large, nationally representative sample with little missing data.
- Due to the limited availability of early prenatal ultrasound, gestational age may be less accurate and we are unable to determine the cause of preterm birth or whether preterm birth is spontaneous.
- We are unable to evaluate the impact of preterm and low birthweight on longer-term outcomes of twins, or estimate perinatal mortality.

Background

Twin pregnancies are universally considered to be 'high-risk'. Maternal complications including hypertensive disorders, anemia, post-partum hemorrhage, and maternal mortality, are more common among twin pregnancies than singleton pregnancies [1]. Twins are also more likely than singletons to be born preterm and to have restricted growth in-utero, thereby increasing their risk for intrauterine demise and neonatal mortality [2]. The majority of existing research on multigravid pregnancies and birth outcomes among twins is from high-resource settings [3]. In these settings, the perinatal mortality rate of twins is 3-7 times higher than that of singletons [4, 5] and up to 60% of twins are born preterm (before 37 weeks) [6, 7].

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In most high resource settings, twin pregnancies are managed by routine prenatal monitoring and neonatal intensive care services, including early identification of twin pregnancies, serial antenatal ultrasound, referral to specialized centers, and prenatal counselling [8]. In lower resource settings data are limited, but twin pregnancies may be at particularly high risk for adverse birth outcomes because of the lack of routine intensive prenatal monitoring and neonatal intensive care services [1, 9]. Additionally, maternal outcomes with multigravid pregnancies may also be worse because of limitations in management of maternal conditions more common in twin pregnancies, such as post-partum hemorrhage and pre-eclampsia [3].

In Southern Africa, where assisted reproductive technology is not commonly available, the incidence of naturally occurring twins is estimated to be high (12-18 per 1000 births) compared to other low- and middle-income countries (LMIC) in East Asia and Latin America where the incidence of twins is as low as 6-9 per 1000 births [10]. This relatively large number of twins may contribute substantially to perinatal mortality in the region [11]. Increased prevalence of preterm delivery and low-birth weight among twins leads to increased risk of under-five mortality due to malnutrition, respiratory disorders, vulnerability to infection, and developmental delays [12, 13].

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It is estimated that one in five twins born in sub-Saharan Africa dies before the age of five [11]. Prior studies of twins in sub-Saharan Africa pre-date implementation of the United Nations Sustainable Development Goals (SDG) in 2015 and do not evaluate specific birth outcomes. This study focuses on Botswana, a country with a rapidly growing economy, strong investment in healthcare, a national program to prevent maternal mortality [14, 15], and a large, nationally-representative birth outcomes surveillance study (Tsepamo). We aim to provide the first published data on the prevalence and outcome of twin pregnancies in Botswana.

Methods

The Tsepamo Study

The Tsepamo Study is a birth outcomes surveillance study in Botswana, where >95% of women deliver in health care settings (not at home) [16]. Details of methodology have been previously published [17, 18]. In summary, deidentified information was abstracted from obstetric cards (antenatal care records used throughout the pregnancy) at the time of discharge from the postnatal ward from women who deliver live-born or stillborn infants at select government maternity hospitals in Botswana. From August 2014-June 2018 data were collected from 8 sites across the country (~45% of births in Botswana) and from July

2018-March 2019 data were collected from up to 18 sites (~72% of births in Botswana).

At each site, data were collected for all in-hospital deliveries with a gestational age greater than 24 weeks. Information included maternal demographics, antenatal care visits, ultrasound reports with date of ultrasound, HIV status, method of delivery, and infant delivery characteristics (including the number of infants delivered, gestational age (GA) at delivery, birthweight, and vital status at birth and discharge).

Outcomes

The primary adverse outcomes assessed were stillbirth (APGAR scores 0,0,0), preterm birth (<37 weeks gestation), very preterm birth (<32 weeks gestation), low birthweight (LBW) (<2500g) and very low birthweight (VLBW)(<1500g). While APGAR scores and birthweights were recorded for each individual twin, neonatal death status was only collected on the first twin and therefore neonatal deaths in twins could not be analyzed. The gestational age was documented by midwives at the time of delivery using the estimated delivery date (EDD), which is calculated at the first antenatal care visit based on the last reported menstrual period and confirmed by ultrasound when available. If the last menstrual period was unknown or suspected to be incorrect, and

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if no ultrasound data was available, midwives occasionally used fundal height measurements to estimate gestational age.

Statistical Analysis

The prevalence of each birth outcome was calculated for singletons and twins and higher order multiples were excluded from analyses. Prevalence of preterm birth and very preterm birth were calculated per pregnancy as both twins were born at the same gestational age. Prevalence of stillbirth, low birthweight and very low birthweight among twins were calculated by pregnancy (defined as either twin with the outcome), and by infant (defined as the outcome in the total number of individual infants). When comparing birth outcomes between singletons vs. twins, we used pregnancy as the unit of analysis (e.g. how many pregnancies ended in at least one stillbirth). Log binomial regression models were fit to determine the relative risk (RR), adjusted risk ratio (aRR), and 95% confidence intervals (95% CI) of adverse birth outcomes among twin compared with singleton pregnancies. Multivariable models were adjusted for maternal age, gravida, educational attainment, and maternal HIV status, which were chosen a priori based on prior analysis of risk factors for adverse birth outcomes in Tsepamo [17-19]. Statistical analyses were performed using SAS software.

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Patient and public involvement

Patients and the public were not involved in the design or conduct of this study.

Results

Study Population

Between August 2014 and March 2019, there were 121,385 infants born to 119,477 women who delivered in the Tsepamo Study, including 117,593 (98.4%) singletons, 3718 twin infants (1859 sets of twins) (1.6%), 69 triplet infants (23 sets of triplets) (0.00%), 4 quadruplets (1 set) (0.00%) and 1 with missing data for delivery number.

Maternal Characteristics and Obstetric Care

Maternal demographics and obstetric care parameters are shown in Table 1. Women with twin pregnancies were older, less likely to be primigravid, and more likely to have had >4 prior pregnancies compared with women with singleton pregnancies. The median

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number of antenatal care visits was similar among women with singletons (10) and women with twins (9). Prenatal ultrasound was performed in 78.5% (980/1248) of women with twin pregnancies at a median gestational age (GA) of 25 [IQR 19, 31] weeks (20.3% prior to 20 weeks GA). In comparison, 66.9% (52,012/77,786) of women with singleton pregnancies received an ultrasound scan, at a median GA of 27 [IQR 20, 33] weeks (14.6% prior to 20 weeks GA). Twins were more likely to be delivered in a tertiary hospital (54.8% vs. 45.1%) and also more likely to deliver via Cesarean-section (54.6% vs. 22.0%).

Birth Outcomes

The median gestational age was 39 weeks [IQR 37,40], and the median birthweight was 3080g [IQR 2750, 3400] among all singletons with 114,749 (97.6%) infants live-born. Among twin pregnancies, the median gestational age was 37 weeks [IQR 34,38] and the median birthweight was 2330g [IQR 1920, 2675] with 3,552 (95.5%) infants live-born. Twin infants had lower mean head circumference than singletons (32.4cm vs. 45.6cm) and lower mean length at birth (34.1cm and 50.1cm).

Twin pregnancies were more likely to result in preterm birth than singleton pregnancies (Table 2). Compared with singletons, twin pregnancies had a higher risk of preterm birth (47.6% vs.

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16.7%, aRR 2.8, 95% CI 2.7,3.2.9) and very preterm birth (11.8% vs. 4.0%, aRR 3.0, 95% CI 2.6, 3.4). C-sections were more common in preterm twins than preterm singletons (47.7% vs. 23.0%), however, among very preterm births (<32 weeks GA), C-section rate was similar (24.9% vs. 22.7%).

Among all 3,718 individual twin infants, 2397 (64.5%) were low birthweight, 508 (13.7%) were very low birthweight and 166 (4.5%) were stillborn. Both twins were low birthweight in 52% of pregnancies, very low birthweight in 10.6% of pregnancies, and stillborn in 2.0%. The first born twin and the second born twin had similar rates of low and very low birthweight, but twin 2 was more likely to be stillborn than twin 1 (5.2% vs. 3.8%) (Table 3). The risk of at least one infant being low birthweight (aRR 4.5, 95% CI 4.3, 4.6), very low birthweight (aRR 5.2, 95% CI 4.7, 5.8) or stillbirth (aRR 2.8, 95% CI 2.3, 3.3) was higher among twin pregnancies than singleton pregnancies (Table 3).

Table 1. Maternal Characteristics and Obstetric Care

	Twin Pregnancies (N=1859)	**Singleton Pregnancies (N=117,593)**

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

*Captured in Tsepamo beginning 3/31/2016

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Table 2. Preterm Birth among twin and singleton pregnancies

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

Table 3. Birth Outcomes among twins by birth order

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

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Missing (N,%)	0	(5.2%) 4	4	0	18	2.4, 3.4)	2.3, 3.3)

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

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Discussion

We performed the first published analysis of the prevalence and outcomes of twin births in Botswana using nationally representative data from 2014-2019. Because of the absence of medically assisted reproduction, Botswana represents a particularly valuable opportunity to study naturally occurring rates and outcomes of twinning. We found that twin births occurred in 16 per 1000 pregnancies, a prevalence much higher than reported twinning rates in LMIC outside of sub-Saharan Africa. We also found that adverse birth outcomes were high among twin pregnancies, including a 6.9% stillbirth prevalence, almost 3-fold higher than stillbirths in singleton pregnancies (2.4%).

The rate of twinning in our study, 16/1000 pregnancies, is consistent with previously reported high rates of naturally

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occurring twins in Southern Africa (12-15/1000 pregnancies in Namibia, South Africa, and Lesotho and 15-18/1000 pregnancies in Zimbabwe) [10]. In contrast, very low twinning rates (6-9/1000 pregnancies) have been documented in LMIC across East Asia and Central and South America [10]. Geographical differences in naturally occurring twin birth rates are primarily due to genetic, racial, and ethnic differences in predisposition to dizygotic (DZ) twin birth [10]. While the rate of twinning in Botswana is high compared to other LMIC, it is lower than that in many high-income countries (HIC), where the introduction of medically assisted reproduction has led to dramatic increases in twinning rates over the past several decades. In the United States, for example, the rate of twin births rose from 18.9 per 1000 births in 1980 to 33.9 per 1000 births in 2014 [20, 21].

The prevalence of stillbirths among twin pregnancies in Botswana (6.9%) is well above the WHO's target of under 12 stillbirths per 1000 births [22, 23]. However, our prevalence is similar to that reported across sub-Saharan Africa, and lower than a reported 10.2% stillbirth prevalence among twins in one Nigerian study [24]. In contrast, the prevalence of stillbirth in twins in Botswana is higher than in high-income countries such as the United States (USA) (0.35%) [25] and South Korea (0.48%) [26]. The prevalence of stillbirth among singletons in our study is

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2.4%, which is nearly 10 times greater than that among singletons in many high-income countries [27, 28]. While twin pregnancies are clearly particularly high-risk and contribute substantially to perinatal mortality, the high prevalence of stillbirths among all pregnancies in Botswana highlights a strong need for interventions to decrease stillbirths in the entire population.

The prevalence of preterm birth among twin pregnancies (46.%) in Botswana is substantially higher than that among singleton pregnancies (16.8%) and slightly higher than reported prevalence of preterm birth among twins in other sub-Saharan countries [24]. However, prevalence of preterm birth with twin pregnancy in Botswana is lower than the United States, where 60% of twin pregnancies have preterm births [6, 28]. While the high prevalence of preterm births among twins in the US may be partially attributed to the increased risk for preterm birth in multiple gestation, it may also be explained by clearer antenatal care guidelines and closer prenatal monitoring among twin pregnancies in the US compared to Botswana. The high prevalence of preterm birth among twins in the US is in line with the American College of Obstetrics and Gynecology's guidelines, which recommend timing delivery based on chorionicity (38 weeks for dichorionic-diamniotic, 34-36 6/7 weeks for monochorionic-diamniotic, 32-34 for monochorionic-monoamniotic) and delivery by 37-38 week's gestation in uncomplicated twin pregnancies to reduce the risk of stillbirth [29-32].

The relatively low rate of preterm birth and high rate of stillbirth among twin pregnancies in Botswana raises the question of whether twin stillbirths in Botswana could be decreased by increasing delivery of high-risk (monochorionic-monoamniotic, monochorionic-diamniotic) twin pregnancies between 34 and 37 weeks [31-33]. To implement this type of change, improvements in proportion of ultrasound in the first trimester of twin pregnancy to identify chorionicity and to improve accuracy of gestational age dating would be needed. Additionally, preterm delivery may carry increased risk in low resource settings like Botswana where intensive neonatal care services are limited. Though delivery by 37 weeks may not be appropriate in all settings, closer surveillance of twin pregnancies after 34 weeks GA, at which time the risk of stillbirth and maternal complications begins to rise, may improve twin outcomes in Botswana [26]. Close monitoring for fetal growth concordance [34], screening for aneuploidy, which is more common among twin pregnancies and associated with higher risk of stillbirth [35, 36], and clear delivery planning during

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antenatal care may also help to reduce adverse perinatal outcomes among twins. In addition to increased antenatal care, improvements in maternal nutrition may help improve outcomes in twin pregnancies in Botswana. Despite the relatively lower prevalence of preterm births in Botswana, we found very high rates of LBW and VLBW among twins (77.2% and 16.8%, respectively, compared to 55.6% and 9.1% in the USA) [7], which is likely multifactorial, but suggests that women with twin pregnancies in Botswana may not receive adequate nutrition to support multiple gestations [37, 38].

Strengths of this study include the large, nationally representative sample with little missing data.

Our study also has several limitations. Due to the limited availability of early prenatal ultrasound, gestational age may be less accurate and we are unable to determine the cause of preterm birth or whether preterm birth is spontaneous. Similarly, we cannot determine the proportion of C-sections that are planned vs. emergent, and do not have data on the indications for delivery via C-section (including presentation of infants). We are also unable to distinguish the proportion of twins that are monochorionic (MC), which is a known risk factor for stillbirth and neonatal death [9, 33]. However, the prevalence of MC twins is thought to be relatively constant

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worldwide (prevalence of DZ twins varies), so this is unlikely to explain the high prevalence of stillbirth that we found among twin pregnancies in Botswana [39, 40]. Finally, due to our study design, we are unable to evaluate the impact of preterm and low birthweight on longer-term outcomes of twins, or estimate perinatal mortality.

Conclusions

In summary, we found that the prevalence of twinning is high in Botswana and adverse birth outcomes are common among twin pregnancies and are often severe. The high rate of stillborn and LBW twins despite a relatively low preterm birthrate compared to twins in high-income countries points to the need for interventions that include early ultrasound and identification of twin gestation and type of twin gestation, improved maternal nutrition, close antepartum surveillance, and advanced delivery planning in order to reduce morbidity and mortality among twin pregnancies in Botswana.

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Abbreviations
GA: Gestational Age
RR: Relative Risk
aRR: Adjusted Relative Risk
IQR: Inter-Quartile Range
LMIC: Low- and Middle-Income Countries
HIC: High-Income Countries

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LBW: Low Birthweight
VLBW: Very Low Birthweight
HIV: Human Immunodeficiency Virus
MZ: monozygotic
DZ: dizygotic
MC: monochorionic
DC: dichorionic

Competing Interests

The authors declare that they have no competing interests.

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Data Availability

Per Botswana IRB regulations, de-identified data from the Tsepamo study can be requested from the Principal Investigator of the Study.

Authors' contributions

AI and RZ designed and performed the analysis and drafted the initial manuscript. RLS, JM, MM, RZ, DJ contributed to the design, data collection and study oversight of the parent study. GM, MD, JM, SD, AI oversaw data collection and data cleaning. All authors contributed substantive feedback, revised and approved the final manuscript.

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References

1. Hanson C, Munjanja S, Binagwaho A, Vwalika B, Pembe AB, Jacinto E, et al. National policies and care provision in pregnancy and childbirth for twins in Eastern and Southern Africa: A mixed- methods multi-country study. PLoS Med. 2019;16(2).
2. Lawn J, Mongi P, Cousens S. Africa's newborns—counting them and making them count. In: Lawn J, Kerber K, editors. Opportunities for Africa's Newborns: Practical data, policy and programmatic support for newborn care in Africa. Cape Town: PMNCH; 2006. p. 11-22.
3. Bellizzi S, Sobel H, Betran AP, Temmerman M. Early neonatal mortality in twin pregnancy: Findings from 60 low-and middle-income countries. J Glob Health. 2018;8(1).
4. Peter C, Wenzlaff P, Kruempelmann J, Alzen G, Bueltmann E, Gruessner SE. Perinatal morbidity and early neonatal mortality in twin pregnancies. Open J Obstet Gynecol. 2013;3(1):78-89.
5. Hack KE, Derks JB, Elias SG, Franx A, Roos EJ, Voerman SK, et al. Increased perinatal mortality and morbidity in monochorionic versus dichorionic twin pregnancies: clinical implications of a large Dutch cohort study. BJOG. 2007;115(1):58-67.
6. Murray SR, Stock JS, Cowan S, Elizabeth SC, Norman JE. Spontaneous preterm birth prevention in multiple pregnancy. Obstet Gynaecol. 2018;20(1):57-63.
7. Martin JA, Hamilton BE, Osterman MJ, Driscoll AK. Births: Final Data for 2018. National Vital Statistics Report, vol 68, no 13. Hyattsville, MD: National Center for Health Statistics; 2019. 47 p.
8. Christensen K, Bjerregaard-Anderson M. Twin-singleton early-life survival in sub-Saharan Africa. Lancet Glob Health. 2017;5(7):e636-7.
9. Glinianaia SV, Obeysekera MA, Sturgiss S, Bell R. Stillbirth and neonatal mortality in monochorionic and dichorionic twins: a population-based study. Hum Reprod. 2011;26(9):2549-57.
10. Smits J, Monden C. Twinning across the Developing World. PLoS One. 2011;6(9).
11. Monden CW, Smits J. Mortality among twins and singletons in sub-Saharan Africa between 1995 and 2014: a

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pooled analysis of data from 90 Demographic and Health Surveys in 30 countries. Lancet Glob Health. 2017;5:e673-9.
12. Justesen A, Kunst A. Postneonatal and child mortality among twins in Southern and Eastern Africa. Int J Epidemiol. 2000;29(4):678-83.
13. Miyahara R, Jasseh M, Mackenzie GA, Bottomley C, Hossain MJ, Greenwood BM, et al. The large contribution of twins to neonatal and post-neonatal mortality in The Gambia, a 5-year prospective study. BMC Pediatr. 2016;16.
14. Mogobe KD, Tshiamo W, Bowelo M. Monitoring Maternity Mortality in Botswana. Reprod Health Matters. 2007;15(30):163-71.
15. Madzimbamuto FD, Ray SC, Mogobe KD, Ramogola-Masire D, Phillips R, Haverkamp M, et al. A root-cause analysis of maternal deaths in Botswana: towards developing a culture of patient safety and quality improvement. BMC Pregnancy Childbirth. 2014;14.
16. Nkhwaluma L, Mashalla Y. Maternal mortality trends at the Princess Marina and Nyangabwe referral hospitals in Botswana. Afr Health Sci. 2019;19(2):1833-40.
17. Zash R, Jacobson DL, Diseko M, Mayondi G, Mmalane M, Essex M, et al. Comparative Safety of Antiretroviral Treatment Regimens in Pregnancy. JAMA Pediatr. 2017;171(10).
18. Zash R, Holmes L, Diseko M, Jacobson DL, Brummel S, Mayondi G, et al. Neural-Tube Defects and Antiretroviral Treatment Regimens in Botswana. New Engl J Med. 2019;381:827-40.
19. Zash R, Jacobson DL, Diseko M, Mayondi G, Mmalane M, Essex M, et al. Comparative safety of dolutegravir-based or efavirenz-based antiretroviral treatment started during pregnancy in Botswana: an observational study. Lancet Glob Health. 2018;6:e804-10.
20. Martin JA, Hamilton BE, Osterman MJ. Three Decades of Births in the United States, 1980-2009. Hyattsville, MD: National Center for Health Statistics; 2012. 8 p. Report No.: 80.
21. Martin JA, Osterman MJ. Is Twin Childbearing on the Decline? Twin Births in the United States, Hyattsville, MD: National Center for Health Statistics; 2019. 8 p. Report No.: 351.
22. Saleem S, Tikmani SS, McClure EM, Moore JL, Azam SI, Dhaded SM. Trends and determinants of stillbirth in developing countries: results from the Global Network's Population-Based Birth Registry. Reprod Health. 2018;15(Suppl 1).

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23. Lawn JE, Blencowe H, Waiswa P, Amouzou A, Mathers C, Hogan D. Stillbirths: rates, risk factors, and acceleration towards 2030. Lancet. 2016;387:587-603.
24. Akaba GO, Agida TE, Onafowokan O, Offiong RA, Adewole ND. Review of Twin Pregnancies in a Tertiary Hospital in Abuja, Nigeria. J Health Popul Nutr. 2013;31(2):272-7.
25. Page JM, Pilliod RA, Snowden JM, Caughey AB. The risk of stillbirth and infant death by each additional week of expectant management in twin pregnancies. Am J Obstet Gynecol. 2015;212(5):630.e1-7.
26. Ko HS, Choi SK, Wie JH, Park IY, Park YG, Shin JC. Optimal Timing of Delivery Based on the Risk of Stillbirth and Infant Death Associated with Each Additional Week of Expectant Management in Multiple Pregnancies: a National Cohort Study of Koreans. J Korean Med Sci. 2018;33(10).
27. Flenady V, Wojcieszek AM, Middleton P, Ellwood D, Erwich JJ, Coory M. Stillbirths: recall to action in high-income countries. Lancet. 2016;387:691-702.
28. MacDorman MF, Gregory EC. Fetal and Perinatal Mortality: United States, 2013. National Vital Statistics Report. 2015;64(8).
29. Committee on Practice Bulletins—Obstetrics; Society for Maternal-Fetal Medicine. Practice Bulletin No. 169: Multifetal Gestations: Twin, Triplet, and Higher-Order Multifetal Pregnancies. Obstet Gynecol. 2016;128(4):e131-46.
30. Dodd JM, Crowther CA, Haslam RR, Robinson SJ. Timing of birth for women with a twin pregnancy at term: a randomized controlled trial. BMC Pregnancy Childbirth. 2010;10.
31. Dodd JM, Crowther CA, Haslam RR, Robinson SJ. Elective birth at 37 weeks of gestation versus standard care for women with an uncomplicated twin pregnancy at term: the Twins Timing of Birth Randomised Trial. BJOG. 2012;119(8):964-74.
32. Wood S, Tang S, Ross S, Sauve R. Stillbirth in twins, exploring the optimal gestational age for delivery: a retrospective cohort study. BJOG. 2014;121(10):1284-93.
33. Cheong-See F, Schuit E, Arroyo-Manzano D, Khalil A, Barrett J, Joseph KS, et al. Prospective risk of stillbirth and neonatal complications in twin pregnancies: systematic review and meta-analysis. BMJ. 2016;354.
34. D'Antonio F, Odibo AO, Prefumo F, Khalil A, Buca D, Flacco ME, et al. Weight Discordance and Perinatal Mortality in Twin Pregnancy: Systemic Review and Meta-Analysis. Ultrasound Obstet Gynecol. 2017;52(1):11-23.

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35. Shi K, Li L, Huang X, Chen B, Zhou Y, Fang Q. Fetal Aneuploidy: A Comparison of Dichorionic Twins and Monochorionic Twins. Fetal Diagn Ther. 2018;44:124-8.
36. Audibert F, Gagnon A. No. 262-Prenatal Screening for and Diagnosis of Aneuploidy in Twin Pregnancies. J Obstet Gynaecol Can. 2017;93(9):e347-61.
37. Luke B. Nutrition in Multiple Gestations. Clin Perinatol. 2005;32:403-29.
38. Goodnight W, Newman R. Optimal Nutrition for Improved Twin Pregnancy Outcome. Obstet Gynecol. 2009;114(5):1121-34.
39. Pison G, D'Addato AV. Frequency of Twin Births in Developed Countries. Twin Res Hum Genet. 2006;9(2):250-9.
40. Hankins GV, Saade GR. Factors influencing twins and zygosity. Paediatr Perinat Epidemiol. 2005;19(Suppl 1):8-9.

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	5,
Study size	10	Explain how the study size was arrived at	4, 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	6
		(*e*) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	10
Outcome data	15*	Report numbers of outcome events or summary measures	10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8, 10

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		(*b*) Report category boundaries when continuous variables were categorized	5
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9, 10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13, 14
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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

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ABSTRACT

Objectives: This study aims to evaluate the prevalence and outcome of twin pregnancies in Botswana.

Setting: The Tsepamo Study conducted birth outcomes surveillance at 8 government-run hospitals (~45% of all births in Botswana) from August 2014-June 2018 and expanded to 18 hospitals (~70% of all births in Botswana) from July 2018-March 2019.

Participants: Data were collected for all live-born and stillborn in-hospital deliveries with a gestational age (GA) greater than 24 weeks. This analysis included 117,593 singleton and 3,718 twin infants (1859 sets [1.6%]) born to 119,477 women between August 2014-March 2019 and excluded 73 higher order multiples (23 sets of triplets and 1 set of quadruplets).

Outcomes Measured: Our primary outcomes were preterm delivery (<37 weeks GA), very preterm delivery (<32 weeks GA) and stillbirth (APGAR score of 0,0,0).

Results: Women with twin pregnancies had a similar median number of antenatal care visits (9 vs. 10), but were more likely to deliver in a tertiary center (54.8% vs. 45.1%, p<0.001) and more likely to have a C-section (54.6% vs. 22.0%, p<0.001) than women with singletons. Compared with singletons, twin pregnancies had a higher risk of preterm delivery (<37 weeks GA) (47.6% vs. 16.7%, aRR 2.8, 95% CI 2.7, 2.9) and very preterm delivery (<32 weeks) (11.8% vs. 4.0%,

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aRR 3.0 95% CI 2.6, 3.4). Among all twin pregnancies, 128 (6.9%) had at least one stillborn infant compared with 2845 (2.4%) stillbirths among singletons (aRR 2.8, 95% CI 2.3, 3.3).

Conclusion:

Adverse birth outcomes are common among twins in Botswana, and are often severe. Interventions that allow for earlier identification of twin gestation and improved antenatal management of twin pregnancies may improve infant and child survival.

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Keywords: Twins, multiple gestation, Botswana, sub-Saharan Africa, stillbirth, preterm, low-birthweight

Strengths and limitations of this study

- Our study uses a large, nationally representative sample with little missing data.
- Due to the limited availability of early prenatal ultrasound, gestational age may be less accurate and we are unable to determine the cause of preterm birth or whether preterm birth is spontaneous.
- We are unable to evaluate the impact of preterm and low birthweight on longer-term outcomes of twins, or estimate perinatal mortality.

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Background

Twin pregnancies are universally considered to be 'high-risk'. Maternal complications including hypertensive disorders, anemia, post-partum hemorrhage, and maternal mortality, are more common among twin pregnancies than singleton pregnancies [1]. Twins are also more likely than singletons to be born preterm and to have restricted growth in-utero, thereby increasing their risk for intrauterine demise and neonatal mortality [2]. The majority of existing research on multigravid pregnancies and birth outcomes among twins is from high-resource settings [3]. In these settings, the perinatal mortality rate of twins is 3-7 times higher than that of singletons [4, 5] and up to 60% of twins are born preterm (before 37 weeks) [6, 7].

In most high resource settings, twin pregnancies are managed by routine prenatal monitoring and neonatal intensive care services, including early identification of twin pregnancies, serial antenatal ultrasound, referral to specialized centers, and prenatal counselling [8]. In lower resource settings data are limited, but twin pregnancies may be at particularly high risk for adverse birth outcomes because of the lack of routine intensive prenatal monitoring and neonatal intensive care services [1, 9]. Additionally, maternal outcomes with multigravid

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pregnancies may also be worse because of limitations in management of maternal conditions more common in twin pregnancies, such as post-partum hemorrhage and pre-eclampsia [3].

In Southern Africa, where assisted reproductive technology is not commonly available, the incidence of naturally occurring twins is estimated to be high (12-18 per 1000 births) compared to other low- and middle-income countries (LMIC) in East Asia and Latin America where the incidence of twins is as low as 6-9 per 1000 births [10]. This relatively large number of twins may contribute substantially to perinatal mortality in the region [11]. Increased prevalence of preterm delivery and low-birth weight among twins leads to increased risk of under-five mortality due to malnutrition, respiratory disorders, vulnerability to infection, and developmental delays [12, 13]. It is estimated that one in five twins born in sub-Saharan Africa dies before the age of five [11]. Prior studies of twins in sub-Saharan Africa pre-date implementation of the United Nations Sustainable Development Goals (SDG) in 2015 and do not evaluate specific birth outcomes [9-12]. This study focuses on Botswana, a country with a rapidly growing economy, strong investment in healthcare, a national program to prevent maternal mortality [14, 15], and a large, nationally-representative birth outcomes surveillance

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study (Tsepamo). We aim to provide the first published data on the prevalence and outcome of twin pregnancies in Botswana.

Methods

The Tsepamo Study

The Tsepamo Study is a birth outcomes surveillance study in Botswana, where >95% of women deliver in health care settings (not at home) [16]. Details of methodology have been previously published [17, 18]. In summary, deidentified information was abstracted from obstetric cards (antenatal care records used throughout the pregnancy) at the time of discharge from the postnatal ward from women who deliver live-born or stillborn infants at select government maternity hospitals in Botswana. From August 2014-June 2018 data were collected from 8 sites across the country (~45% of births in Botswana) and from July 2018-March 2019 data were collected from up to 18 sites (~72% of births in Botswana).

At each site, data were collected for all in-hospital deliveries with a gestational age greater than 24 weeks. Information included maternal demographics, antenatal care visits, ultrasound reports

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with date of ultrasound, HIV status, method of delivery, and infant delivery characteristics (including the number of infants delivered, gestational age (GA) at delivery, birthweight, and vital status at birth and discharge).

Outcomes

The primary adverse outcomes assessed were stillbirth (APGAR scores 0,0,0), preterm birth (<37 weeks gestation), very preterm birth (<32 weeks gestation), low birthweight (LBW) (<2500g) and very low birthweight (VLBW)(<1500g). 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. The gestational age was documented by midwives at the time of delivery using the estimated delivery date (EDD), which is calculated at the first antenatal care visit based on the last reported menstrual period and confirmed by ultrasound when available. If the last menstrual period was unknown or

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suspected to be incorrect, and if no ultrasound data was available, midwives occasionally used fundal height measurements to estimate gestational age.

Statistical Analysis

The prevalence of each birth outcome was calculated for singletons and twins and higher order multiples were excluded from analyses. Prevalence of preterm birth and very preterm birth were calculated per pregnancy as both twins were born at the same gestational age. Prevalence of stillbirth, low birthweight and very low birthweight among twins were calculated by pregnancy (defined as either twin with the outcome), and by infant (defined as the outcome in the total number of individual infants). When comparing birth outcomes between singletons vs. twins, we used pregnancy as the unit of analysis (e.g. how many pregnancies ended in at least one stillbirth). Log binomial regression models were fit to determine the relative risk (RR), adjusted risk ratio (aRR), and 95% confidence intervals (95% CI) of adverse birth outcomes among twin compared with singleton pregnancies. Multivariable models were adjusted for maternal age, gravida, educational attainment, and maternal HIV status, which were chosen a priori based on prior analysis of risk factors for adverse birth outcomes in Tsepamo [17-19]. Statistical analyses were performed using SAS software.

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Patient and public involvement

Patients and the public were not involved in the design or conduct of this study.

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Results

Study Population

Between August 2014 and March 2019, there were 121,385 infants born to 119,477 women who delivered in the Tsepamo Study, including 117,593 (98.4%) singletons, 3718 twin infants (1859 sets of twins) (1.6%), 69 triplet infants (23 sets of triplets) (0.00%), 4 quadruplets (1 set) (0.00%) and 1 with missing data for delivery number. Gestational age was known in 98.8% of singletons and 98.7% of twins.

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Maternal Characteristics and Obstetric Care

Maternal demographics and obstetric care parameters are shown in Table 1. Women with twin pregnancies were older, less likely to be primigravid, and more likely to have had >4 prior pregnancies compared with women with singleton pregnancies. The median number of antenatal care visits was similar among women with singletons (10) and women with twins (9). Prenatal ultrasound was performed in 78.5% (980/1248) of women with twin pregnancies at a median gestational age (GA) of 25 [IQR 19, 31] weeks (20.3% prior to 20 weeks GA). In comparison, 66.9% (52,012/77,786) of women with singleton pregnancies received an ultrasound scan, at a median GA of 27 [IQR 20, 33] weeks (14.6% prior to 20 weeks GA). Twins were more likely to be delivered in a tertiary hospital (54.8% vs. 45.1%) and also more likely to deliver via Cesarean-section (54.6% vs. 22.0%).

Birth Outcomes

The median gestational age was 39 weeks [IQR 37,40], and the median birthweight was 3080g [IQR 2750, 3400] among all singletons with 114,749 (97.6%) infants live-born. Among twin

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pregnancies, the median gestational age was 37 weeks [IQR 34,38] and the median birthweight was 2330g [IQR 1920, 2675] for the first twin and 2290g [IQR 1830, 2610] for the second twin with 3,552 (95.5%) infants live-born. The mean head circumference was 32.4cm for both the first and second twin, and slightly lower than the mean head circumference for singletons (34.2cm). Mean length at birth was similar for the first and second twin (45.7cm and 45.4cm, respectively) but lower than the mean length of singletons (50.1cm).

Twin pregnancies were more likely to result in preterm birth than singleton pregnancies (Table 2). Compared with singletons, twin pregnancies had a higher risk of preterm birth (47.6% vs. 16.7%, aRR 2.8, 95% CI 2.7,3.2.9) and very preterm birth (11.8% vs. 4.0%, aRR 3.0, 95% CI 2.6, 3.4). C-sections were more common in preterm twins than preterm singletons (47.7% vs. 23.0%), however, among very preterm births (<32 weeks GA), C-section rate was similar (24.9% vs. 22.7%).

Among all 3,718 individual twin infants, 2397 (64.5%) were low birthweight, 508 (13.7%) were very low birthweight and 166 (4.5%) were stillborn. Both twins were low birthweight in 52% of

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pregnancies, very low birthweight in 10.6% of pregnancies, and stillborn in 2.0%. The first born twin and the second born twin had similar rates of low and very low birthweight, but twin 2 was more likely to be stillborn than twin 1 (5.2% vs. 3.8%) (Table 3). The risk of at least one infant being low birthweight (aRR 4.5, 95% CI 4.3, 4.6), very low birthweight (aRR 5.2, 95% CI 4.7, 5.8) or stillbirth (aRR 2.8, 95% CI 2.3, 3.3) was higher among twin pregnancies than singleton pregnancies (Table 3).

Table 1. Maternal Characteristics and Obstetric Care

	Twin Pregnancies (N=1859)	**Singleton Pregnancies (N=117,593)**
Maternal Characteristics		
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%)

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Missing	17	934
Obstetric Care		
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

	Twin Pregnancies (N=1859)	Singleton Pregnancies (N=117,593)	Relative Risk (RR)	Adjusted Relative Risk*

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				(ARR)
Preterm birth (<37 wks) Missing (%)	873 (47.6%) 25 (1.3%)	19462 (16.7%) 1391 (1.2%)	2.8, 95% CI 2.7, 3.0	2.8, 95% CI 2.7,2.9
Very Preterm birth (<32 wks) Missing (%)	217 (11.8%) 25 (1.3%)	4664 (4.0%) 1391 (1.2%)	2.9 95% CI 2.6, 3.4	3.0, 95% CI 2.6, 3.4

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Table 3. Birth Outcomes among twins by birth order

	Twin Pregnancies (N=1859, resulting in 3718 Infants)				**Singleton Pregnancies(N=117,594)**	**RR (singleton vs. any twin)**	**aRR* (singleton vs. any twin)**
	Twin 1 N=1859 (Infants)	**Twin 2 N=1859 (Infants**	**Any Twin N=1859 (Pregnancies)**	**Both Twins N=1859 (Pregnancies)**			
Low birthweight (<2500g)	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		
Very Low birthweight (<1500g)	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		
Stillbirth	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		

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

Discussion

We performed the first published analysis of the prevalence and outcomes of twin births in Botswana using nationally representative data from 2014-2019. Because of the absence of medically assisted reproduction, Botswana represents a particularly valuable opportunity to study naturally occurring rates and outcomes of twinning. We found that twin births occurred in 16 per 1000 pregnancies, a prevalence much higher than reported twinning rates in LMIC outside of sub-Saharan Africa. We also found that adverse birth outcomes were high among twin pregnancies, including a 6.9% stillbirth prevalence, almost 3-fold higher than stillbirths in singleton pregnancies (2.4%).

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The rate of twinning in our study, 16/1000 pregnancies, is consistent with previously reported high rates of naturally occurring twins in Southern Africa (12-15/1000 pregnancies in Namibia, South Africa, and Lesotho and 15-18/1000 pregnancies in Zimbabwe) [10]. In contrast, very low twinning rates (6-9/1000 pregnancies) have been documented in LMIC across East Asia and Central and South America [10]. Geographical differences in naturally occurring twin birth rates are primarily due to genetic, racial, and ethnic differences in predisposition to dizygotic (DZ) twin birth [10]. While the rate of twinning in Botswana is high compared to other LMIC, it is lower than that in many high-income countries (HIC), where the introduction of medically assisted reproduction has led to dramatic increases in twinning rates over the past several decades. In the United States, for example, the rate of twin births rose from 18.9 per 1000 births in 1980 to 33.9 per 1000 births in 2014 [20, 21].

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The prevalence of stillbirths among twin pregnancies in Botswana (6.9%) is well above the WHO's target of under 12 stillbirths per 1000 births [22, 23]. However, our prevalence is similar to that reported across sub-Saharan Africa, and lower than a reported 10.2% stillbirth prevalence among twins in one Nigerian study [24]. In contrast, the prevalence of stillbirth in twins in

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Botswana is higher than in high-income countries such as the United States (USA) (0.35%) [25] and South Korea (0.48%) [26]. The prevalence of stillbirth among singletons in our study is 2.4%, which is nearly 10 times greater than that among singletons in many high-income countries [27, 28]. While twin pregnancies are clearly particularly high-risk and contribute substantially to perinatal mortality, the high prevalence of stillbirths among all pregnancies in Botswana highlights a strong need for interventions to decrease stillbirths in the entire population.

The prevalence of preterm birth among twin pregnancies (46.%) in Botswana is substantially higher than that among singleton pregnancies (16.8%) and slightly higher than reported prevalence of preterm birth among twins in other sub-Saharan countries [24]. However, prevalence of preterm birth with twin pregnancy in Botswana is lower than the United States, where 60% of twin pregnancies have preterm births [6, 28]. While the high prevalence of preterm births among twins in the US may be partially attributed to the increased risk for preterm birth in multiple gestation, it may also be explained by clearer antenatal care guidelines and closer prenatal monitoring among twin pregnancies in the US compared to Botswana. The high

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prevalence of preterm birth among twins in the US is in line with the American College of Obstetrics and Gynecology's guidelines, which recommend timing delivery based on chorionicity (38 weeks for dichorionic-diamniotic, 34-36 6/7 weeks for monochorionic-diamniotic, 32-34 for monochorionic-monoamniotic) and delivery by 37-38 week's gestation in uncomplicated twin pregnancies to reduce the risk of stillbirth [29-32].

The relatively low rate of preterm birth and high rate of stillbirth among twin pregnancies in Botswana raises the question of whether twin stillbirths in Botswana could be decreased by increasing delivery of high-risk (monochorionic-monoamniotic, monochorionic-diamniotic) twin pregnancies between 34 and 37 weeks [31-33]. To implement this type of change, improvements in proportion of ultrasound in the first trimester of twin pregnancy to identify chorionicity and to improve accuracy of gestational age dating would be needed. Additionally, preterm delivery may carry increased risk in low resource settings like Botswana where intensive neonatal care services are limited. Though delivery by 37 weeks may not be appropriate in all settings, closer surveillance of twin pregnancies after 34 weeks GA, at which time the risk of stillbirth and maternal complications begins to rise, may improve twin outcomes in Botswana [26]. Close

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monitoring for fetal growth concordance [34], screening for aneuploidy, which is more common among twin pregnancies and associated with higher risk of stillbirth [35, 36], and clear delivery planning during antenatal care may also help to reduce adverse perinatal outcomes among twins. In addition to increased antenatal care, improvements in maternal nutrition may help improve outcomes in twin pregnancies in Botswana. Despite the relatively lower prevalence of preterm births in Botswana, we found very high rates of LBW and VLBW among twins (77.2% and 16.8%, respectively, compared to 55.6% and 9.1% in the USA) [7], which is likely multifactorial, but suggests that women with twin pregnancies in Botswana may not receive adequate nutrition to support multiple gestations [37, 38].

Strengths of this study include the large, nationally representative sample with little missing data. Our study also has several limitations. Due to the limited availability of early prenatal ultrasound, gestational age may be less accurate. 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.

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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. We are also unable to distinguish the proportion of twins that are monochorionic (MC), which is a known risk factor for stillbirth and neonatal death [9, 33]. However, the prevalence of MC twins is thought to be relatively constant worldwide (prevalence of DZ twins varies), so this is unlikely to explain the high prevalence of stillbirth that we found among twin pregnancies in Botswana [39, 40]. Due to our study design, we are unable to evaluate the impact of preterm and low birthweight on longer-term outcomes of twins, or estimate perinatal mortality. Finally, we do not have data from deliveries occurring outside the hospital, though this is rare (<5%) in Botswana [15, 16].

Conclusions

In summary, we found that the prevalence of twinning is high in Botswana and adverse birth outcomes are common among twin pregnancies and are often severe. The high rate of stillborn and LBW twins despite a relatively low preterm birthrate compared to twins in high-income

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countries points to the need for interventions that include early ultrasound and identification of twin gestation and type of twin gestation, improved maternal nutrition, close antepartum surveillance, and advanced delivery planning in order to reduce morbidity and mortality among twin pregnancies in Botswana.

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Abbreviations

GA: Gestational Age
RR: Relative Risk
aRR: Adjusted Relative Risk
IQR: Inter-Quartile Range
LMIC: Low- and Middle-Income Countries
HIC: High-Income Countries
LBW: Low Birthweight
VLBW: Very Low Birthweight
HIV: Human Immunodeficiency Virus
MZ: monozygotic
DZ: dizygotic
MC: monochorionic
DC: dichorionic

Competing Interests

The authors declare that they have no competing interests.

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Data Availability

Per Botswana IRB regulations, de-identified data from the Tsepamo study can be requested from the Principal Investigator of the Study.

Authors' contributions

AI and RZ designed and performed the analysis and drafted the initial manuscript. RLS, JM, MM, RZ, DJ contributed to the design, data collection and study oversight of the parent study. GM, MD, JM, SD, AI oversaw data collection and data cleaning. All authors (AI, MD, GM, JM, SD, MM, JM, DJ, RL, RLS, RZ) contributed substantive feedback, revised and approved the final manuscript.

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Ethics Statement

Not Applicable

References

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1. Hanson C, Munjanja S, Binagwaho A, Vwalika B, Pembe AB, Jacinto E, et al. National policies and care provision in pregnancy and childbirth for twins in Eastern and Southern Africa: A mixed- methods multi-country study. PLoS Med. 2019;16(2).
2. Lawn J, Mongi P, Cousens S. Africa's newborns—counting them and making them count. In: Lawn J, Kerber K, editors. Opportunities for Africa's Newborns: Practical data, policy and programmatic support for newborn care in Africa. Cape Town: PMNCH; 2006. p. 11-22.
3. Bellizzi S, Sobel H, Betran AP, Temmerman M. Early neonatal mortality in twin pregnancy: Findings from 60 low-and middle-income countries. J Glob Health. 2018;8(1).
4. Peter C, Wenzlaff P, Kruempelmann J, Alzen G, Bueltmann E, Gruessner SE. Perinatal morbidity and early neonatal mortality in twin pregnancies. Open J Obstet Gynecol. 2013;3(1):78-89.
5. Hack KE, Derks JB, Elias SG, Franx A, Roos EJ, Voerman SK, et al. Increased perinatal mortality and morbidity in monochorionic versus dichorionic twin pregnancies: clinical implications of a large Dutch cohort study. BJOG. 2007;115(1):58-67.
6. Murray SR, Stock JS, Cowan S, Elizabeth SC, Norman JE. Spontaneous preterm birth prevention in multiple pregnancy. Obstet Gynaecol. 2018;20(1):57-63.
7. Martin JA, Hamilton BE, Osterman MJ, Driscoll AK. Births: Final Data for 2018. National Vital Statistics Report, vol 68, no 13. Hyattsville, MD: National Center for Health Statistics; 2019. 47 p.
8. Christensen K, Bjerregaard-Anderson M. Twin-singleton early-life survival in sub-Saharan Africa. Lancet Glob Health. 2017;5(7):e636-7.
9. Glinianaia SV, Obeysekera MA, Sturgiss S, Bell R. Stillbirth and neonatal mortality in monochorionic and dichorionic twins: a population-based study. Hum Reprod. 2011;26(9):2549-57.
10. Smits J, Monden C. Twinning across the Developing World. PLoS One. 2011;6(9).
11. Monden CW, Smits J. Mortality among twins and singletons in sub-Saharan Africa between 1995 and 2014: a pooled analysis of data from 90 Demographic and Health Surveys in 30 countries. Lancet Glob Health. 2017;5:e673-9.

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12. Justesen A, Kunst A. Postneonatal and child mortality among twins in Southern and Eastern Africa. Int J Epidemiol. 2000;29(4):678-83.
13. Miyahara R, Jasseh M, Mackenzie GA, Bottomley C, Hossain MJ, Greenwood BM, et al. The large contribution of twins to neonatal and post-neonatal mortality in The Gambia, a 5-year prospective study. BMC Pediatr. 2016;16.
14. Mogobe KD, Tshiamo W, Bowelo M. Monitoring Maternity Mortality in Botswana. Reprod Health Matters. 2007;15(30):163-71.
15. Madzimbamuto FD, Ray SC, Mogobe KD, Ramogola-Masire D, Phillips R, Haverkamp M, et al. A root-cause analysis of maternal deaths in Botswana: towards developing a culture of patient safety and quality improvement. BMC Pregnancy Childbirth. 2014;14.
16. Nkhwaluma L, Mashalla Y. Maternal mortality trends at the Princess Marina and Nyangabwe referral hospitals in Botswana. Afr Health Sci. 2019;19(2):1833-40.
17. Zash R, Jacobson DL, Diseko M, Mayondi G, Mmalane M, Essex M, et al. Comparative Safety of Antiretroviral Treatment Regimens in Pregnancy. JAMA Pediatr. 2017;171(10).
18. Zash R, Holmes L, Diseko M, Jacobson DL, Brummel S, Mayondi G, et al. Neural-Tube Defects and Antiretroviral Treatment Regimens in Botswana. New Engl J Med. 2019;381:827-40.
19. Zash R, Jacobson DL, Diseko M, Mayondi G, Mmalane M, Essex M, et al. Comparative safety of dolutegravir-based or efavirenz-based antiretroviral treatment started during pregnancy in Botswana: an observational study. Lancet Glob Health. 2018;6:e804-10.
20. Martin JA, Hamilton BE, Osterman MJ. Three Decades of Births in the United States, 1980-2009. Hyattsville, MD: National Center for Health Statistics; 2012. 8 p. Report No.: 80.
21. Martin JA, Osterman MJ. Is Twin Childbearing on the Decline? Twin Births in the United States, Hyattsville, MD: National Center for Health Statistics; 2019. 8 p. Report No.: 351.
22. Saleem S, Tikmani SS, McClure EM, Moore JL, Azam SI, Dhaded SM. Trends and determinants of stillbirth in developing countries: results from the Global Network's Population-Based Birth Registry. Reprod Health. 2018;15(Suppl 1).
23. Lawn JE, Blencowe H, Waiswa P, Amouzou A, Mathers C, Hogan D. Stillbirths: rates, risk factors, and acceleration towards 2030. Lancet. 2016;387:587-603.

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24. Akaba GO, Agida TE, Onafowokan O, Offiong RA, Adewole ND. Review of Twin Pregnancies in a Tertiary Hospital in Abuja, Nigeria. J Health Popul Nutr. 2013;31(2):272-7.
25. Page JM, Pilliod RA, Snowden JM, Caughey AB. The risk of stillbirth and infant death by each additional week of expectant management in twin pregnancies. Am J Obstet Gynecol. 2015;212(5):630.e1-7.
26. Ko HS, Choi SK, Wie JH, Park IY, Park YG, Shin JC. Optimal Timing of Delivery Based on the Risk of Stillbirth and Infant Death Associated with Each Additional Week of Expectant Management in Multiple Pregnancies: a National Cohort Study of Koreans. J Korean Med Sci. 2018;33(10).
27. Flenady V, Wojcieszek AM, Middleton P, Ellwood D, Erwich JJ, Coory M. Stillbirths: recall to action in high-income countries. Lancet. 2016;387:691-702.
28. MacDorman MF, Gregory EC. Fetal and Perinatal Mortality: United States, 2013. National Vital Statistics Report. 2015;64(8).
29. Committee on Practice Bulletins—Obstetrics; Society for Maternal-Fetal Medicine. Practice Bulletin No. 169: Multifetal Gestations: Twin, Triplet, and Higher-Order Multifetal Pregnancies. Obstet Gynecol. 2016;128(4):e131-46.
30. Dodd JM, Crowther CA, Haslam RR, Robinson SJ. Timing of birth for women with a twin pregnancy at term: a randomized controlled trial. BMC Pregnancy Childbirth. 2010;10.
31. Dodd JM, Crowther CA, Haslam RR, Robinson SJ. Elective birth at 37 weeks of gestation versus standard care for women with an uncomplicated twin pregnancy at term: the Twins Timing of Birth Randomised Trial. BJOG. 2012;119(8):964-74.
32. Wood S, Tang S, Ross S, Sauve R. Stillbirth in twins, exploring the optimal gestational age for delivery: a retrospective cohort study. BJOG. 2014;121(10):1284-93.
33. Cheong-See F, Schuit E, Arroyo-Manzano D, Khalil A, Barrett J, Joseph KS, et al. Prospective risk of stillbirth and neonatal complications in twin pregnancies: systematic review and meta-analysis. BMJ. 2016;354.
34. D'Antonio F, Odibo AO, Prefumo F, Khalil A, Buca D, Flacco ME, et al. Weight Discordance and Perinatal Mortality in Twin Pregnancy: Systemic Review and Meta-Analysis. Ultrasound Obstet Gynecol. 2017;52(1):11-23.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

35. Shi K, Li L, Huang X, Chen B, Zhou Y, Fang Q. Fetal Aneuploidy: A Comparison of Dichorionic Twins and Monochorionic Twins. Fetal Diagn Ther. 2018;44:124-8.
36. Audibert F, Gagnon A. No. 262-Prenatal Screening for and Diagnosis of Aneuploidy in Twin Pregnancies. J Obstet Gynaecol Can. 2017;93(9):e347-61.
37. Luke B. Nutrition in Multiple Gestations. Clin Perinatol. 2005;32:403-29.
38. Goodnight W, Newman R. Optimal Nutrition for Improved Twin Pregnancy Outcome. Obstet Gynecol. 2009;114(5):1121-34.
39. Pison G, D'Addato AV. Frequency of Twin Births in Developed Countries. Twin Res Hum Genet. 2006;9(2):250-9.
40. Hankins GV, Saade GR. Factors influencing twins and zygosity. Paediatr Perinat Epidemiol. 2005;19(Suppl 1):8-9.

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	5,
Study size	10	Explain how the study size was arrived at	4, 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	6
		(*e*) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	10
Outcome data	15*	Report numbers of outcome events or summary measures	10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8, 10

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		(*b*) Report category boundaries when continuous variables were categorized	5
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9, 10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13, 14
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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