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Double-timed artificial insemination along with gonadorelin acetate injection improved the fertility in repeat breeder cows

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ABSTRACT

Repeat breeding syndrome (RBS)is a multifactorial disorder in dairy cows, which cause a serious economic loss. The study was intended to know the effects of single-timed artificial insemination (SAI) and double-timed artificial insemination (DAI) with and without injection of gonadorelin acetate (GnRH analogue) for the improvement of fertility in repeat breeder **(**RB) cows. A total of 120 RB cows were selected in this study. Firstly, all cows were deworming and then divided into equal six groups namely; Group–ST1 (SAI), Group–ST2 (SAI with single injection of GnRH), Group–ST3 (SAI with double injection of GnRH), Group–DT1 (DAI), Group–DT2 (DAI with single injection of GnRH) and Group–DT3 (DAI with double injection of GnRH). The corpus luteum (CL) diameter and serum progesterone (P_4) and estrogen (E_2) hormone were estimated at day 18 post AI of the cows. Pregnancy rate was recorded at day 45–90 post AI. Diameter of CL and serum P_4 were significantly ($P<0.05$) higher and serum E_2 was significantly ($P<0.05$) lower in the RB cows with double injection of GnRH than others. Pregnancy rate was significantly ($P<0.05$) higher jointly in Group–DT2 and DT3 than other groups. Pregnancy was significantly ($P<0.05$) higher in DAI compared to SAI irrespective of GnRH injection strategies. It was also significantly ($P<0.05$) higher in AI with double injection of GnRH than AI without injection of GnRH irrespective of insemination strategies. Results confirm that double injection of GnRH and DAI improve the fertility in RB cows and the study helps Veterinarians and researchers to describe the insemination strategies for the prevention of RBS in dairy cows.

Keywords: GnRH injection, insemination strategies, ovarian and hormonal parameters, pregnancy rate, repeat estrus cows.

How to Cite

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INTRODUCTION

In the repeat breeding syndrome (RBS), dairy cows exhibit the hormonal and physiological asynchronies including subnormal plasma estrogen (E_2) and suprabasal progesterone (P_4) concentration, delayed surge of luteinizing hormone (LH) and delayed ovulation during estrus [1,2,3]. The poor endometrial environment and altered contractility hampers the sperm endurance and transportation of gametes during delayed ovulation [3]. Early insemination in estrus decreases the pregnancy due to fertilization failure or early embryonic mortality (EEM) [4]. Single artificial insemination (AI) following a routine AM/PM schedule may decreases the fertilization due to the deficiency of spermatozoa numbers and fertilizing ability by the moment ovulation occurs [3]. The AI timing with respect to ovulation and high numbers of fertilizable spermatozoa is very important for ensuring the fertilization in dairy cows [3,5]. Re-inseminations or double insemination during estrus may improve the fertility in repeat breeding (RB) dairy cows due to the correction of hidden factors of RBS or error in heat detection [6,7].

The P_4 dictate the significant role in favoring the conceptus development and the secretion of interferon-τ (IFN-τ) for maintenance of pregnancy [8]. In high yielding dairy cows the increased milk production is associated with elevated metabolism as well as P_4 metabolism in the liver, which reduces P_4 in the plasma [9]. Lower P_4 concentration related to the delayed embryo development, which decreases the signalling for the maternal detection of pregnancy, results the embryonic loss [10,11]. The EEM is a prime cause of lower conception rate and the EEM and fetal mortalities are predicted to be 50% in dairy cows and 70–80% of these fatalities occur in the first 16 days after insemination [12,13]. A number of strategies have been used to increase the plasma P_4 in order to reduce the proportion of embryonic loss, and most likely the administration of natural gonadotropin releasing hormone (GnRH), GnRH agonists or human chorionic gonadotropin (hCG) through the insemination in dairy cows [14,15].

The GnRH is secreted by hypothalamus which regulates the synthesis of LH and follicular stimulating hormone (FSH) and combindly these two hormones control the follicular development, ovulation and corpus luteum (CL) functions [16]. The efficiency of synthetic GnRH analogue imitates the action of natural GnRH in modifying reproductive physiology of cows [6]. Hormonal problem or insufficiency of hormones in cows may rectify by the exogenous use of GnRH throughout the estrus [17]. After insemination at particular period, immediate with the existence of dominant follicle (DF) of first and second waves in cows, the application of GnRH, a GnRH agonist or hCG could stimulate the role of CL, increase the size of CL, induce accessory CL formation, increase plasma P_4 and decrease E_2 in plasma, which have positive effect on the proportion of conception or existence of live fetus in the uterus [18,19,20,21,22]. It has been hypothesized that exogenous administration of GnRH in a cows at the time of AI and at day 12 post estrus may regulate the concentrations of P_4 closer to physiological level of healthy cows [14]. Use of GnRH prior to AI or at day 12 post AI may increase the fertility and reduce the embryonic mortality specially the beneficial effects of GnRH have been observed in high yielding cows during heat stress [23,24]. Double insemination and GnRH injection at day 12 post AI of RB cows were suggested for dairy management [25].

Limited reports were available about the use of synthetic hormones and double insemination for management of RB dairy cows in particular regions of Bangladesh. Nothing was done to investigate the problems of RBS using GnRH analogue at the time of AI and at day 12 post AI with double insemination for enhancement of conception rate of crossbred RB cows in Bangladesh. Now a day, it is very essential to know the effect of gonadorelin acetate (GnRH analogue) injection with double insemination on pregnancy rate in RB cows for successful dairy farming.

Considering these facts the experiment was designed to know the effects of single-timed AI (SAI) and double-timed AI (DAI) with and without injection of GnRH for the improvement of fertility in RB cows.

MATERIALS AND METHODS

Ethical approval

This experiment was approved by Animal Welfare and Ethical Committee, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh (Approval No. 05/AWEC).

Study period and selection of study areas

The research work was conducted from January 2016 to December 2019 throughout the study areas in Bangladesh and the desired populations of dairy cows were selected from the study areas. To achieve best possible target of the research there are about five clusters of study areas were selected in Bangladesh, (i) Gazaria (23°32.5′N90°36.5′E), Munshiganj (ii) Sreenagar (23°32.2′N90°17.5′E), Munshiganj (iii) Shahjadpur (24°10.2′N89°35.3′E), Sirajgonj and Rural Development Academy (RDA), Sherpur (24°40′N89°25′E), Bogura (iv) Karnaphuli (22°13′N91°48′E) and Patiya (22°30′N 91°98′E), Chattogram (v) Mymensingh Sadar (24°45′N90°25′E) including BAU Veterinary Teaching Hospital, Mymensingh; Gazipur Sadar (24°0′N90°25.5′E), Gazipur and Savar Military Farm, Savar (23°51′30″N90°16′00″E), Dhaka.

Selection of experimental RB cows

A total of 120 RB cows with cyclic ovaries, well body condition and clear vaginal mucus at estrus were randomly selected from the surveyed cows throughout the study areas. The cows were selected as RB cows in the study, which were failed to get pregnancy by three or more breeding with regular interval without noticeable genital problems as described by Gustafsson and Emanuelson [26].

Experimental design

Routine deworming in RB cows was done using broad spectrum anthelmintic Trilev-Vet® Bolus. This anthelmintic was administered in the RB cows @ two boluses per 100 kg body weight and the second dose was applied at seven days interval. After 15 days of deworming, RB cows were randomly assigned into six groups (ST1, ST2, ST3, DT1, DT2 and DT3) and each group was comprised with 20 cows. The RB cows of Group–ST1, Group–ST2, Group–ST3, Group–DT1, Group–DT2, Group–DT3 were allocated for SAI, SAI with single injection of GnRH, SAI with double injection of GnRH, DAI, DAI with single injection of GnRH and DAI with double injection of GnRH, respectively. The experimental RB cows were reared under traditional dairy management in the owner farms.

Implementation of GnRH injection and insemination strategies

The RB cows of Group–ST1 (N=20), Group–ST2 (N=20) and Group–ST3 (N=20) were inseminated through SAI at 12–13 hours after onset of estrus, but the RB cows of Group–DT1 (N=20), Group–DT2 (N=20) and Group–DT3 (N=20) were inseminated through DAI at 12–13 hours and 22–23 hours after onset of estrus. The RB cows of Group–ST2 and Group–ST3 were injected with Ovurelin® Injection @ one mL per cow intramuscularly just prior to AI. Simultaneously, the RB cows of Group–DT2 and Group–DT3 were also injected with Ovurelin® Injection @ one mL per cow intramuscularly just prior to first AI. After that, RB cows of Group–ST3 and Group–DT3 were also injected with second Ovurelin® Injection with same dose intramuscularly at day 12 post AI. The SAI and DAI was done by 0.5 mL frozen semen of Holstein Friesian crossbred (75%) bull.

Measurement of corpus luteum (CL)

Diameter of CL in the experimental RB cows was measured at day 18 post AI through ultrasonography by the use of portable ultrasound scanner (Bionet® MU1V, Korea) assembled with a real time B mode 7.5 MHz linear transrectal probe. The transrectal ultrasonography was performed as described by Sharma et al. [27]. Measurement of diameter of each and every CL in the cows was performed by intrinsic measuring calipers of ultrasound scanner for three times and the mean value (mm) was noted for further analysis.

Quantitative estimation of serum progesterone (P_4) and estrogen (E_2) hormones

Four mL of blood sample was collected from experimental RB cows at day 18 post AI by the sterile clot activator vacuum tube and blood collecting needle (VIP™, Zhejiang Gongdong Medical Technology Co., Ltd. (South Gate), China) for quantitative determination of serum P_4 and E_2hormones. The blood sample containing tubes were placed in normal temperature at 45°angles for serum separation. After four to five hours the upper most light yellowish liquid (serum) of the tubes were collected into clean and dry sterile centrifuge tubes by the help of individual sterile dropper. Then the serum was centrifuged at 3000 rpm for 15 minutes using centrifuge machine then upper clearest liquid was collected into another centrifuge tubes. This process was repeated for three times and the pure serum samples were collected in the properly labeled eppendorf tube. These eppendorf tube containing serum samples were transferred to the laboratory by maintaining cool chain using thermo flask containing ice cubes and stored in the deep freezer at –20^0C. The serum P_4 and E_2 (estradiol 17β) hormones were estimated from the preserved serum samples using specific hormone ELISA kit (Nova Tec Immunodiagnogtica GmbH, Technologio and Waldpark, Dietzenbach, Germany) and microplate reader (SPECTRAmax® 340PC384, USA) in the laboratory of Community Based Dairy Veterinary Foundation, BAU, Mymensingh-2202, Bangladesh. Detailed procedure for the estimation of optical density (OD) value as 'x' of P_4 and E_2 hormones were performed as per prescribed literature supplied with the specific test kits. Procedure was repeated three times for each sample and average OD value was recorded for the calculation of serumP_4 and E_2 concentration. Concentrations were calculated by the use of formula $y = -0.14\ln(x) + 0.794$for P_4 hormone and $y = -0.36\ln(x) + 2.663$ for E_2 (estradiol 17β) hormone, which were obtained from the standard (STD) samples OD curves. Figure 1 represents the P_4 STD samples OD curve and Figure 2 represents E_2 STD samples OD curve.

Monitoring of non-return rate and pregnancy diagnosis

Non-return rate in the inseminated RB cows was recorded at day 21 after insemination. Pregnancy diagnosis was also performed by rectal palpation and transrectal ultrasonography at day 45–90 after insemination to enumerate the effects of GnRH injection and insemination strategies on the fertility in RB cows. The transrectal ultrasonography was done as described in earlier section.

Statistical analysis

The Chi-square test was done to find out the significant variation in pregnancy rate in the RB cows through the effects of GnRH analogue injection and insemination strategies. One-way "ANOVA" using Post Hoc test was also done to compare the mean value of observed parameters among the GnRH injected and non-injected experimental groups to enumerate the factors affecting in pregnancy rate. The statistical approaches in this study were done by the use of computerized IBM SPSS statistics software version 20 (UK).

RESULTS

The effect of GnRH injection and insemination strategies on pregnancy rate in RB cows is shown in Figure 3. Pregnancy rate in Group–ST1, Group–ST2, Group–ST3, Group–DT1, Group–DT2 and Group–DT3 were 15.00%, 40.00%, 50.00%, 70.00%, 85.00% and 90.00%, respectively. Pregnancy rate in Group–ST3 and Group–DT1 were significantly ($P<0.05$) higher compared to Group–ST1. Pregnancy rate in Group–DT2 and Group–DT3 were significantly ($P<0.05$) higher compared to Group–ST1, Group–ST2 and Group–ST3. Pregnancy rate was non-significantly ($P>0.05$) higher in Group–ST2 than Group–ST1; Group–ST3 than Group–ST2; Group–DT1 than Group–ST2 and Group–ST3; Group–DT2 than Group–DT1; Group–DT3 than Group–DT1 and Group–DT2. However, the pregnancy rate was higher in the Group–DT3 than all other groups.

Effect of insemination strategies on pregnancy rate in RB cows irrespective of GnRH injection strategies is presented in Table 1. The pregnancy rate in SAI and DAI in RB cows were 35.00% and 81.67%, respectively. The pregnancy rate in DAI was significantly ($P<0.05$) higher compared to SAI in RB cows irrespective of GnRH injection strategies.

Table 1. Effect of insemination strategies on pregnancy rate in repeat breeder (RB) cows irrespective of gonadotropin releasing hormone (GnRH) injection strategies

Experimental groups	Number of pregnant RB cows	Pregnancy rate (%)
SAI in RB cows	21	35.00[a]
DAI in RB cows	49	81.67[b]

N=60 RB cows in each experimental group. [a,b]Values within the column differ significantly ($P<0.05$).

Effect of GnRH injection strategies on pregnancy rate in RB cows irrespective of insemination strategies is presented in Table 2. The pregnancy rate in AI without injection of GnRH, AI with single injection of GnRH and AI with double injection of GnRH were 42.50%, 62.50% and 70.00%, respectively irrespective of insemination strategies. Pregnancy rate in AI with double injection of GnRH was significantly ($P<0.05$) higher than AI without injection of GnRH. Pregnancy rate was also non-significantly ($P>0.05$) higher in AI with single injection of GnRH than AI without injection of GnRH; and in AI with double injection of GnRH than AI with single injection of GnRH. Pregnancy rate was higher in AI with double injection of GnRH than other groups.

Table 2. Effect of gonadotropin releasing hormone (GnRH) injection strategies on pregnancy rate in repeat breeder (RB) cows irrespective of insemination strategies

Experimental groups	Number of pregnant RB cows	Pregnancy rate (%)
AI without injection of GnRH	17	42.50[a]
AI with single injection of GnRH	25	62.50[a,b]
AI with double injection of GnRH	28	70.00[b]

N=40 RB cows in each experimental group. [a,b]Values within the column differ significantly ($P<0.05$).

Effect of GnRH injection strategies on ovarian activity at day 18 post AI in RB cows irrespective of insemination strategies is presented in Table 3. Diameter of CL, serum P_4andserum E_2 at day 18 post AI in RB cows without injection of GnRH were 16.23±0.37 mm, 5.77±0.15 ng/mL and2.69±0.25 pg/mL, in RB cows with single injection of GnRH were 20.90±0.30 mm, 7.90±0.15 ng/mL and 2.23±0.24 pg/mL, and in RB cows with double injection of GnRH were 23.37±0.35 mm, 9.42±0.15 ng/mL and 1.38±0.18 pg/mL, respectively. The diameter of CL and serum P_4 were significantly ($P<0.05$) higher and the serum E_2 was non-significantly ($P>0.05$) lower in the RB cows with single injection of GnRH in comparison to RB cows without injection of GnRH at day 18 post AI. Likely, the diameter of CL and serum P_4 were also significantly ($P<0.05$) higher and the serum E_2 was significantly ($P<0.05$) lower in the RB cows with double injection of GnRH compared to RB cows with single injection of GnRH and RB cows without injection of GnRH at day 18 post AI.

Table 3. Effect of gonadotropin releasing hormone (GnRH) injection strategies on ovarian activity at day 18 post artificial insemination (AI) in repeat breeder (RB) cows irrespective of insemination strategies

Parameters	Experimental groups		
	RB cows without injection of GnRH	RB cows with single injection of GnRH	RB cows with double injection of GnRH
Diameter of CL (mm)	16.23±0.37[c]	20.90±0.30[b]	23.37±0.35[a]
Serum P_4 (ng/mL)	5.77±0.15[c]	7.90±0.15[b]	9.42±0.15[a]
Serum E_2 (pg/mL)	2.69±0.25[a]	2.23±0.24[a]	1.38±0.18[b]

N=40 RB cows in each experimental group. Values on each groups are Mean±SE. [a,b,c]Values within the row differ significantly (P<0.05).

DISCUSSION

The pregnancy rate was higher in the RB cows with single injection of GnRH just prior to AI compared to the RB cows that are inseminated without injection of GnRH irrespective of insemination strategies. This result is in agreement with the finding that the use of GnRH at the time of AI increases conception rate in dairy cows [28,29,30]. Similarly, Stevenson et al. [7] also reported that GnRH increased the conception rates of RB cows than the control groups given only a single AI. Defiantly, this finding differed from the inspection of other researchers [31] who concluded that GnRH had no affect the conception rate if inserted at the time of AI.

The effect of single injection of GnRH at the time of AI on ovarian activity in the RB cows at day 18 post AI illustrated that the diameter of CL and serum P_4 were significantly (P<0.05) higher and the serum E_2 was non-significantly (P>0.05) lower than the RB cows that are inseminated without injection of GnRH. This elevated diameter of CL and more circulating P_4 (due to super function of CL) and lower circulating E_2 may influence the fertility in the experimental RB cows. However, the improvement of fertility in the RB cows due to GnRH treatment just prior to AI in the present study has two probable clarifications as accounted by Jaswal and Singh [28]. Firstly, application of GnRH just prior to AI at estrus improved the ovulation rate [32]. The GnRH injection stimulate the LH surge after maximum two hours of treatment [16], which increase the plasma LH concentration after 2–2.5 hours [33]. Since the preovulatory LH surge normally happens about six hours after onset of estrus [34]. Application of GnRH at insemination may have provoked the secondary LH surge before or after the spontaneous preovulatory LH surge [28]. That added increment of LH may be beneficial to the events associated with more conception in RB cows. Secondly, circulating P_4 promotes the development of embryo and regulates the mechanism of luteolysis [35]. GnRH injection during estrus results the LH surge and following ovulation LH increases blood flow to ovaries, causing ovarian hyperemia. Therefore, CL formation occurs rapidly and significantly increases the P_4 production [29]. Moreover, single GnRH dose at the time of AI can increase the subsequent plasma P_4 concentration [29], which is related to hypertrophy and hyperplasia of the luteal cells.

The pregnancy rate was increased in the RB cows with double injection of GnRH (just prior to AI and at day 12 post AI) (70.00%) than other strategies of GnRH injection irrespective of insemination strategies. The present findings is resembles with many earlier reports [6,28,36]. Though Beckers et al. [37] observed that there was no significant difference between GnRH injected and controlled groups which were given at day 12 post insemination. Whereas, the previous studies showed that application of GnRH after AI at specific times coincident with the presence of DF of the first and second follicular waves may stimulate the CL activity, increase the circulatory P_4 level and decrease E_2 production which have positive effect on embryo survival [19].

Result showed that in the GnRH injected cows the size of CL was significantly (P<0.05) increases. This increase in luteal tissue area is most likely responsible for higher (significant at P<0.05) serum concentration of P_4 observed in the RB cows with double injection of GnRH than without injection of GnRH and single injection of GnRH prior to AI. Similar to the present study, several authors [38,39,40,41] detected higher P_4 concentrations in dairy cows after GnRH injected between day 11 and 15 post AI. Simultaneously, GnRH administration on day 12 is similar as early application of GnRH for enhancing P_4 production, which is necessary for maternal recognition of pregnancy and beginning of luteolytic mechanism characterized by the embryonic secretion of anti-luteolytic factors [10].Thus the higher circulatory serum P_4 concentration decreases the embryonic mortality in the early stages [6]. Furthermore, the serum E_2 at day 18 post AI was significantly (P<0.05) lower in the RB cows with double injection of GnRH than other groups, might be due to enhance the regression of follicles in the second or third follicular waves as described by Šuluburić et al. [20] and Thatcher et al. [21].

Remarkably, the double injection of GnRH (prior to AI and at day 12 post AI) increases the circulating P_4, enhances the embryonic secretion of anti-luteolytic factors and decreases the circulating E_2. All of which are necessary for successful maternal recognition of pregnancy, reducing EEM, embryonic development and embryo survival. Therefore, the administration of GnRH at the time of AI and at day 12 post AI could increases the pregnancy in the experimental RB cows.

The results in this experiment also showed that the pregnancy rate was significantly ($P<0.05$) higher in DAI compared to SAI in the RB cows, which is coincide with Singh et al. [3] and Hailu et al. [6]. The results showed that repeated inseminations at 12–13 hours and at 22–23 hours after onset of estrus increases the pregnancy rate in the RB cows with DAI comparable to that of RB cows with SAI irrespective of GnRH administration strategies. Similar to the present study, Singh et al. [3] reported that the second AI in RB animals increases pregnancy rates. Double inseminations with the few hours interval during estrus may increase pregnancy rate notably for RB cows with silent heat and/or for the missed detection of estrus or may be due to delayed ovulation [6]. From the earlier studies as reviewed by Singh et al. [3], it may be speculated that the DAI could increases the pregnancy in the dairy cows through a number of possible causes. Firstly, in DAI the second AI might be closer to ovulation, which may provide enough sperms with elevated fertilizing ability at the site of fertilization and thus lead to increased pregnancy rates. Secondly, this improvement could be the shorter revelation to an unfavorable maternal environment before they effort the fertilization. Thirdly, the DAI would have refilled the sperm pool, increasing the availability of fertile sperms at the time of ovulation. Furthermore, the DAI with few hours interval reduce the occurrence of RBS by increasing possibility of site and time of semen deposition and estrus recognition [6]. Though Stevenson et al. [7] found a marginal increase in pregnancy rate from 32.1–33.5% when a single second AI was performed 12–16 hours after first AI. The current experiment revealed that higher pregnancy (81.67%) in DAI was found in the experimental RB cows. This significant difference from earlier studies might be due to the administration of GnRH at the time of AI and at day 12 post AI. Thus the improvement of pregnancy rate following DAI in the RB cows should therefore not be disregarded.

CONCLUSION

Results clearly mentioned that GnRH analogue (gonadorelin acetate) injections could increase the diameter of CL and serum P_4 and decrease the serum E_2 at day 18 post AI in the RB cows. Thus the DAI (at 12–13 and 22–23 hours after onset of estrus) with double injection of GnRH (at AI and at day 12 post AI) may increase the pregnancy rate in the RB cows. Animal breeder may use the strategies like double injection of GnRH analogue along with DAI during the estrus in RB cows for the prevention of RBS in the dairy herds.

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COMPETING INTERESTS

The authors declare that there is no potential competing interests pertinent to this article was reported.

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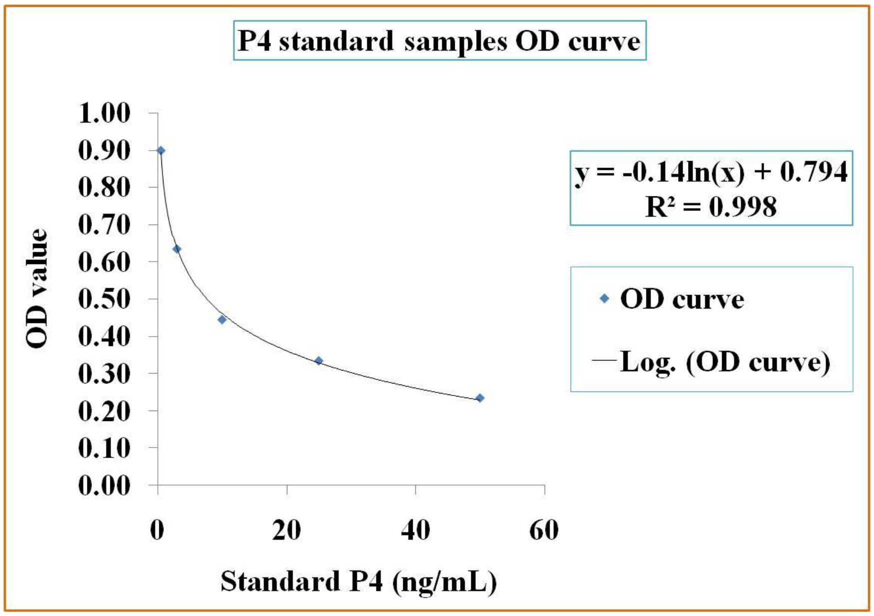

Figure 1. P_4 standard (STD) samples optical density (OD) curve

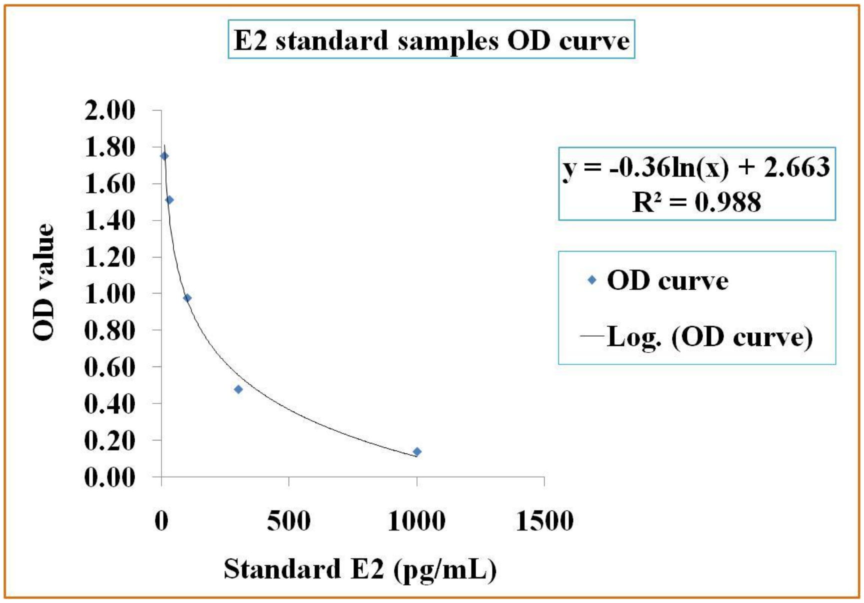

Figure 2. E_2 standard (STD) samples optical density (OD) curve

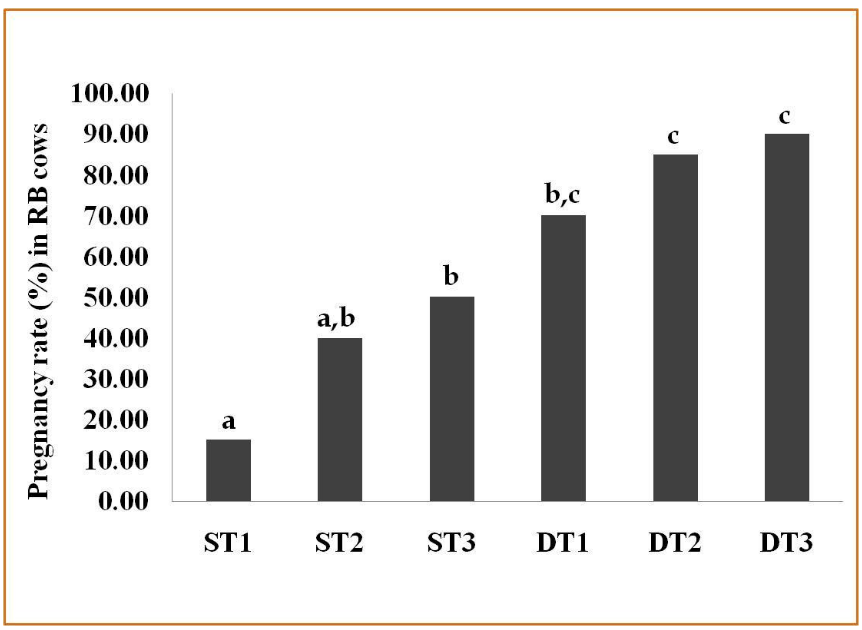

Figure 3. Effect of gonadotropin releasing hormone (GnRH)injection and inseminationstrategies on pregnancy rate in repeat breeder (RB) cows