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# Insulin Pretreatment During Mid-Cycle and Programmed Breeding on Conception of Repeat Breeding Crossbred Cows

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## *ABSTRACT*

The effect of insulin pretreatment during mid-cycle on conception rate of repeat breeding (RB) cows subjected for programmed breeding was studied. A total of 24 RB crossbred cows which were found to be free of subclinical endometritis (SCE) were randomly divided into two groups. Group I (Control; n=12): Animals were inseminated on oestrus (Day 0) without any treatment. Group II – (INS; n = 12): RB cows were administered with Biphasic Insulin product (0.25units / kg BW s.c.) for three days from Day 8 to Day 10 of the cycle (Day 0 – oestrus). Prostaglandin (PG) (Cloprostenol: 500 mcg, i.m.) was administered on Day 11. On the day of induced oestrus (Day 0i) i.e., 48 h post PG, the 12 cows were divided into two subgroups and inseminated with GnRH (n=6) or GnRH + Ins (n=6). Blood samples were collected on Day 0 and 10 in both groups and on the day of induced oestrus (Day 0i) in INS group for Insulin estimation. Insulin concentration was significantly ($P < 0.05$) increased in INS group on Day 10 and Day 0i in response to Ins treatment. In control group, the conception rate among the RB cows was found to be 33.33 per cent. A significantly ($P < 0.01$) high conception rate (66.67%) was recorded in the INS group among RB cows, when inseminated with or without INS during the induced oestrus, than the Control group. It could be concluded that INS therapy during the mid-cycle prior to PG induced programmed breeding can improve the conception rate in RB cows.

***Key words:*** Repeat breeding, Insulin pretreatment, Programmed breeding, Cattle

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# INTRODUCTION

Fertility of the crossbred cows is the backbone of profitable dairy industry, but it is frequently compromised by infertility conditions especially repeat breeding syndrome. The etiology for the infertility was obscure in 37.80 per cent of the repeat breeding (RB) cows (Perez-Marin and Espana, 2007). Apart from the endocrine or ovulatory perturbations, oocyte quality and embryo development were also considered to have a role in infertility (Ferreira *et al.* (2011). Ferreira *et al*. (2016) observed aberrations of oocyte ultra-structures, especially mitochondria, and greater expression of apoptotic genes in oocytes from RB cows than normal cows. A dynamic relationship exists between the follicular micro-environment constituting the follicular fluid (FF) and the developing oocyte. Composition of the FF could influence the oocyte quality (Revelli *et al*., 2009). Altered intra-follicular steroid environment could possibly affect the oocyte quality. Sood *et al.* (2017) recorded 73.00 per cent higher oestradiol concentration in FF of RB cows than normal cows, which would compromise the oocyte quality. Reduced plasma concentrations of metabolic hormone (insulin) and related growth factors (insulin-like growth factor-IGF) were reported to have direct effects on follicular dysfunction (Spicer and Echternkamp, 1995). Satheshkumar *et al.* (2019 and 2021) also reiterated that failure of the intra-follicular IGF / IGFBP system, probably due to deficient nutritional status, lead to ovulatory disturbances and follicular dysfunction in infertile buffaloes. Based on these observations, it was hypothesized that, insulin pretreatment in programmed breeding could improve the conception rate by improving the follicular and oocyte quality in RB cows. Hence the study was conducted to study the effect of insulin pretreatment during mid-cycle on conception rate in RB cows subjected for programmed breeding.

# MATERIALS AND METHODS

Crossbred cows brought with the history of infertility to the Large Animal Gynaecology Unit of Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu, Thanjavur, from in and around Thanjavur district of Tamil Nadu were utilized for the study. Pluriparous ($2^{nd}$ – $6^{th}$ parity) crossbred cows which were reported to be in oestrus and failed to conceive after three or more consecutive inseminations were selected and subjected for thorough gynaeco-clinical and ultrasonographic examination. The regularly cycling RB cows which were in oestrus were subjected for White Side Test (WST) and endometrial cytology to rule out subclinical uterine infection. Animals that were positive for subclinical endometritis (SCE) were not included in the study. A total of 24 RB crossbred cows which were found to be free of SCE were randomly divided into two groups.

## Experimental groups:

Group I (Control; n=12): The RB cows were not subjected for any treatment protocol. Animals were confirmed for oestrus (Day 0) and inseminated. They were examined at 24 hrs. interval for the occurrence of ovulation and reinseminated on second and / or third day until ovulation

Group II (INS; n = 12): RB cows were administered with Biphasic Insulin product (0.25units /kg BW s.c.) for three days from Day 8 to Day 10 of the cycle (Day 0 – oestrus). Prostaglandin (PG) (Cloprostenol Injection I.P, Pragma, Intas pharmaceutical Ltd) 500 mcg, i.m. was administered on Day 11. On the day of induced oestrus (Day 0i) i.e., 48 h post PG, all the 12 cows in the group were randomly divided into 2 subgroups.

INS-Gn (n=6): On the day of induced oestrus (Day 0i), GnRH analogue (Busereline acetate 10 mcg i.m.) was administered along with insemination.

INS-Gn-Ins (n=6): GnRH analogue (10 mcg i.m.) and Insulin (0.25 units /kg BW s.c.) was administered along with the insemination. In both the subgroups, animals were reinseminated on the second day and if the DF persisted, they were inseminated on third day also.

## Conception rate

All the inseminated cows were confirmed for pregnancy by rectal palpation and ultrasound scanning at 60 days post insemination. The conception rates were calculated for each group.

## Estimation of sero-insulin concentrations

Blood samples (3 ml) were collected from all the Control and INS group on Day 0 and Day 10 in the clotting activator vaccutainer by direct jugular vein puncture with the help of sterile 18 G needle. In INS group, blood samples were collected on Day 0i also. The serum was separated by centrifuging blood samples at 1500 rpm for 10 minutes and the serum samples were stored at (-20$^{O}$ C) for further estimation of insulin concentrations. Insulin concentration was estimated by immunoradiometric assay based on coated-tube separation using CT IRMA Kit.

**Table 1: Sero-insulin (µIU/ml ) concentrations in control and treatment groups of repeat breeding cows**

| | Insulin (µIU/ml) | | | Significance |
|---|---|---|---|---|
| | Day 0 | Day 10 | Day 0i | |
| **Control (n=12)** | 8.62 ± 0.42[a] | 7.19 ± 0.26[a] | -- | NS |
| **INS (n=12)** | 8.37 ± 0.84[a] | 9.75 ± 0.26[b] | 9.82 ± 0.47[b] | * |
| Significance | NS | * | | |

Values within the column / row with different superscripts differ significantly * (P < 0.05) NS: Not significant (P > 0.05)

### Statistical analysis

The data concerning the insulin concentrations on various days of the cycle and conception rate in both the groups were subjected to statistical analysis using one way Analysis of Variance (ANOVA) with a completely randomized design using SPSS® 28.0. Software package. The analysis of the data followed the methods outlined by Snedecor and Cochran (1994).

## RESULTS AND DISCUSSION

The data on insulin concentrations on various days of study in control and treatment groups are presented in Table 1. Perusal of the data revealed that the Ins concentration was significantly (P < 0.05) increased in INS group on Day 10 and Day 0i in response to Ins treatment which was in concurrence with the findings of Sheetal *et al.* (2018).

**Table 2: Conception rate between control and treatment groups of repeat breeding crossbred cows**

| S. No. | Groups | Total no. of animals | No. of animals conceived | Conception rate (%) |
|---|---|---|---|---|
| **1** | **Control** | 12 | 4 | 33.33[a] |
| **2** | **INS-Gn** | 6 | 4 | 66.67[b] |
| **2** | **INS-Gn-Ins** | 6 | 4 | 66.67[b] |
| | **Significance** | | | ** |

Values within the column with different superscripts differ significantly** (P < 0.01)

The data on insulin concentrations on conception rate in control and treatment groups are presented in Table 2. In control group, the conception rate among the RB cows was found to be 33.33 per cent. Even without any treatment intervention, the conception rate achieved in the present study could be attributed to repeated inseminations at 24 h intervals for two to three days, based on ultrasonographic follow-up of preovulatory follicles. The approach of repeated inseminations would have settled the conception among the RB cows which experienced delayed ovulation. Thus repeated inseminations at 24 h intervals for two days could be recommended as simple therapeutic approach for the RB as suggested by Arthur *et al.* (1989). A significantly high conception rate (66.67%) was recorded in the INS group among RB cows, when inseminated with or without INS during the induced oestrus, than the Control group. The increase in insulin and IGF-1 levels was reported to have stimulatory effect on follicular growth and regulation of luteal function, through glucose availability and hormone production in cattle (Ponsart *et al.*, 2014). Even though we haven't conducted follicular micro-environment study, based on the observations of improved sero-insulin concentrations, it could be concluded that INS therapy would have potentiated the follicular health and oocyte quality and embryonic development thereon.

## CONCLUSION

From the findings of the study, it could be concluded that INS therapy during the mid-cycle prior to PG induced programmed breeding can improve the conception rate in RB cows.

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## CONFLICT OF INTEREST

None.

## REFERENCES

Arthur, G.H., Noakes, D.E. and Pearson, H. (1989). Ovulatory defects. In: Veterinary Reproduction and Obstetrics, 6th Edn, ELBS, London, Pp.357.

Ferreira, R. M., Ayres, H., Chiaratti, M. R., Ferraz, M. L., Araujo, A. B., Rodrigues, C. A., Watanabe, Y. F., Vireque, A. A.,

Joaquim, D. C., Smith, L. C., Meirelles, F. V. and Baruselli, P. S. (2011). The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. *J. Dairy Sci*., **94:**2383–2392.

Ferreira, R. M., Chiaratti, M. R., Macabelli, C. H., Rodrigues, C. A., Ferraz, M. L., Watanabe, Y. F., Smith, L. C., Meirelles, F. V. and Baruselli, P. S. (2016). The infertility of repeat-breeder cows during summer is associated with decreased mitochondrial DNA and increased expression of mitochondrial and apoptotic genes. *Biol. Reprod*. **94**:66.

Perez-Marin, C. C. and F. Espana. (2007). Oestrus expression and ovarian function in repeat breeder cows, monitored by ultrasonography and progesterone assay. *Reprod. Domest. Anim*., **42:**449–456.

Ponsart, C., Gamarra, G., Lacaze, S. and Ponter A.A. (2014). Nutritional status of donor cows: Insulin related strategies to enhance embryo development. *Anim. Reprod.,* **11**:195–198.

Revelli, A., Piane, L. D., Casano, S., Molinari, E., Massobrio, M. and Rinaudo, P. (2009). Follicular fluid content and oocyte quality: From single biochemical markers to metabolomics. *Reprod. Biol. Endocrinol*., **7**:40.

Satheshkumar, S., Brindha, K., Srinivasa Prasad, Ch., Sakthivel, S., Namitha, B. and Meenambigai, T.V. (2021). Proteo-biochemical characterization of ovarian follicular cysts in buffaloes *(Bubalus bubalis). Ind. J. Vet. & Anim. Sci. Res.,* **50**: 48-56.

Satheshkumar, S., Revathipriya, B., Sakthivel, S., Brindha, K. and Parthiban, M. (2019) Protein Profile of Granulosa Cells in Cyclic and Acyclic Buffaloes. *Ind. J. Vet. & Anim. Sci. Res.,* **48**: 45-51.

Sheetal, S. K., Prasad, S. and Gupta H. P. (2018). Effect of insulin or insulin-like growth factor-I administration at mid-luteal phase of the estrous cycle during superovulation on hormonal profile of Sahiwal cows. *Vet World*., **11:** 1736–1741.

Snedecor, G. W. and Cochran, W.G. (1994). Statistical Methods [8th ed]. Iowa State University Press, USA. pp.

Sood, P., Zachut, M., Dekel, I., Dube, H., Jacoby, S. and Moallem, U. (2017) Preovulatory follicle characteristics and oocyte competence in repeat breeder dairy cows. J. Dairy Sci. 100: 9372–9381.

Spicer, L.J and Echternkamp, S.E. (1995). The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animal. *Dom. Anim. Endocrin*., **12**:223–245.