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Original Research

Ovarian and Fertility Responses Following Administration of GnRH During Estrus and PGF2α during Diestrus in Repeat Breeder Cattle

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Abstract

The study was conducted on crossbred dairy cows (n=30) presented at Veterinary Clinical Complex, with a history of repeat breeding. The study compared ovarian and fertility responses in such animals while using exogenous Gonadotropin Releasing Hormone GnRH, Prostaglandin F (PGF2 α), and double Artificial Insemination (AI) in three groups viz; Group I- (n=10), administered GnRH (Buserelin Acetate @ 20μg total dose) at spontaneous estrus followed by double AI, Group II (n=10)- administered PGF2 α (Cloprostenol Sodium@ 500μg total dose) on day 12 of the cycle, followed by double AI at induced estrus and Group III (n=10) subjected to double AI without any hormonal drug, at spontaneous estrus. The second AI performed 18 hrs of the first insemination in all three groups. Ovulation and conception rates were significantly higher and the duration between mid-estrus (mounting and copious mucus discharge) to ovulation was shorter in Group I ($p<0.05$), compared to Groups II and III. In conclusion, the use of GnRH at the time of estrus in repeat breeder cows is more efficient in terms of inducing faster ovulation with higher ovulation and conception rates. Also breaking the estrus cycle during the luteal phase by PGF2 α, followed by double insemination using frozen semen has an advantage over double insemination in repeat breeders.

Keywords: Cattle, GnRH, PGF2 α, Repeat Breeding

Introduction

Repeat breeding is an established challenge for fertility and production in cattle. Infact, low conception and long calving interval in repeat breeders is a major bottleneck in cattle dairy industry. A study by Fitsum (2017) indicates a key role played by ovaries in reproduction-sterility or infertility. Timely intervention in diagnosis and treatment of ovarian functional abnormalities in repeat breeders may help to mitigate problem of repeat breeding in dairy cows. As a better alternative to rectal palpation, diagnosis can be made if the ovaries are examined using ultrasonography for two successive examinations, one at peak estrus and another 24 hours later. Diagnostic ultrasonography aids in the study of follicular and luteal dynamics in repeat breeders and accordingly an appropriate treatment strategy to control estrus cycle may be devised.

Controlling estrus cycle in repeat breeders with hormone analogues is a potential alternative for improving fertility and production in repeat breeder cattle. Pearson and England, 1993 suggested that delayed ovulation is common causes of failure of conception in cyclic animals. GnRH analogues have been suggested to hasten the timing of ovulation through rapid rise in FSH and LH concentrations, 30 to 60 min post administration (Cunningham, 2002). Also, PGF2α has been suggested to initiate ovulation through demise of the CL- withdrawal of progesterone's negative feedback mechanism (Charlton, 2008). Moreover, in case of mature corpus luteum on ovary of non-pregnant animals, PGF2α analogue is indicated to induce new cycle (Fitsum, 2017). But the comparison of the use PGF2α at diestrus and GnRH at estrus on ovulation and conception has sparse findings in literature. Keeping this in view, the present study will be focused on application of GnRH administration at the time of estrus or $PGF_{2\alpha}$ analogue administration during diestrus to control estrus cycle and improve conception rate in repeat breeder cattle. The study hypothesized to find the superiority of GnRH and $PGF_{2\alpha}$ at different stages of estrous cycle in repeat breeders. Our objectives were to 1) investigate the effect of exogenous GnRH at the time of insemination on ovarian follicular phase dynamics in repeat breeder cattle, 2) investigate the effect of exogenous $PGF_{2\alpha}$ on ovarian luteal phase dynamics in repeat breeder cattle and 3) compare fertility responses in repeat breeders following use of exogenous GnRH or $PGF_{2\alpha}$

Materials and Methods

The animals under study were assigned three groups viz; Group I- treatment animals (repeat breeder cows administered GnRH, n=10), Group II- treatment animals (repeat breeder cows administered $PGF_{2\alpha,,}$ n=10) and Group III- Control animals (repeat breeder cows with no treatment at insemination, n=10). All animals were selected from the cases presented at referral veterinary hospital, Veterinary Clinical Complex, FVSc & AH, Shuhama. Ultrasonography was carried out on the day of estrus/insemination as well as a 12 (n=6) and 24hours after insemination in Group I and III while on day 12 of cycle, day of insemination on induced estrus and 12 (n=5) and 24hours of insemination in group II. The animals of Group I were administered commercially available Buserelin acetate (GnRH analogue) @ 20 µg total dose, whereas animals of Group II were given commercially available Cloprostenol sodium (PGF analogue) @ 500 µg total dose on day 12 of cycle. While Group III were subjected to two inseminations 24 hours later, without any drug. Ovarian and fertility responses were compared between the groups. Ovulation rate was determined based on number of animals ovulated upto 24 hours of peak of estrus (midestrus). Conception rate was determined by diagnosing pregnancy at 45 days of insemination using ultrasonography. Statistical analysis was carried out using t-test and chi-squared test.

Results

Ovulation and conception rates are presented in table. Dominant follicle ovulated Dominant follicle ovulated after 12 hours (n=8) and not ovulated after 24 hours (n=2), in animals subjected to GnRH at estrus. In animals, given $PGF_{2\alpha}$ at diestrus, Mature CL was regressed, dominant follicle was observed 48 hours later (n=8) and ovulated in next 18 hours (n=5). Using only double insemination, dominant follicle was ovulated 18 hours later in 40 % animals (4/10)

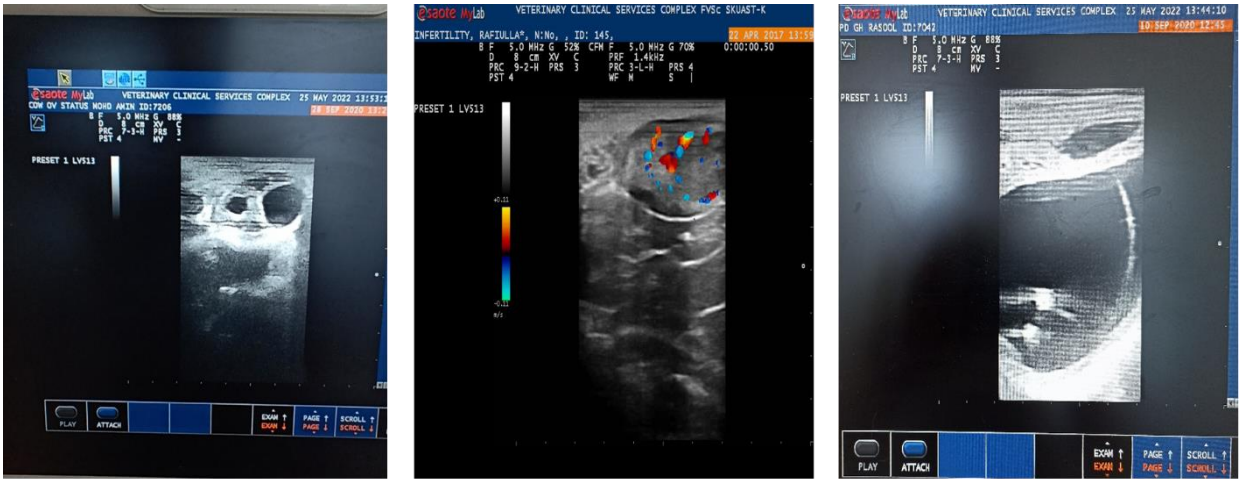
Table 1: Ovarian and fertility responses observed by reproductive ultrasonography

Group	Protocol	Ovulation rate (%)	Conception rate (%)
I (n=10)	GnRH at estrus + Double AI	80^{A}	50^{a}
II (n=10)	$PGF_{2\alpha}$ at diestrus + Double AI	62.5^{B}	30^{b}
III (n=10)	Double AI at estrus	40^{C}	20^{c}
$P<0.05^{A \text{ vs B and C}}$, $P<0.05^{a \text{ vs b and c}}$			

Discussion

The animals were bred 6-7 times before and failed to conceive. All animals otherwise exhibited regular estrus cycles. Thus, in all repeaters, hormonal balance disruptions around ovulation time leading to fertilization failure was expected as a cause of failure of conception. Therefore, owners were advised for the hormonal treatment. Based on these assumptions we, in this study, used GnRH analogue to induce ovulation of a dominant follicle. The dominant follicle and a mature CL are shown in Fig 1A and 1B, respectively. $PGF_{2\alpha}$ was used on day 12 of the estrus cycle, as majority of studies in literature indicate better response of corpus luteum at mid luteal phase. $PGF_{2\alpha}$ terminates luteal phase of estrus cycle by causing lysis of the corpus luteum (CL), thereby inducing new estrus. The animals were scanned on or after day 45 of insemination for pregnancy. The pregnant uterus containing conceptus is shown in Fig. 1C.

Fig. 1: Scanning of ovaries and uterus through ultrasonography, using recto-linear probe (5/7.5MHz)



A-dominant follicle on ovary B-mature corpus luteum on ovary C-conceptus with amniotic membrane

The findings of the present study showed that in repeat breeder cows, GnRH in follicular phase leads to higherovulation and conception rates compared to the use $PGF_{2\alpha}$ in luteal phase. Our findings are supported by a number of previous studies;GnRH administration at estrus has already been found to have significant effect on conception rates in repeat breeders (Morgan and Lean, 1993). Also, other studies reveal that GnRH treatment during estrus prevents failure of ovulation in repeat breeders (Nakao *et al* 1984) and elevates progesterone levelin subsequent stages of cycle (Kaim*et al* 2003) which could be responsible for improving conception rate.Moreover, GnRH at estrus has been documented to induced faster and intense LH peak (Rosenberg *et al* 1991; Ryan *et al* 1994). The findings could better explain our good conception and ovulation rates happening while using GnRH at estrus in clinical cases of repeat breeders. Our findings thus indicate a potential of GnRH at estrus to increase fertility in repeat breeder cows presented at field as well as institutional levels. Furthermore, GnRH, promotes ovulation of Graafian follicle (Mussard *et al* 2007) through hastening of LH surge (Kaim *et al* 2003).In fact, GnRH and LH pulse frequencies, act as a major drive for ovulation of a dominant follicle Crowe *et al* 2014). We, in this study, also hypothesized the breaking/shortening of estrus cycle using $PGF_{2\alpha}$ may promote timed ovulation and improve conception rate in repeat breeders. We achieved 60 and 30% ovulation and conception rates, respectively, while using prostaglandin at luteal phase of repeat breeders, presented at our hospital. An optimal size of dominant follicle was achieved in next 48 hours of prostaglandin shot in animals. In cattle, varying results of conception (in a range

of 13-60 %) have been achieved using one shot of prostaglandin (Adebabay *et al* 2013; Tewodros et al 2015; Weldyesus et al 2016). But, a more supportive report in this regard in sheep reveals that use of PG in luteal phasepromoted follicular growth andgenerated a preovulatory follicle with larger diameter and improved fertility in ewes (Fierro *et al* 2013). In cattle, improvement of conception following estrus synchronization with PGF2α in the mid to late luteal phase is not studied extensively. Although,a number of studies have used prostaglandin in combination with other hormones for effective synchronization of estrus and ovulation followed by acceptable conception rates in cattle (reviewed by Bihon and Assef2021). Therefore, we in the present study, aimed to induce luteolysis by PG in luteal phase to generate a dominant follicle capable of timed ovulation. We obtained an acceptable improvement in ovulation as well as conception rates compared to control animals where no treatment was used. However, this method of treatment was less efficient as compared to use of GnRH at estrus.

Double AI, used to cover ovulation window in animals, was hypothesized to maintain availability of sperm for prolonged duration in the tract, to improve conception rate. Following two inseminations at about 18 hours interval improved fertility, although results were lesser compared to other groups in the study (data presented in table). Pregnancy rate improvements have been reported long back by Bostedt (1976) in animals subjected to re-insemination after 24 hours. Similarly, Singh *et al* (2005) reported higher pregnancy rate in repeat breeding heifers where inseminations were repeated at 6 hours interval in a spontaneous estrus. Although results obtained by double AI in our study were lesser,but, it can be speculated that repeated inseminations might prove effective in enhancing fertility in cows especiallyin case of delayed ovulations. However, hormonal therapy with GnRH or LH may be more effective in solving the problem.

Conclusion

Use of GnRH at the time of estrus in repeat breeders is more efficient in terms of more conception rate, Use of $PGF_{2\alpha}$ during luteal phase has acceptable ovulation and conception rates and use of GnRH and $PGF_{2\alpha}$+ double AI have advantage over only double AI procedure

Contribution by authors

All the authors contributed equally to writing the manuscript. The final manuscript was read by all others and consented to publication.

Conflict of Interests

There is no conflict of interest.

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