

Original Article

A comparative study on productive, reproductive and ovarian features of repeat breeder and normal cyclic cows in the selected areas of Bangladesh

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• Received: May 15, 2018 • Revised: July 26, 2018 • Accepted: Aug 2, 2018 • Published Online: Aug 10, 2018



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ABSTRACT

Objective: The research was accomplished to appraise the productive and reproductive physiology of repeat breeder (RB) cows and compare to normal cyclic (NC) cows.

Methodology: A total of 366 RB cows were surveyed from 1859 crossbred and indigenous cows using a questionnaire upon positive sampling of RB syndrome in dairy farms throughout the selected areas of Bangladesh. Out of 366 RB cows, 170 were randomly selected, which were at day 0 of estrous cycle. To compare the physiological characteristics, 170 NC cows at day 0 of estrous cycle were also selected randomly.

Results: Results showed that age, BCS, and parity were higher in RB cows than NC cows. Survey on productive characteristics illustrated that milk production ($P<0.05$) and lactation length ($P<0.01$) was significantly higher in RB cows compared with NC cows. The study on reproductive features exposed that age at puberty and the first calving age was significantly ($P<0.01$) lower but the number of service, gestation length, calving interval, and voluntary waiting period were significantly ($P<0.01$) higher in RB cows than that of NC cows. Ovarian physiological characteristics revealed that estrus duration was significantly ($P<0.05$) lower in RB cows in comparison with NC cows. In addition, the largest follicular diameter was significantly ($P<0.01$) lower but vaginal electrical resistance (VER) was significantly ($P<0.01$) higher in RB cows compared with NC cows at day 0 of the estrous cycle.

Conclusion: Results indicate hurly-burly characteristics of ovarian physiology in RB cows. This study could help veterinarians and researchers to define various risk factors associated with RB cows.

KEYWORDS

Estrous cycle; Follicular diameter; Repeat breeder; VER

How to cite: Hasan MMI, Hassan MM, Mohanta RC, Miah MAH, Harun-Or-Rashid M, Juyena NS. A comparative study on productive, reproductive and ovarian features of repeat breeder and normal cyclic cows in the selected areas of Bangladesh. Journal of Advanced Veterinary and Animal Research. 2018; 5(3):324-331.

INTRODUCTION

The occurrences of many reproductive diseases and disorders in cows and goats have been reported in Bangladesh ([Shamsuddin et al., 2001](#)) and the reproductive performance of dairy cows directly influenced by repeat breeding ([Khair et al., 2013](#)). Repeat breeder (RB) is the cow that has returned to estrus subsequent to a third or more inseminations, is showing regular periods between estruses, has birth minimum once, is less than 10 years of age, has no indication of aberrations in the reproductive tracts identified by rectal palpation and has no anomalous genital ejections ([Royal et al., 2000](#)).

Prevalence of RB cows were 11.5% ([Asaduzzaman et al., 2016](#)) and 22.3% ([Hassan, 2017](#)) at different areas of Bangladesh, where 11.3% in commercial dairy farms in Chittagong ([Nath et al., 2014](#)). There are about 10.1% occurrence of repeat breeding found in Swedish dairy cows due to different causes including extrinsic and intrinsic issues allied with individual cows ([Gustafsson and Emanuelson, 2002](#)). Uterine infection, inheritance, ovulatory malfunctions, faulty heat detection and indecent timing of insemination predisposed the fertilization disappointment and premature embryonic loss in dairy cows those are the foremost reasons of repeat breeding ([Asaduzzaman et al., 2016](#)). Among the bredable cows, some of the cows forcedly inseminated at an erroneous time during artificial insemination ([Shamsuddin et al., 2001](#)) leading to amplify the occurrence of repeat breeding in Bangladesh ([Asaduzzaman et al., 2016](#)). Non-infectious causes of repeat breeding syndrome are bad management, chromosomal aberrations, hormonal imbalance, insufficient estrus recognition, unacceptable timing of service, offensive semen management, infertile bulls, poor nourishment, and warmth stress ([El-Khadrawy et al., 2011](#)). Many risk factors may affect the occurrence of RB in cows such as age, parity, BCS and milk yield ([Asaduzzaman et al., 2016](#)) physiologic status, milk yield, types of feeds, and peripartum events ([Nath et al., 2014](#)).

Fertilization rate might be reduced due to an oocyte problem ([Britt, 1994](#)). Follicular diameter (FD) positively improved the pregnancy rates ([Meneghetti et al., 2009](#); [SáFilho et al., 2009](#)). Likewise, most favorable size of ovulatory follicles is still a chief apprehension in beef ([Busch et al., 2008](#)) and dairy farming ([Vasconcelos et al., 2013](#); [Bisinotto et al., 2014](#)). [Perry et al. \(2005\)](#) observed that a higher ovulatory follicular diameter (OFD) significantly increases the conception rate. Prediction of the ovarian condition has been studied in cows and in

other females by vaginal electrical resistance (VER) without any illustration of estrus exposure ([Schams et al., 1977](#)), and the VER value gradually decreases through the estrous cycle, and finally the value is lower at day 0 of the estrous cycle.

The objectives of the present study was to investigate the impending variation in different productive (milk production and lactation length), reproductive (age at puberty and calving, the number of service on the last conception, gestation length of last pregnancy, voluntary waiting period after the last parturition, and calving interval), and ovarian physiological features (estrus duration, FD, and VER at day 0 of estrous cycle) in RB and normal cyclic cows to find out the potential causes and risk factor(s) of RB syndrome in dairy cows in the chosen areas of Bangladesh.

MATERIALS AND METHODS

Study area, duration and ethical approval: The dairy cows were comprised from Gozaria and Srinagar of Munshiganj; Shahzadpur of Sirajgonj; Karnaphuli of Chittagong; and different areas of Mymensingh and Savar Military Farm, Bangladesh. The study was conducted during the period from January 2016 to December 2017. This study was approved by Animal Welfare and Ethical Committee, Bangladesh Agricultural University, Mymensingh (No. 05/AWEC/2018).

Design of questionnaire: To obtain farm- and cow-level variables, a well-stipulated questionnaire was assembled. The questionnaire was designed accompanied with various parameters such as breed, age, body weight, body condition score (BCS), milk production, lactation length, age at puberty, first calving age, number of service per conception, gestation length, calving interval, and voluntary waiting period (VWP).

Selection of farms, animals and data collection: The desired dairy cows in the study were selected from smallholding and commercial dairy farms throughout the mentioned study areas. Farms having ≥ 5 dairy cows including at least one (1) RB cows having ≥ 1 parities with normal post partum and regular estrous cycle were considered for the study. A total of 1859 crossbred and indigenous dairy cows were surveyed from the 188 preferred farms throughout the study areas and 366 RB cows were selected. Out of 366 RB cows, 170 were randomly selected which were in standing heat at day 0 of the estrous cycle. To compare physiological characteristics, 170 NC cows with the standing heat of the first estrous cycle after the last parturition were also selected randomly from the surveyed cows. Out of 170 cows, 135 Holstein-Frisian crossbred, 15 Jersey

crossbred, 10 Shahiwal crossbred, and 10 indigenous cows were selected in each group (RB and NC cows). Data were collected from the desired farms and the cow's level projected variables using the stipulated questionnaire. Randomly selected RB and NC cows were also used for the measurement of FD and VER.

Determination of body condition score (BCS):

Nutritional status in the selected cows were scored into the following grades using the lumbar vertebral process (LVP) as land mark as described by [Rahman \(1996\)](#), such as, Grade 1: cows had so much muscle that LVP not only invisible but also difficult to palpate, Grade 2: LVP not visible but easily palpable, Grade 3: only the head of LVP visible from close distance and easily palpable, and Grade 4: LVP clearly visible even from long distance with rough hair coat and thin back muscle.

Measurement of the largest ovarian FD:

Examination of ovaries and FD was measured in the selected cows at day 0 of the estrous cycle by transrectal ultrasonography using Ultrasound Machine (Bionet® MU1V, Korea) equipped with a 7.5-MHz linear probe. The operational procedure of the ultrasound machine was executed as described by [Islam \(2012\)](#) and the measurement of FD was done by the inherent measuring calipers of the ultrasound machine. It was repeated for three times and the average diameter of follicle was recorded for further analysis in the present study. The measured diameter of the largest follicle symbolically presented in **Figure 1**.

Recording of VER:

VER values were recorded in the selected cows at day 0 of the estrous cycle by the use of electronic heat detector (DRAMINSKI® Owocowa 17, Poland), and the procedure was completed as expressed by [Malakar \(2014\)](#). Estimation of VER symbolically presented in **Figure 2**.

Statistical analysis:

Data from the questionnaires and measurement values of FD and VER were entered into Microsoft Excel-2007 work sheet and analyzed by SPSS software version 20 using descriptive statistics. Continuous data from the ovarian, reproductive, and productive physiological characteristics of RB and NC cows were analyzed by ANOVA.

RESULTS AND DISCUSSION

General survey throughout the randomly selected cows:

Results in the general survey throughout the selected RB cows (n=170) and NC cows (n=170) are presented in

Table 1, which revealed that age and BCS were significantly ($P<0.01$) higher in RB cows than that of NC cows but the parity number was showed insignificantly ($P>0.05$) higher in RB cows compare with NC cows.

The present findings of higher age in RB cows comparison with NC cows, resembles to the findings of [Gustafsson and Emanuelson \(2002\)](#), [Nath et al. \(2014\)](#) and [Asaduzzaman et al. \(2016\)](#). Similar to the present study, [Shamsuddin et al. \(2001\)](#) reported that poor body condition decreases the conception rate



Figure 1. Largest Ovarian Follicle



Figure 2. Estimation of VER

Table 1. General survey on the selected cows

Survey parameters	RB cows (n=170)	NC cows (n=170)
Age (months)	91.65±26.19 ^a	78.69±17.71 ^a
BCS (On the basis of LVP)	2.94±1.21 ^a	2.14±0.92 ^a
Parity number	4.37±1.67 ^c	4.24±1.49 ^c

^a values with superscript indicates 1% level of significant within the rows, where ($P<0.01$). ^c values with superscript indicates insignificance within the rows, where ($P>0.05$).

Table 2. Productive characteristics of RB cows (RBCs) and NC cows (NCCs)

Parameters (Mean±SD)	Holstein-Frisian crossbred cows		Jersey crossbred cows		Shahiwal crossbred cows		Indigenous cows	
	RBCs (n=135)	NCCs (n=135)	RBCs (n=15)	NCCs (n=15)	RBCs (n=10)	NCCs (n=10)	RBCs (n=10)	NCCs (n=10)
Average milk production in last lactation (Litre/Day)	22.22 ±5.54 ^a	18.22 ±3.58 ^a	14.13 ±2.70 ^b	12.07 ±1.67 ^b	10.10 ±1.45 ^b	8.70 ±1.16 ^b	2.75 ±0.72 ^b	2.05 ±0.50 ^b
Last lactation length (Days)	343.54 ±14.51 ^a	297.15 ±13.47 ^a	338.33 ±15.89 ^a	292.67 ±11.00 ^a	325.00 ±32.06 ^a	275.50 ±11.66 ^a	310.50 ±28.72 ^a	240.50 ±6.86 ^a

^a values with superscript indicates 1% level of significance within the rows and breeds, where ($P<0.01$). ^b values with superscript indicates 5% level of significance within the rows and breeds, where ($P<0.05$).

Table 3. Reproductive characteristics of RBCs and NCCs

Parameters (Mean±SD)	Holstein-Frisian crossbred cows		Jersey crossbred cows		Shahiwal crossbred cows		Indigenous cows	
	RBCs (n=135)	NCCs (n=135)	RBCs (n=15)	NCCs (n=15)	RBCs (n=10)	NCCs (n=10)	RBCs (n=10)	NCCs (n=10)
Age at puberty (months)	22.04 ±4.98 ^a	25.79 ±1.34 ^a	26.47 ±2.59 ^a	29.20 ±0.86 ^a	27.00 ±3.23 ^a	30.90 ±1.10 ^a	30.00 ±5.66 ^b	35.10 ±1.10 ^b
Age at first calving (months)	31.61 ±4.96 ^a	35.18 ±1.37 ^a	36.33 ±2.72 ^a	38.67 ±1.11 ^a	36.85 ±3.02 ^a	40.10 ±1.20 ^a	39.50 ±5.66 ^b	44.20 ±1.32 ^b
No. of service on last conception	3.44 ±1.50 ^a	1.62 ±0.49 ^a	3.40 ±1.06 ^a	1.53 ±0.52 ^a	2.90 ±0.88 ^a	1.40 ±0.52 ^a	2.80 ±1.40 ^a	1.20 ±0.42 ^a
Gestation length on last pregnancy (Days)	284.46 ±9.39 ^a	269.19 ±7.24 ^a	279.87 ±7.75 ^a	268.67 ±5.77 ^a	278.10 ±9.28 ^c	272.50 ±6.95 ^c	280.40 ±6.47 ^a	273.00 ±5.79 ^a
Calving interval between last two pregnancy (Days)	463.85 ±38.05 ^a	365.00 ±15.25 ^a	456.33 ±22.40 ^a	361.33 ±37.96 ^a	433.50 ±29.26 ^a	373.00 ±12.52 ^a	458.00 ±25.63 ^a	375.00 ±17.16 ^a
Voluntary waiting period after last parturition (Days)	107.22 ±19.50 ^a	61.39 ±10.66 ^a	104.40 ±12.84 ^a	67.13 ±10.52 ^a	94.50 ±15.46 ^a	71.10 ±9.69 ^a	118.80 ±11.41 ^a	76.60 ±6.77 ^a

^a values with superscript indicates 1% level of significance within the rows and breeds, where ($P<0.01$). ^b values with superscript indicates 5% level of significance within the rows and breeds, where ($P<0.05$). ^c values with superscript indicates insignificant within the rows and breeds, where ($P>0.05$).

Table 4. Ovarian physiological characteristics of RBCs and NCCs at day 0 of the estrus cycle

Parameters (Mean±SD)	Holstein-Frisian crossbred cows		Jersey crossbred cows		Shahiwal crossbred cows		Indigenous cows	
	RBCs (n=135)	NCCs (n=135)	RBCs (n=15)	NCCs (n=15)	RBCs (n=10)	NCCs (n=10)	RBCs (n=10)	NCCs (n=10)
Estrus duration (hours)	11.25 ±3.92 ^a	14.97 ±3.06 ^a	11.33 ±4.76 ^b	15.27 ±3.08 ^b	11.50 ±4.79 ^b	15.40 ±2.46 ^b	11.70 ±5.87 ^b	16.10 ±2.33 ^b
Largest follicular diameter (mm)	9.62 ±2.37 ^a	13.79 ±2.14 ^a	9.73 ±1.70 ^a	13.88 ±1.67 ^a	9.77 ±3.56 ^a	13.90 ±1.52 ^a	9.93 ±2.21 ^a	14.09 ±1.51 ^a
Vaginal electrical resistance (Ω)	264.74 ±41.332 ^a	211.28 ±28.61 ^a	256.67 ±26.58 ^a	208.22 ±21.56 ^a	256.00 ±59.46 ^b	207.67 ±17.85 ^b	240.67 ±33.33 ^a	197.00 ±20.76 ^a

^a values with superscript indicates 1% level of significance within the rows and breeds, where ($P<0.01$). ^b values with superscript indicates 5% level of significance within the rows and breeds, where ($P<0.05$).

of the dairy cattle. Effects of higher number of parity on repeat breeding syndrome were negative in the present study. It is very conceivable that as a cow gets older and parity increases, the uterine tissue becomes senile and the organ becomes more prone to infections and ultimately increasing the low conception rate in dairy cows ([Hassan, 2017](#)). Correspondingly, [Bonnevillie-Hébert et al. \(2011\)](#) reported that the RB syndrome increases if the cow has a peripartum reproductive problem and also increases with parity.

Productive characteristics of the selected cows:

The numerical analysis of the productive characteristics of the selected cows is presented in **Table 2**. Results

showed that average milk production was significantly ($P<0.05$) higher in RB cows of all breeds except Frisian crossbred cows, where its significant level was $P<0.01$, but the lactation length was significantly ($P<0.01$) higher in RB cows of all breeds in comparison to NC cows.

Elevated milk production and higher lactation length in RB cows compare with NC cows clearly indicate that the occurrence of repeat breeding syndrome (RBS) was higher in cows having higher milk production and larger lactation length, which resembles with the findings of [Hassan \(2017\)](#). Similarly, the number of services per conception increases in high-yielding cows due to changes in reproductive physiology ([Royal et al., 2000](#)).

Furthermore, it was also reported that an increasing milk production and higher lactation length are found to be risk factors for being an RB ([Gustafsson and Emanuelson, 2002](#)) and RBS increases in cows due to higher milk yield in Bangladesh ([Asaduzzaman et al., 2016](#)). On the other hand, milk production and lactation length of NC cows in the present findings were relative with the results of a study completed by [Hauque et al. \(2011\)](#).

Reproductive characteristics of the selected cows:

Survey results in the reproductive characteristics of the selected cows are presented in **Table 3**. Results exposed that age at puberty and first calving age were significantly ($P<0.01$) lower in all crossbred RB cows except indigenous RB cows, where its significant level was $P<0.05$. Results also uncovered that the number of service (AI) on the last conception, gestation length on the last pregnancy, calving interval between the last two pregnancies, and voluntary waiting period (VWP) after the last parturition were significantly ($P<0.01$) higher in all RB cows in comparison to NC cows, except the gestation length on the last pregnancy in Shahiwal crossbred cows, where it was insignificantly ($P>0.05$) higher in RB cows than NC cows.

Early puberty and early first calving increase the lifetime productivity in cows ([Lesmeister et al., 1973](#)) to be an economic beneficiary fact of dairy farming. However, there is a natural reality that all the animals including cattle attain their pubertal age and subsequently the first calving at the optimum physiological growth of the body. According to the report of [Hauque et al. \(2011\)](#), the age of puberty and first calving age are attained normally in the dairy cows in Bangladesh, which relatively resembles with the findings of the present study in normal cyclic cows, and this report coincides with the results in the research of [Ashraf \(1998\)](#). Even though the current research revealed that age at puberty and the first delivery age in RB cows were lower than the normal cyclic cows, which may be a fact in the repeat breeding syndrome, the cause is not clear due to the limited reference. [Gupta et al. \(2016\)](#) report the many factors, which have a direct or indirect effect on growth, puberty, and sexual maturity in animals such as species, genetic potentiality, the plane of nourishment, growth, body weight, functions of different hormones, and health and other management circumstances. Here, it is necessary to study the facts of the correlation between early puberty consequently the early first calving and the incidence of RB syndrome in dairy cows.

Significantly, a higher number of inseminations on the last conception in RB cows than NC cows indicate that the conception rate of the RB cows was low, which may be due to the faulty AI or any other factors of RB syndrome. Here, it is possible that the cow gets an injury in the cervix and/or the uterus and the uterine tissue becomes favorable to infections, leading to endometritis by repeated services (AI) on the last conception in cows. Similarly, [Singh et al. \(1996\)](#) reported that intrauterine infectious agents interfere the implantation of fertilized ova due to creating a mark changes in pH of the intrauterine environment and inflammation in the endometrium and denudation of the uterine lining. Unbalanced intrauterine atmosphere and endometritis is one of the significant causes of repeat breeding syndrome in dairy cows ([Ahmadi and Dehghan, 2007](#)). However, within the NC cows, the number of services in conception was relatively similar in all breeds in the study, which is comparatively alike with the findings of [Hauque et al. \(2011\)](#).

Due to the list reference, the present findings in the correlation between the higher gestation length and the incidence of RBS in dairy cows is still obscure. However, [Jenkins et al. \(2016\)](#) reported that animals born following long gestation lengths decrease the fertility.

A remarkable difference of voluntary waiting period (VWP) in between RB and NC cows indicates a fact in the occurrence of RBS. In early lactation, most of the dairy cows produce a higher volume of milk than the later stage. Results in **Table 2** showed that the higher volume of milk production is a significant causal factor of RB syndrome in dairy cows, resulting in a comprehensible positive correlation among the higher VWP and milk production on RB syndrome. Feasible delaying effects upon the post-partum gap to ovulation and consequently estrus and conception are influenced by several physiological aspects of high lactation in the high-yielding dairy cows ([Edgerton, 1980](#)). Moreover, similar to the present study, [Burke et al. \(1995\)](#) reported that in a prolonged negative energy balance (NEB) in the dairy cows, the energy crisis relative to milk yield extends the period of post-partum anestrus.

In the present findings, the calving interval was higher in the RB cows compare with normal cows, which is a normal fact in relation to the cumulative measuring of the duration of a higher number of services, higher gestation length, and higher VWP in the repeat breeders.

Ovarian physiological characteristics of the selected cows:

Fallout in the study on ovarian characteristics of the selected cows at day 0 of the estrous cycle is presented in **Table 4**. Results showed that estrus duration was significantly ($P<0.05$) lower in all RB cows than NC cows except Frisian crossbred cows, where its significant level was $P<0.01$. Results also illustrated that the largest follicular diameter was significantly ($P<0.01$) lower in all RB cows than NC cows at day 0, but the VER was significantly ($P<0.01$) higher in RB cows in comparison with NC cows of all breeds, except Shahiwal crossbred cows, which showed 5% level of significant ($P<0.05$).

Lower estrus duration in RB cows compares with NC cows undoubtedly specify that the occurrence of RBS was higher in the cows with lower estrus duration. However, the estrus duration in both the RB and NC cows were also lower in high yielding than in low-yielding cows, which coincides with the report of [Lopez et al. \(2004\)](#). Ovulatory FD influences the intensity of estrus expression ([Ferraz et al., 2017](#)) and the superior size of ovulatory follicles at the time of insemination associate with an elevated concentration of estrogen in the circulation that influences the higher expression of estrus ([SáFilho et al., 2009](#)). The previous study showed that heat detection accuracy is 30% in Bangladesh ([Shamsuddin et al., 2001](#)) and this accuracy is very low, which may be due to the shorter estrus duration and/or lower expression of estrus, inability of farmers to detect heat, lack of modern tools for heat detection, and silent heat in cows. Inadequate estrus detection ([El-Khadrawy et al., 2011](#)) and improper timing of insemination ([Shamsuddin et al., 2001](#); [El-Khadrawy et al., 2011](#)) manifest the higher number of repeat breeder in dairy cows. Similarly, [Ferraz et al. \(2017\)](#) also reported that the high intensity of estrus expression increases the conception rate in comparison to the low intensity of estrus expression. Moreover, there is a positive association between the conception and estrus expression ([Roelofs et al., 2010](#)).

The inferior diameter of the largest follicles at estrus in RB cows than in NC cows evidently point toward the lower FD increases the occurrence of RBS, which resembles the findings of [de Tarso et al. \(2016\)](#). In a research article, [de Tarso et al. \(2016\)](#) reported that a larger ovulatory follicle is essential for pregnancy in cows, but smaller ovulatory follicle ($<11\text{mm}$) decreases the pregnancy rate and increases the embryonic or fetal mortality.

Normally, the VER value is a bio-physiological fact in females, and [Tadesse et al. \(2011\)](#) have stated that the VER acceptably used to evaluate the ovarian conditions and the period of estrous cycle. It is published that through the estrus, the estrogen level is higher, which causes the NaCl level to be high, resulting in a significant lower level of electrical resistance ([Fehring, 1996](#)). From the above statements, the present research outcome may be hypothesized that the synergistic interlink between the higher values of VER and lower estrus expression and/or lower duration at estrus in all breeds of RB cows in comparison to NC cows. Furthermore, in the present study, the estimated VER values during the standing heat of the estrous cycle of NC cows were relatively greater than the previous findings of [Tadesse et al. \(2011\)](#). This difference may be due to the intravaginal probe design and the intensity of practice of the application of VER machine.

CONCLUSION

In conclusion, the research work demonstrates that RBS in dairy cows is a multifactorial crisis involving the partum, peri-partum, and post-partum factors. Age, BCS, parity number, milk production, lactation length, age of puberty and calving age, number of services, gestation length, and VWP may influence the occurrence of RBS. Lower estrus duration and lower FD subsequently higher VER values in RB cows comparison to NC cows at day 0 of the estrous cycle indicate the turbulence of ovarian synchrony and estrus, resulting in increased incidence of RBS. Results suggest that the disturbed ovarian synchrony in RB cows should be considered to make up a proper treatment regimen for RB syndrome as well as to improve the fertility of RB cows.

ACKNOWLEDGEMENT

The authors express their deepest sense of gratitude and appreciation to project TF 24 - EM/15 funded by Krishi Gobeshona Foundation (KGF), BKGET, BARC, Bangladesh for generous the opportunity and by providing all kinds of facilities required to conduct this research work smoothly.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

NSJ and MMIH designed the study, interpreted the data and drafted the manuscript. MMIH, MMH, RCM and

MAHM were involved in the collection of data and measurement of FD and VER. NSJ, MMIH, and MHR acquire a part in critical checking of this manuscript.

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