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A Comparative Study On Reproductive Hormones Of Repeat Breeding And Synchronized Repeat Breeding Dairy Cows Under Bathan Rearing System At Baghabari Milk Shed Areas In Bangladesh

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Abstract:*Repeat breeding (RB) is a major problem that causes a great economic loss in dairy herds. Farmers cannot get benefit from it as it increases production cost by insemination, treatment,feed,labor,calving interval,culling rates and decreased calf and milk production.A total of thirty (30) dairy cows ofrepeat breeder at the different sheds of dairy farmers at the Bathanrearing area of ShahjadpurUpazilla under Sirajgonj district in Bangladesh used for this study.The cows were synchronized in a standard heat period in which GnRH and PGF2α were treated.Blood samples were collectedand separated serum samples wereexamined for the comparative study of the reproductive hormonal level of repeat breeder andsynchronized repeat breeder dairy cows.The level of luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone were significantly higher in synchronized repeat breeder cows than repeat breeder cows. The level of estradiol was also significantly higher in synchronized repeat breederCows. The mean value of four reproductive hormonesofnormal repeat breeder cows were consequently (LH=0.0825mIU/mL, FSH=4.647mIU/mLProgesterone =1.807 ng/mL,estradiol=82.23pg/mL) and in synchronizedrepeat breeder cows (LH=2.481mIU/mL, FSH=17.04mIU/mL, Progesterone=5.927 ng/mL, estradiol=107.3pg/mL).The results from the present findings clearly indicated that reproductive hormonal aberration might be one of the major cause of repeat breeding in dairy cows at Baghabari milk shed areas.*

Kew words:*Reproductive hormone,synchronization, artificial insemination, repeat breeder cows.*

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I. Introduction

Hormones are chemical substances produced in the body that controls and regulates the activity of certain cells or organs[1] .Many hormones are secreted by special glands, such as thyroid hormone produced by the thyroid gland[2].Dairy cattle reproduction is controlled by several hormones which are produced by numerous endocrine glands[3]. These hormones are secreted by the glandular cells and pass into the blood where they are transported throughout the body to complete their respective functions. Reproductive hormones can be used in the supervision of animal production[4].Hormones can be used both diagnostically and therapeutically[5]Prompt advances in technology allow on-the-farm diagnostic kits to be economical and accurate. Milk Progesterone kits are used to determine the fixed stage of reproductive cycle or as a preliminary diagnosis of pregnancy[6].

Luteinizing Hormone (LH)is a glycoprotein consisting of two subunits[7].A linear decrease in serum LH occurred in cows throughout the onset of pregnant, whereas serum LH remained unaffected in non-pregnant cows[8].LH is responsible for causing the follicle to rupture (ovulate) by acting on the ovary which will release the egg. Following ovulation the LH also stimulates the development of the corpus luteum[9] (CL).

Follicle stimulating hormone (FSH) is a potentially important hormone in initiating reproductive activity[10].FSH stimulates the growth, development and function of the follicle[11].

Progesterone is a steroid hormone produced by the corpus luteum (CL) on the ovary which forms after ovulation of the oocyte[12].Progesterone marks uterine epithelium, myometrium and hypothalamus causing endometrial secretion, inhibition of GnRH release and stimulates maintenance of pregnancy. The level of progesterone in the serum reveals the activity of the CL on the ovary. The level of progesterone rises for the first 4 to 6 days following ovulation. Maximum levels are reached between days 10-17. High levels of progesterone are produced which helps to maintain pregnancy while cows become pregnant. If the cow does not become

pregnant, the CL begins to degenerate and the level of progesterone abruptly decreases on days 18 or 19 andallowing the cow to return to estrus on day 21-23th[13].

Besides Estrogens (Estradiol) plays a critical role in most metabolic, behavioral and morphological requirements, which are essential in reproduction of the female vertebrates[14] .There is no doubt that estrogens govern important activities including metabolic reactions in male, too.

The estrous cycleencompasses the recurring physiologic changes that are induced by reproductive hormones in most mammalian including females. Estrous cycles start after sexual maturity in females and are interrupted by anestrous phases or pregnancies[15].Typically, estrous cycles continue until death[16].The estrus cycle is governed by the complex interactions of various hormones that are produced in the brain and ovaries; progesterone and estrogen being two of these[17]The follicle (egg) grows throughout the cycle and ovulation (the release of the egg) occurs when the progesterone levels drop and the estrogen rises[18] A structure called the corpus luteum then forms on the ovary, which then produces progesterone[19]. Any cows that haven't cycled after 35-42 days should be examined for any abnormalities and to help maximize her chances of early conception[20].

Repeat-breeder cows are commonly referred to sub-fertile animals without any anatomical or infectious abnormality that do not become pregnant until the third or subsequent breeding or remain infertile after numerous services[21].Repeat breeding is one of the major problems affecting the reproductive yield. The reason of repeat breeding is multifaceted; most common causes are genetical, anatomical defects of the reproductive regions hormonal imbalances, infections such as clinical, subclinical endometritis and poor management[22].The objective of this study was to investigate the effectiveness of plasma hormones like FSH, LH, progesterone and estradiol in both repeat breeder cows and synchronized repeat breeder cows at Baghabari milk shed areas in Bangladesh to find out the conception aberrationamong the repeat breeder cows.

II. Materials and Methods

Study design: Prospective open label observational study

Study location:Baghabari milk shed areas of Sirajgonj district and in the Department of Biochemistry and Molecular Biology ofJahangirnagar University, Savar, Dhaka, Bangladesh.

Study duration:October 2017 to October 2018

Sample size: 30 repeat breeder cows with defects in pregnancy.

Sample size calculation:The sample size was estimated on the basis of a single proportion design.The target population from which we randomly selected our sample was considered 30.

Subjects & selection method: The study population was drawn from consecutive repeat breeder cows.These selected repeat breeder cows were marked by ear tag and collected breeding history of each individual cows.

Inclusion criteria:

1. Repeat breeder cows
2. Female
3. Mature aged
4. Symptomatically healthy

Exclusion criteria:

1. Normal breeding cows
2. Feeble cows
3. Cows which are physically inactive

Procedure methodology:

A total of thirty (30) blood samples were collected into vacutainer tube from the jugular vein of each experimental cow. Blood samples were collected before synchronization from all experimental cows. Poor rates of estrous detection combined with poor conception rates (CR) make management of reproduction in lactating dairy cows a challenge in most dairy herds. Blood samples were collected after synchronization measured by Estrous Detector Device for AI in appropriate time. The serum samples were prepared from all experimental cows. The serum samples were prepared in the animal Health Laboratory of Bangladesh Livestock Research Institute, Regional station, Baghabari, Shahjadpur, Sirajgonj in Bangladesh. The plasma serum was separated by centrifugation (1500 rpm for 20 min).The separated serum was collected in a sterile vial and preserved at −20°C Laboratory Deep freezer until analysis. All serum samples were analyzed in the laboratory at the department of Biochemistry and Molecular Biology, Faculty ofBiological Sciences, Jahangirnagar University (JU), Savar, Dhaka, Bangladesh by using endocrine detection kits (Mono LelacR USA).

Synchronization of experimental dairy cows:

Experimental dairy cows were reared under the bathan feeding and management system. In addition, mineral supplement were given to all experimental cows. All experimental cows were synchronized by the treatment of GnRH (day-0), PGF2α (day-7) and Artificial insemination -AI + GnRH (day-9) by following the

below estrous synchronization protocol for more efficient reproductive management of Repeat breeder cows. To help producer's reproduction more efficiently, protocols for synchronization of estrus have been developed. The dairy cows those were identified as repeat breeder cows had been synchronized with the treatment of GnRH and PGF2α as described by Pursley, 1995[23].

Statistical analysis:
Statistical analysis was performed with the two-tailed student's t test. P values <0.05 were considered statistically significant.All statistical analysis was done using Graph Pad Prism 7 (La Jolla,CA).

III. Results

The mean value of four types of reproductive hormone is summarized in a table.

Table: Hormonal values in repeat breeder and synchronized repeat breeder cows

Parameters	Repeat breeder cows	Synchronized repeat breeder cows	Level of significance
	Mean ± SE	Mean ± SE	
LH(mIU/mL)	0.8251 ± 0.08153	2.481 ± 0.4806	****
FSH(mIU/mL)	4.647 ± 0.7999	17.04 ± 2.743	****
Progesterone (ng/mL)	1.807 ± 0.2148	5.927 ± 0.4219	****
Estradiol (E2)(pg/mL)	82.23 ± 2.402	107.3 ± 6.974	**

SE=Standard error, **=highly significant, NS=Non-significant, LH=Luteinizing hormone, FSH=Follicle stimulating hormone**

3.1 Luteinizing Hormone (LH)

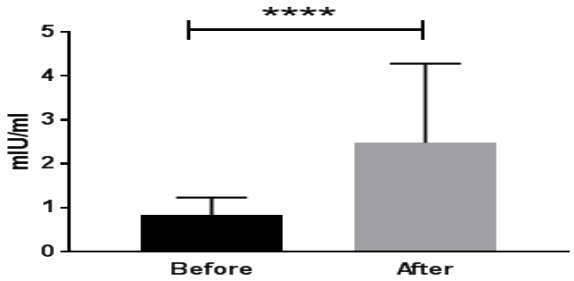

Fig.1:Comparison of mean concentration of LH betweenrepeat breeder and synchronized repeat breeder cows.

The mean concentrationof LH in synchronized repeat breeder cows was about2.481 mIU/mLand the mean concentrationof LH in repeat breeder cows was about 0.387mIU/mL.From the figure 1 it is evident that the mean concentration of luteinizing hormone was higher in synchronized repeat breeder cows than repeat breeder cows.Data are presented as mean value levels ± SEM. Here, p<0.0001. Here; Before= Repeat breeder cows, After= Synchronized repeat breeder cows.

3.2 Follicular Stimulating Hormone (FSH)

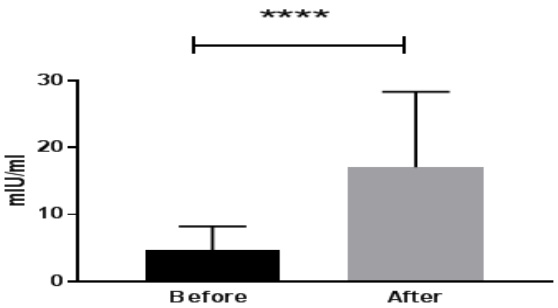

Fig 2:Comparison of mean concentration of FSH betweenrepeat breeder and synchronized repeat breeder cows.

The mean concentration of FSH in synchronized repeat breeder cows was about17.04 mIU/mLand the mean concentrationof FSH in repeat breeder cows was about 4.647 mIU/mL.From the figure 2 it is evident that the mean concentrationof follicular stimulating hormone was higher in synchronized repeat breeder (After) cows than repeat breeder cows (Before).Data are presented as mean value levels ± SEM. Here, $p<0.0001$.Here; Before= Repeat breeder cows, After= Synchronized repeat breeder cows.

3.3 Progesterone Hormone:

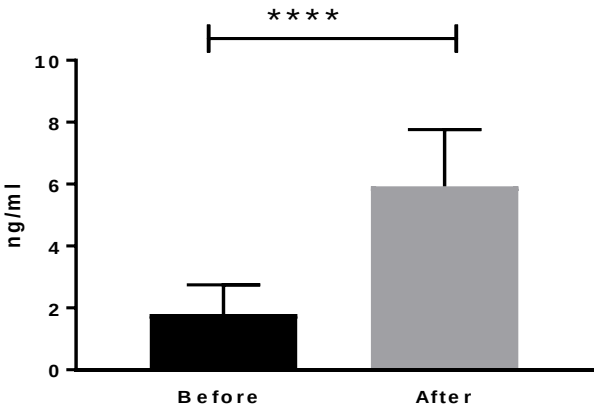

Fig.3:Comparison of mean concentration of Progesterone betweenrepeat breeder and synchronized repeat breeder cows.

The mean concentration of progesterone in synchronized repeat breeder cows was about5.927 ng/mL. Besides, the mean concentrationof progesterone in repeat breeder cows was about 1.807 ng/mL.From the figure 3 it is evident that the mean concentrationof progesterone was higher in synchronized repeat breeder (After) cows than repeat breeder cows (Before).Data are presented as mean value levels ± SEM. Here $p<0.0001$.Here; Before= Repeat breeder cows, After= Synchronized repeat breeder cows.

3.4 Estradiol (E2):

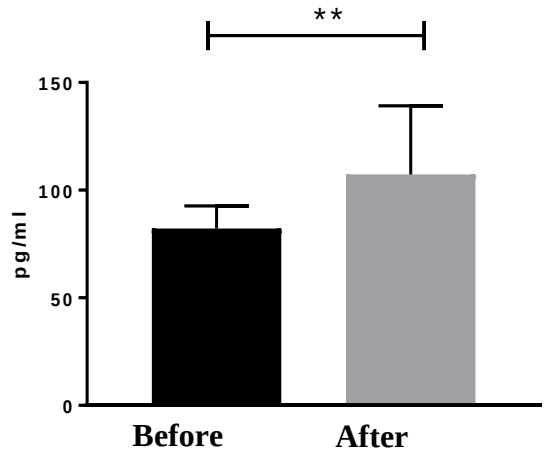

Fig. 4: Comparison of mean concentration of Estradiol between repeat breeder and synchronized repeat breeder cows.

The mean concentration of estradiol in synchronized repeat breeder cows was about116.4 pg/mL and the mean concentration of estradiol in repeat breeder cows was about 81.59 pg/mL.From the figure 4 it is evident that the mean concentrationof estradiol wasslightly higher in synchronized repeat breeder (After) than repeat breeder cows (Before).Data are presented as mean value levels ± SEM. Here, $p<0.01$.Here $p<0.0001$. Here; Before= Repeat breeder cows, After= Synchronized repeat breeder cows.

IV. Discussion:

Regulation of estrous cycles (17 to 25 days) in clinically normal cows has been associated with improved conception efficiency[24].There is a general agreement that estrous cycles terminate in most unmated cows within 3 to 4 days after corpus luteum start to regress[25]. Plasma progesterone decreases from near maximum to near minimum concentrations in about two days[26].Onset of estrus and the LH surge occurs in another 24 hr[27] followed by ovulation in 20 to 26 hr[28].Increased secretion of FSH and E, during the follicular phase of the menstrual cycle has been reported in women during late reproductive cycle[29].During the estrous cycle, secretion of ovarian follicular estradiol is thought to be responsible for priming pituitary gonadotrophs[30] as well as for inducing the gonadotropin surge in cattle[31].

Ovariectomy of immature rats significantly rises the concentration of plasma LH[32,33]and FSH[34]. The sensitivity of the hypothalamus-pituitary axis to estradiol must thus decline prior to puberty in the heifer[35]. As a result this permits LH to stimulate follicular growth and leads to increased estrogen production[36]and ovulation.

In the normal estrous cycle the decrease in progesterone concentrations is followed by an increase in the frequency of LH pulses[37].High circulating levels of LH stimulate the follicle to grow and secrete estradiol, which in turn elicits the surge of LH that causes ovulation[38].

In our study, the level of luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone hormone were significantly different between repeat breeder cows and synchronized repeat breeder cows. From the results it is demonstrated that the three reproductive hormone consecutively LH, FSH, progesterone were significantly higher in the synchronized repeat breeder cows than in the repeat breeder cows. Furthermore, estradiol concentration was slightly higher in the synchronized repeat breeder cows than in the repeat breeder cows.

V. Conclusion:

Repeat breeding is an important factor affecting economic success in dairy management.Whatever the cause of low conception, our result confirmed that synchronized repeat breeder cowshadhigher reproductive hormones as compared torepeat breeder cows.It is clearlydemonstrated that the reproductive hormones play significant role in solving repeat breeding problems of dairy cows. Use of these reproductive hormones may minimize the repeat breeding problems in dairy cows.

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Declaration:

There is no conflict of interest among the authors regarding this article and all authors contributed equally.

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