Efficacy of the progesterone impregnated intra-vaginal sponge on fertility in repeat breeding cows

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Abstract
Supplementing exogenous Progesterone (P₄) during the early luteal phase shall increase the conception rate among the repeat-breeding cows. The present study was undertaken to compare the efficacy of the P₄ impregnated intra-vaginal sponge (PIVS) which were used fresh and after storage, on fertility in repeat breeding cows. A total of 40 repeat breeding cows were selected and divided into four groups randomly. Group I (n=10): Freshly purchased PIVS were used; Group II (n=10): PIVS were stored for 6 months after purchase and utilized; Group III (n=10): PIVS were stored for 12 months after purchase and utilized; Group IV (n=10): No P₄ supplementation (Control). The sponges were kept intravaginally from Day 6-16 post insertion. Pregnancy diagnosis was performed 28-35 days after insemination. Blood samples were collected on Day 6 and Day 10 post insertion of the vaginal sponge for P₄ hormone estimation. There was a significant decrease (P<0.05) in P₄ concentration on day 16 in both the pregnant and non pregnant animals of Group III when compared to Group I. There was no significant increase in P₄ concentration in the control group in both pregnant and non-pregnant animals when compared between days 6 and 16. Even though higher fertility rates were observed in Group I, when compared to the other groups, it does not differ significantly at P<0.05 level. Based on the study, Progesterone impregnated intra-vaginal device may be used for increasing the conception rate in repeat breeding cows which are better to use within 6 months after production. Suitable adjuvant may be added to improve the keeping quality of the Progesterone impregnated intra-vaginal device for more than 6 months.

Keywords: progesterone impregnated intra-vaginal sponge, conception rate, repeat breeder, serum progesterone

Introduction
Reproductive efficiency is determined by the number of cows within a herd that become pregnant, give birth, and produce calves each year. The incidence of repeat breeding cows has been reported to be 15–20% [1]. Causes of repeat breeding have been attributed to factors which are nutritional, hormonal, abnormalities in the gamete, delayed ovulation, inadequate luteal function, infection and managemental causes and compromises in artificial insemination (AI) procedures [2,3].

Progesterone (P₄) is essential for orchestrating the histotrophic environment for the nourishment of the conceptus. Embryonic deaths are caused by genetic and environmental factors. Most embryonic deaths, especially due to hormonal disorders, are the result of luteal insufficiency [3]. For this reason, P₄ or its analogues are supplemented to reduce embryonic losses before or after artificial insemination. Controlled breeding using progestagens such as Progesterone Releasing Intra-vaginal Device (PRID), Controlled Internal Drug Release Device (CIDR) or Norgestomet ear implants was effective in achieving good fertility in normally cycling dairy cattle [4].

In repeat breeding cows, controlled breeding using a CIDR was found to be an effective tool to achieve a high pregnancy rate through a fine regulation of folliculogenesis [5]. Some researchers report an overall increase of 5% in pregnancy rates following P₄ administration. P₄ treatment for 6 days after artificial insemination can increase pregnancy rates (10% more) [6]. The concept of supplementing repeat-breeding cows with exogenous P₄ during the early luteal phase could possibly increase conception rate per insemination was reported [7].
However, there are no reports on the efficacy of the P₄ devices which were stored for variable periods. Hence, the present study was undertaken to compare the efficacy of the P₄ impregnated intra-vaginal sponge (PIVS) which were used fresh and after storage, on fertility in repeat breeding cows.

Materials and Methods
Crossbred cows which were brought for insemination /infertility investigation to Gynaecology Unit of Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu were utilized for the study. A total of 40 repeat breeding cows which were diagnosed as oestrus, free from uterine infections, without palpable genital tract abnormalities and having good body condition were selected for this study. All the selected animals were inseminated twice on subsequent days and divided into four groups randomly based on the application of PIVS insert. Group I (n=10): Freshly purchased PIVSs were used; Group II (n=10): PIVS were stored for 6 months after purchase and utilized; Group III (n=10): PIVS were stored for 12 months after purchase and utilized; Group IV (n=10): No P₄ supplementation (Control).

The sponges were kept intravaginally for 10 days from Day 6-16 post artificial insemination (Day 0- oestrum). Pregnancy diagnosis was performed 28-35 days after AI by trans-rectal ultrasound. Blood samples were collected on Day 6 and Day 10 post insertion of the vaginal sponge for P₄ hormone estimation using a commercial Radioimmunoassay (RIA) progesterone kit (M/s Beckman Coulter). The data were compiled, expressed as a percent for better interpretation and analyzed statistically. The conception rates in the treatment cycle were compared between different groups by completely randomized design [8].

Results and Discussion
The conception rates in repeat breeding cows treated with PIVS are presented in Figure 1. The conception rates in Group-I, II, III and IV were observed to be 60.00, 50.00, 40.00 and 30.00%, respectively. The conception rate was higher in group-I, followed by group-II and III and lowest in untreated control group-IV. The results were statistically non-significant, except between group-II and IV.

Exogenous P₄ supplementation has been shown to improve conception rates in cows when administered early in pregnancy, of course not earlier than 3 days following insemination [5]. The results of conception rate revealed the first service conception rate was the highest in the progesterone sponge treated group while it was the lowest in the progesterone depot injected group [9].

In the present study, the mean plasma P₄ levels on days 6 and 16 of the cycle in repeat breeder cows increased significantly (P<0.01) as days progressed (Table 1). There is a significant decrease (P<0.05) in P₄ concentration on Day 16 in both the pregnant and non pregnant animals of Group III when compared to Group I. There is no significant increase in P₄ concentration in the control group in both pregnant and non pregnant animals when compared between Day 6 and 16. Even though higher conception rates were observed in Group I, when compared to the other groups, it does not differ significantly at P<0.05 level.

Table 1: Comparison of mean serum progesterone level in Repeat Breeding Cows on day 6 and 16 post insemination in different groups

| Pregnancy Status | Day 6 | Day 16 |
|------------------|-------|--------|
|                  | Group I | Group II | Group III | Group IV | Group I | Group II | Group III | Group IV |
| Pregnant         | 2.25 ± 0.08ab | 2.24 ± 0.22ab | 1.34 ± 0.03ab | 1.48 ± 0.02ab | 6.53 ± 0.10ab | 5.30 ± 0.15bc | 4.78 ± 0.42bc | 5.55 ± 0.12ab |
| Non-Pregnant     | 1.38 ± 0.04abc | 1.60 ± 0.17abc | 1.24 ± 0.06abc | 1.47 ± 0.11abc | 5.48 ± 0.08abc | 5.13 ± 0.07abc | 4.69 ± 0.10abc | 3.93 ± 0.04abc |

Within columns means with different letters (A, B) differ significantly (p<0.05) between pregnant and non-pregnant groups for the experimental group within the days.

Within rows means with different letters (a, b, c, d) differ significantly (p<0.05) between the different experimental groups in pregnant and non-pregnant groups for days.

A low increase [15] and moderate increase (1 ng/ml) above that in the control cows, has been reported previously in high milk producing cows treated with CIDR [16] whereas a high increase (4.2 ng/ml and 2.4 ng/ml) in plasma P₄ concentrations were achieved by CIDR treatment. No difference in progesterone levels of conceived and non-conceived buffaloes following CIDR treatment [17]. However, the exogenous P₄ supplementation like intravaginal device during early luteal phase post insemination increased blood concentration of P₄ and conception rate [18].

Fig 1: Conception rate in Repeat Breeding cows treated with post AI Progesterone impregnated vaginal sponge (different day of manufacture) and Untreated control group
Administration of P₄ has been revealed to increase endometrial protein secretion and growth factors concerned in the control of early embryo development [19] and can improve both secretions of anti-luteolytic interferon-s and embryo survival [20]. The higher conception rate in animals by using P₄ may be due to an improved uterine environment for embryo survival and development [11,12].

Conclusion

The present study supports the hypothesis that supplemental progesterone in dairy cows from day 6 to day 16 post insemination will decrease pregnancy loss and increase conception rate. Progesterone impregnated intra-vaginal device may be used for increasing the conception rate in repeat breeding cows which are better to use within 6 months after production. A Suitable adjuvant may be added to improve the keeping quality of the Progesterone impregnated intra-vaginal device for more than 6 months.

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