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## Ovarian physiology of repeat breeder cows at Bathan area of Bangladesh

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### Abstract

The experiment was implemented at farmer's house and Bathan areas of Shahjadpur Upazila in Sirajganj and Bera Upazila under Pabna districts of Bangladesh. The aim of the study was to know the incidence of repeat breeding (RB), physiology of different follicles and deformity of ovary and uterus in Holstein Frisian (HF) cows. Forty (40) RB HF cows (>50% HF Genetic) were selected on the basis of age and parity. Pyometra, endo-metra, cervicitis, Salpingitis and tumor like growth etc. in uterine wall were observed. Luteal and follicular cyst was also identified. Number of growing follicle appeared ultrasonically, size of Graafian follicle, breed, cow's body size, and inheritance Torrele appeared unrasonicarly, size of Graanan Fonce, breed, cow's body size, and innernance level and parity, nutritional factors and days calving to first service were recorded properly. All collected data were statistically analyzed by the SPSS soft-ware program using completely randomized design (CRD). Results revealed that, significantly (P<0.05) highest Body Condition Score (BCS) was  $3.47\pm0.05$  observed in the cows of group-D whose age were more than 8 years of old compared to others group (table 1). In-case of average daily milk yield and remained dry condition differences were highly significant (P<0.001). Maximum milk yield (20.41\pm0.82 l/day) was observed in group C appared to others group but minimum dry condition remaining period was observed in group-C compared to others group but minimum dry condition remaining period was observed in group-A than others. Days of heat showed number of AI service were non-significant (P>0.05) in the whole experiment (table-1). The percentage of infection (Pyometra, Endometra) in the uterine wall differed non-significantly (P>0.05) among all the groups and minimum occurrence were observed in group-A (figure 1). Highest percent of cyst observed in the right ovary than left of the cows of group-D compared to others group but the differences were non-significant (P>0.05).Comparatively higher percentage (58.82±12.30) of follicles observed in left ovary of group-D than right ovary of others group (table 3). Non-significantly (P>0.05) the highest average number  $(2.40\pm0.44)$  of follicles was observed in left ovary of the group-C than the cows of others group. Though the follicle size in the end of luteal phase differed non-significantly (P>0.05) but comparatively larger ( $14.90\pm1.54$ mm) follicle in diameter were observed in group-C, than the follicles of others groups.

Key words: RB, OFC, Uterine Infection, Diameter, Follicle

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## Introduction

Baghabari is one of the most economically

important place for milk production and it meets up two-third demand for milk of the

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whole country (Mohammed et al., 2017). The farmers of these areas rear about 98% crossbred cows and most of the cows are Holstein Friesian (HF) x Local crossbred. Their conception rate is 50-60% and require 1-3 insemination for a conception (Mohammed et al., 2017). In most cases, repeat breeding in cows are responsible for poor conception rate. About 29 percent dairy cattle in the Baghabari milk shed areas are repeat breeder (Mohammed et al., 2017). Khair et al. (2013) stated that Repeat Breeding (RB) directly influences the reproductive performance of dairy cows. The prevalence of RB in cows varied in different places in Bangladesh like 11.5% (Asaduzzaman et al., 2016) and 22.3% (Hasan et al., 2017) and 29% in Baghabari Milk shed areas of Bangladesh (MS Islam et al. 2018). In Swedish dairy farm occurrence of RB is about 10% (Gastafsson and Emanuelson et al. 2002). . RB is a multi factorial problems and increased production cost(Tk. 11051 /farm/year) in dairy farm through increasing insemination, treatment, feed, labour and management cost (Ali et al., 2013), increase calving interval, culling rates and decreased calf and milk production (Lafi et al., 1992). Variation of the hormonal constituents of the animal body has been reported for the reproductive failure (Adler et al., 2009, Lequin. 2005). Uterine infection, inheritance and hormonal imbalance may lead to RB(Asaduzzaman et al., 2016). Many scientists observed others risk factors of RB like age, parity, BCS, milk yield(Asaduzzaman 2016, Islam et al., 2012), physiological status ,milk yield, feed (Nath et al., 2014). Pesánteza et al. (2016) reported that ovarian follicular cysts (OFC) in dairy cattle cause economic losses as a result of extension of the open period, cost of treatment, and risk of elimination of cows

from the herd. OFC incidence risen with increasing milk production and in these conditions, between 15 to 30% of cows develop this ovarian condition within 60 days postpartum (Peter, 2004). Cystic ovarian disease (COD) is responsible for frequent reproductive failure in cattle, resulting in significant economic losses for the dairy industry and COD incidence within dairy farms is variable, ranging between 5 and 25%, which is most likely due to different management strategies (Silviu Ionut BORS et al., 2018). This disease is characterized by the failure of a mature follicle to ovulate at the appropriate time during the estrous cycle (Ortega, et al., 2015). Conception rate in cows might be fluctuated due to the O-cyte problems (Britt, 1994). Follicular diameter at specific stage of estrus cycle generally plays a vital role in the fertilization/conception rate (Meneghetti et al., 2009). Busch et al. (2008) stated that most favorable size of ovulatory follicles is still a chief apprehension in beef and dairy farming (Vas concelos et al., 2013, Bisinotto et al., 2014). The higher follicular diameter significantly increases the pregnancy /conception rate (Perry et al., 2005). Although global scientific literature offers outstanding reviews and original articles about RB and OFC, to our knowledge, there are very little scientific studies describing their clinical presentation in a herd. That's why this study was undertaken with the objective to identify the uterine infections, ovarian physiological features of Holstein Friesian RB cows in the selected areas.

#### **Materials and Methods**

#### Location of the experiment

The experiment was implemented at farmer's

house and Bathan areas of Shahjadpur and Bera under Sirajganj and Pabna district of Bangladesh. The areas located at the northern part of the country under Sirajganj (23°41′ N-90°20′ E) and Pabna (24°00′ N-89°10′ E) district of Bangladesh (which known as milk pocket area of Bangladesh). About two-third of cattle reared and most of them are crossbred of Holstein Friesian (HF) with indigenous cattle.

#### **Grouping of animals**

There were forty (n=40) Holstein-Freisian (>50% Blood) repeat breeding (RB) Cows of 02 to 08 years-old were randomly selected for this study. A herd comprising 10 cows at farmer's house and another 30 cows at Bathan areas were maintained under this study. They were divided in to four groups on the basis of age like A (2-4 years), B (5-7 years), C (7-8 years) and D (>8 years). All RBC were marked by ear tag and maintained breeding record by Cattle Herd Book. These animals had an average number of service required for conception were 5.79 to 8.67 no per conception.

# Measurement of body condition score (BCS)

The body condition score (BCS) implied the nutritional and health status of the selected cows and were scored considering the following grades using the lumber vertebral process (LVP) as described by Rahman (*et al.*1996), like 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 (Hasan *et al*, 2018).

# Measurement of the ovarian follicular diameter

The examination of ovaries and follicular diameter (FD) were measured in the selected cows initially by rectal palpation. Finally at days 10 of the estrous cycle the ovarian follicles were visualized by ultrasonography machine (Dus 60 Vet, Edan, USA). The feces were removed from the rectum. cleaned and the surrounding areas of vulva and rectum were swabbed with disinfectant to prevent infection in the reproductive tract. The ovary was grasped by inserting left hand through the rectum and the follicles were visualized by inserting a sectorial probe through the vagina. Before inserting the probe, it was disinfected by swabbing with 70% alcohol. Follicle numbers were recorded by counting on the screen of ultrasonography machine. The diameters of follicles were recorded by measuring length and breadth of each follicle on the screen (Figure 2). All visible follicles (>2.0 mm) were counted and recorded. The follicles were measured 3 times at a 3-day interval period without considering the stage of the reproductive cycle of the experimental animals (Deb et al. (2018).

#### Data recording

Oestrus detection was performed by monitoring the daily oestrus behaviour of the selected animals, 3 times each day for 30 minutes. After selecting the RB cows, infection Pyometra. like Endometra. Salpingitis and Tumor like Cervicitis, growth among others in uterine wall were identified with the help of modern veterinary approaches (Ultrasound machine). The status of ovarian follicles was observed at 10th day of estrous cycle. Finally, the ovaries were examined for

presence or absence of Luteal and follicular cysts. Ultrasound scan with a 5 MHz, linear-array, vaginal transducer was used, obtaining static images. The ovulatory follicles were studied on day 0 – the first day of study (n = 18) and until those formed during the following cycle (n = 22). Follicle size. location right ovary/left ovary (RO/LO), and number of follicles per ovary were studied in all studied cows. The following parameters were evaluated: the examination day, day of maximum size, period of follicular growth and follicular atresia and atresia rate. Moreover, cows body size, and inheritance level and parity, nutritional factors, heat stress (record temperature and Relative Humidity at the time of estrus), days calving to first service and different reproductive diseases were recorded properly.

#### Statistical analysis

Data from the measurement values of FD, uterine infection and cysts 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 cows were analyzed following principle of ANOVA.

#### **Results and Discussion**

#### Productive physiology of RB cows

The productive characteristics of the selected cows are presented in Table 1. Results showed that average milk production was higher (P<0.001) in group C  $(20.41\pm0.82)$ , than all the others groups (A, B and D) and lower (15.27±0.76) in group B compared to others groups. Present findings resembled with the findings of Hasan et al. (2018). Similarly Hasan et al., (2017) stated that higher repeat breeding syndrome(RBS) was found in cows having higher milk production and lactation period. However, 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). Table 1 results also showed that significantly (P<0.01) BCS observed in group higher D  $(3.47\pm0.05)$  compared to others groups (Hasan et al., (2018) but did not found positive relationship with repeat breeding in cows.

Table 1: F	Phenotypic para	ameter of repeat	breeder cows
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Parameter	Group (Mean±SE)				Р	Level of
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	A(2-4	B(2-/	C(7-8	D(>8	value	significance
	years)	years)	years)	years)		
BCS(0-5)	2.94±0.17	3.00±0.14	3.22±0.06	3.47±0.05	0.01	*
Milk yield(liter/day)	18.15±1.52	15.27±0.76	20.41±0.82	20.06±0.94	0.00	**
Dry period (month)	20.66±1.36	31.07±1.32	30.27±1.12	30.23±1.98	0.00	**
Interval between	31.50±6.91	47.56±7.71	44.90±4.11	41.47±9.28	0.71	NS
two estruses (days)						
Service given (No)	8.67±1.95	$6.74 \pm 0.95$	$5.79 \pm 0.62$	8.18±2.34	0.37	NS

#### **Uterine Infection**

There were no statistical variation of infection in the uterine wall with minimum  $(22.22\pm14.69\%)$  in group A and maximum (29.41±11.39%) in group D (Figure 1). The animals of group A, having 2 to 4 years of age which is smaller than that of others groups whose age >4 years of old. 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 et al., 2017). Correspondingly, Bonneville-Hébert et al. (2011) reported that the RB syndrome increases if the cow has per- partum reproductive problem and also increases with parity (Hassan et al., 2018). So it is clear that with the increases of age there will be increased the incidence rate of RB syndrome in cows.

The results of our experiment in table 2 reviled the percentage of Ovarian Follicular Cyst (OFC) through Ultrasonography in both ovaries. There was no statistical variation of cysts percentage among the groups (table-2). Whole the experiments the percentage differences of OFC among the groups were non-significant (P>0.05). JL Pesánteza et al., 2016 observed that the incidence rate of follicular Cyst 23% in Swedish dairy farm, which slightly more than our findings  $(11.11\pm5.11)$  of group -A, but smaller than Group- D  $(70.59 \pm 11.39)$ . Our observed values, consistent with other observations in comparatively better conditions (Melendez et al., 2003, Vanholder et al., 2006), contrasts with the findings (1 to 16%)1980s, (Kesler and recorded in the Garverick 1982, Bartlett et al., 1986) which were more similar. The differences in the incidence of Cystic Ovarian Follicle (COF)



Figure 1: Percentage of uterine infection incidence in RB cows

#### **Ovarian Follicular Cyst**

Different researchers stated the appearance of OFC in different ways among them Kesler *et al.* 1981 described OFC as may be observed as thin-walled, fluid filled structures on one or both ovaries and be associated with high plasma estradiol but Farin and colleagues (1992) described the appearance of follicular cysts on ultrasound screens as "an anechoic antrum and a smooth, thin wall with few or no gray patches. may due to the parity number because Group A contained the cows whose age ranged from 2 to 4 years where as Group D possessed more than 8 years old. Short and irregular estrus and age were regarded as the major clinical signs of cows with OFC; in this study, however, cows diagnosed with OFC were submitted to reproductive examination due to absence of estrus or failure to return to estrus after insemination.

Parameter	Group(Mean±SE)				Р	Level of
	А	В	С	D	Value	significance
	(2-4 years)	(5-7 years)	(7-8 years)	(>8 years)		
Ovarian	66.67±16.66	48.15±9.79	48.84±7.71	70.59±11.39	0.35	NS
follicular cyst in						
right ovary (%)						
Ovarian	11.11±5.11	14.81±6.96	23.26±6.51	17.65±9.53	0.76	NS
follicular cyst in left ovary (%)						

Table 2: Cysts presented (%) of repeat breeder cows

NS= Non significant

#### **Evaluation of follicles**

The results obtained from Ultrasonography (EDAN Dus-60) examination of RB Cows presented comparatively higher average percentage of follicles( $58.82 \pm 12.30$ ) in the left ovary of group-D than the right ovary of the animals of others groups (A, B and C) in the whole duration of the experiment (Table 3). The highest average number  $(2.40\pm0.44)$ of follicles were observed in left ovary of the cows of group-C than others group but the differences were non-significant (P>0.05). The follicle size at 10th days of estrus cycle differed non-significantly (P>0.05) but comparatively larger (14.90±1.54) follicle in diameter was observed in group-C, than the follicles of the cows of others groups.

During our study we considered large, medium and small follicles in the ovary under Ultrasonography examination a few of them represented large follicles which was taken under our main consideration. The average numbers of follicles according to their size and ovarian positions were given in table 3. Numbers of small follicles per ovary in our experimental cows were more or less similar to GK dab et al (2018). In general majority of the follicles were small to medium in cows (GK dab et al, 2018). On the contrary Hasan et al 2018 observed in RB Holstein Friesian Cows the largest follicles size  $9.62 \pm 2.37$  and in normal cows was  $13.79 \pm 2.14$ . The largest follicles found in our experiment in RB cows slightly larger than found Hasan et al 2018. The number of follicles in an ovary did not vary (P>0.05) between right and left ovary of RBC. In general the size of follicle depends on many factors and hormonal status as well as stage of estrus cycle (Figure 2). Follicle numbers and sizes continuously and considerably oscillate during the cycle due to wave-like development pattern in cattle (Adams et al., 1992).

Parameter	Group(Mean±SE)				Р	Level of
	А	В	С	D	Value	significance
	(2-4 years)	(5-7 years)	(7-8 years)	(>8 years)		
Measurable	33.33±16.66	25.93±8.59	37.21±7.45	11.76±8.05	0.26	NS
follicles in right						
ovary (%)						
Measurable	44.44±17.56	29.63±8.95	37.21±7.45	58.82±12.30	0.27	NS
follicles in left ovary (%)						
Follicles in right ovary (no)	2.00±0.80	2.00±0.52	2.40±0.44	1.33±0.80	0.31	NS
Follicles in left ovary (no)	1.67±0.70	0.85±0.46	0.90±0.38	1.00±0.70	0.38	NS
Largest follicular diameter (mm)	13.50±1.04	10.50±0.53	14.90±1.54	10.60±0.67	0.17	NS

Table 3: Physiological parameter of the reproductive tract and ovaries of repeat breeder cows

NS= Non significant



Figure 2: Different images of follicle and CL on the screen of Ultra sound machine

#### Conclusion

It is concluded as, the present study developed guide line information on ovarian follicular size, cystic status and physical condition of RB cows that may be associated with repeat breeding in cows. Different types of follicles more or less evenly distributed in both ovaries, although large number of follicles and follicular diameter are higher in left ovaries. This information will be helpful for the researcher as well as the veterinary personal for early detection and treatment of repeat breeder cows.

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