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Research Article

FERTILITY ASSESSMENT IN RELATION TO OVARIAN FOLLICULAR DIMENSIONS AND GnRH THERAPY IN DAIRY CATTLE

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ABSTRACT

The present study was carried out to observe the effects of GnRH at the time of AI and mid luteal phase on conception rate in dairy cows. The cows were randomly divided in 4 treatment and 2 control groups. Group I (n=10) cows were injected with 10 µg Buserelin acetate intramuscularly at the time of AI. Group II (n=10) cows were inseminated twice with AM/PM rule and injection Buserelin acetate was administered intramuscularly at the time of first AI after detection of oestrus. Group III (n=10) cows were inseminated after estrus detection and 10 µg Buserelin acetate was injected intramuscularly on day 12 post-insemination. Group IV (n=10) cows were inseminated twice with AM/PM rule after detection of estrus and 10 µg Buserelin acetate was injected intramuscularly on day 12 post-insemination. Group V (Control group; n=10) cows inseminated once after detection of oestrus; and Group VI (Control group; n=10) inseminated twice after detection of oestrus. The percentage of cows with ovulatory follicles on the day of observed oestrus were ranging between 1.0 and <1.3 cm; ≥1.3 and <1.5 cm; ≥ 1.5 cm was found to be 47.76 %, 37.31 % and 14.92 %, respectively in all groups ($p>0.05$). The mean diameter of ovulatory follicles including all groups of experimental animals was found to be 1.29±0.019 cm. Out of 47 cows treated, 27 (57.45 %) cows were found to be pregnant. The average conception rate in control group was 50 %. The study observed that there was no significant difference ($p>0.05$) on conception rate among all six groups of dairy cows including treated and control groups, when GnRH injection was administered @ 10 µg i.m. on the day of insemination during natural estrus or on day 12 days post AI by adopting either single or double insemination.

Keywords: Dairy cattle, Fertility, Follicular dimensions, GnRH, Insemination

It has become evident that the success of gestation establishment in dairy animals is directly associated to the competence and diameter of the ovulatory follicle. Bovine follicles achieve ovulatory capacity at a diameter of approximately 10 mm. In an attempt to increase pregnancy rate and to reduce embryonic losses, various hormonal treatments like GnRH and its analogues have been administered to normal cyclical cows prior to artificial insemination or at the time of artificial insemination or during the mid cycle following artificial insemination. Administration of GnRH during estrus in cattle induce pre-ovulatory peak of LH from the pituitary within 2 -4 h and ovulation within 24 -36 h, depending on the developmental stage of the follicle. GnRH analogue treatment twelve days post AI of cattle is the most suggested solution to reduce the incidence of repeat breeding and to enhance conception rate in cows (Hailu *et al.*, 2014). With the aim of enhancing conception rate, the present study was conducted with specific objectives of evaluating effects of GnRH analogue at the time of insemination and 12 days post insemination, assessment of ovarian follicular dimensions ultrasonographically during estrus before artificial insemination and to evaluate relationship of fertility in dairy cattle.

MATERIALS AND METHODS

The sixty normal cyclic dairy cows presented for artificial insemination (AI) at college hospital were included in this research work. The cows were divided in six treatment groups. The reproductive status of all the experimental cows was assessed by performing rectal and ultrasonographic examination. Cervical mucus of each cow was collected during estrus when presented for AI and white side test (WST) was performed to assess the possibility of uterine infection. The animals with a positive result for infection on white side test were not included in this study. The onset of estrus in each experimental cow was recorded based on the information provided by owners which was further monitored by observing behavioral signs viz., homosexual behavior, bellowing, frequent urination etc.

Ovarian structures were evaluated by transrectal ultrasonographic imaging using real-time B-mode instrument with a multi-frequency curvilinear array transducer (Hitachi-Aloka F-31 equipped with a 7.5 MHz transducer) to monitor ovarian follicular dimensions The ultrasound image was frozen when the Graafian follicle (GF) was visualized to be at its maximal size and the GF was determined by taking the mean of 2 perpendicular diameter measurements length (L) and width (W). The dominant follicle of the wave was defined as the one that grew to at least 1 cm and exceeded the diameter of other follicles in the wave. The treatment protocol is depicted in Table 1.

Cows were inseminated using recto-vaginal method with frozen thawed semen of respective breed straws.

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Table 1

Protocol and treatment wise random distribution of experimental cows

Group of Animals	Treatment*	Dosage	Day of Treatment	Insemination
Group – I (n=10)	Inj. Buserelin acetate	2.5ml (10µg), IM	Time of AI	Single
Group – II (n=10)	Inj. Buserelin acetate	2.5ml (10µg), IM	Time of AI	Double (AM/PM)
Group – III (n=10)	Inj. Buserelin acetate	2.5ml (10µg), IM	Day 12 post A.I.	Single
Group – IV (n=11)	Inj. Buserelin acetate	2.5ml (10µg), IM	Day 12 post A.I.	Double (AM/PM)
Group – V (n=10)	No treatment given (Control Group)			Single
Group – VI (n=10)				Double (AM/PM)

*Inj. Zydarelin (Each ml contains 4.2 mcg Buserelin acetate; equivalent to Buserelin 4mcg)

Records were maintained of dates of return to estrus. Pregnancy status of all the experimental cows was determined using real-time B-mode ultrasound scanner with multi-frequency curvilinear probe (7.5 MHz) from day 30-40 post AI. Cows diagnosed pregnant were re-examined four weeks later to confirm pregnancy status and to identify pregnancy loss. The conception rate was calculated by dividing the total number of pregnant cows by the number of inseminated cows. Chi-square test and completely randomized designs (CRD) – ANOVA was used to evaluate significant differences for parameters.

RESULTS AND DISCUSSION

Ultrasonographic Measurements of Ovarian Follicles in Cows

Ovaries were scanned by trans-rectal ultrasonography for the measurement of pre-ovulatory follicular size on the day of natural estrus concurrent with AI in each experimental cow. The diameter and location of all antral ovarian follicles ≥ 0.5 cm within the ovaries were measured and recorded. Diameters of individual ovulatory follicles ranged from 1.00 cm to 2.00 cm including all animals of treatment and control groups. The percentage of cows with ovulatory follicles ranging between 1.00 and <1.3 cm; ≥1.3 and <1.50 cm; ≥ 1.50 cm were found to be 47.76, 37.31 and 14.92 %, respectively including cows of all groups. The mean diameters of the ovulatory follicles among different groups of animals on the day of natural estrus concurrent with artificial insemination did not differ significantly ($p>0.05$). However, no literature is available to compare the effect of ovulatory follicle sizes on conception rates in cows in the present study.

Effects of ovarian follicular dimensions, GnRH administration during estrus or on day 12 post AI on conception rate in cows

It become evident that the success of gestation establishment is directly associated with the competence and diameter of the ovulatory follicles. Therefore, the study was aimed to evaluate relationship of fertility with preovulatory ovarian follicle size in normal cyclic cows. The results pertaining to fertility on account of ovarian follicular size on the day of oestrus in different groups of cows are presented in Table 2.

Table 2

Analysis of ovarian follicular dimensions and conception rate in different groups of dairy cows

Group of Cows	Follicle Diameter (cm)	AI	No. of Cows Inseminated	GnRH Treatment (10 µg i.m.)	No. of Cows Conceived	Conception Rate (%)
I	1.37±0.77	Single	12	Day 0	7	58.33
II	1.32±0.06	Double (AM/PM)	13	Day 0	9	69.23
III	1.24±0.07	Single	11	Day 12	7	63.63
IV	1.26±0.05	Double (AM/PM)	11	Day 12	4	36.36
V	1.28±0.05	Single	10	Control	5	50.00
VI	1.30±0.04	Double (AM/PM)	10	Control	5	50.00
Avg	1.29±0.019	-	67 (Treatment Group : 47 & Control Group: 20)	-	37 (Treatment Group : 27 & Control Group: 10)	55.22 (Treatment Group: 57.45 % & Control Group: 10 %)

No significant differences ($p>0.05$) ovulatory follicles among different groups

No significant differences ($p>0.05$) on conception rates among different treatment groups

The mean diameter of ovulatory follicles including all groups of experimental animals was found to be 1.29±0.019 cm. The mean diameters of the ovulatory follicles among different groups of animals on the day of natural estrus concurrent with artificial insemination did not differ significantly ($p>0.05$). There were no significant differences ($p>0.05$) in conception rates among all six groups of dairy cows including treatment and control groups, when GnRH injection was administered @ 10 µg i.m. on the day of insemination during natural estrus or on day 12 post AI by adopting either single or double insemination.

Out of 67 dairy cows including all six groups (treatment and control) in this study, 37 cows were found to be pregnant with an overall conception rate of 55.22 %. The study observed that there were no significant differences ($p>0.05$) on conception rates among all six groups of dairy cows including treatment and control groups, when GnRH injection was administered @ 10 µg i.m. on the day of insemination during natural oestrus or on day 12 days post AI by adopting either single or double insemination during observed oestrus.

The conception rate in cows subjected to single artificial insemination during observed estrus including treatment and control groups (Group I, III and V: n=33) was recorded as 57.57 %, whereas conception rate in cows subjected to double insemination in treatment and control groups (Group II, IV and VI: n=34) was found to be 52.94 %. Overall pregnancy rates in cows treated with GnRH agonist (Groups I, II, III and IV: n= 47) either on day 0 or 12 post AI were recorded as 57.48 % compared with 50% in control groups (Groups V and VI: n= 20) when no treatment was advocated. In the present study, overall pregnancy rate when GnRH treatment was administered on the day of observed oestrus (Group I- single AI and group II- double AI.: n=25) was recorded as 64.00% whereas the overall pregnancy rate in the cows when treated with GnRH on day 12 post AI (Group III- single AI and group IV- double AI.: n=22) was found to be 50 %. The pregnancy rate in control non-treated group (Group V) when the cows were subjected to single AI was recorded as 50 % whereas in cows in control non-treated group (Group VI) subjected to double AI was also found to be 50%. Conception rate including four treated groups (Group I, II, III and IV) was higher than the conception rate in two control groups (Group V and VI).

The study demonstrated that supplementation of Buserelin acetate to AI (single or double) on day 0 or day 12 post A.I. improved pregnancy rate. Perry *et al.* (2005) reported a tendency of cows that ovulated follicles <12.1 mm in diameter to be less likely to support pregnancy after insemination compared with cows that ovulated with 14.7 mm follicles. No cows ovulated with follicles >17 mm or <10 mm in diameter. Echternkamp *et al.* (2014) observed that ovulatory follicles ranging in diameter from 14 -17.9 mm yielded the greatest conception rates in cattle. Pfeifer *et al.* (2012) stated in their study that cows with ovulatory follicles ranging from 13-15 mm of diameter presented higher pregnancy rate than those detected with larger or smaller follicles. Perry *et al.* (2007) concluded that small follicles were likely to ovulate in response to exogenous GnRH treatment than larger follicles, with anovulated follicles having a diameter of 10.1±0.7 compared with the 12.1±0.2 mm diameter of ovulated follicles. Perry *et al.* (2007) reported maximum pregnancy rate of 68% at a follicular size of 12.8 mm in cows.

The conception rates lower than the present results were reported by Stevenson *et al.* (1990) when cows were inseminated once and administered with 100 µg GnRH (Cystorelin) during estrus with conception rate of 41.6%. When cows were inseminated twice on the day of estrus and treated with 100 mcg of GnRH, the conception rate was 37.5%. Gumen *et al.* (2011) performed single insemination in cows on the day of estrus and administered treatment of buserelin acetate @ 10 µg intramuscular and observed conception rate of 44.3% whereas they reported conception rate of 47.6% in control groups of animals. Kantharaj *et al.* (2015) reported 18.18 % conception rate in repeat breeder cow subjected to single AI and 27.27% conception rate when subjected to multiple AI during observed estrus in cows. In the present study, the results of conception rates (63.78%) when cows were treated with GnRH agonist on the day of estrus corroborate with the reports of Ryan *et al.* (1994) (61.6%). The results of conception rates in the present study when cows were treated with GnRH agonist on the day of estrus were higher than the conception rates reported in GnRH treated cows on day 0 by Gumen *et al.* (2011) (44.3%); Hailu *et al.* (2015) (59%) and Mehrotra *et al.* (2015) (32%). Whereas higher conception rates were recorded by Parikh *et al.* (2018) (66.66%) than the results of the present study when cows were treated with GnRH agonist on day 0.

When the cows were treated with 10 µg of GnRH agonist on day 12 post AI, the conception rates (50%) recorded in the present study were higher than the reports of Dirandeh *et al.* (2014) (20%) in cows, whereas, Ryan *et al.* (1994) (60.6%) and Jaswal and Singh (2013) (67.69%) observed higher conception rates than the conception rates observed in the present study in cows.

The pregnancy rates in the present study in control

groups of cows (50%) when no treatment was administered are in agreement with the results of Yildiz *et al.* (2009) (50%). The conception rates were higher than the present results when the cows were untreated (control) as investigated by Ryan *et al.* (1994) (62.3%) and Jaswal and Singh (2013) (55.38%) whereas lower results in cows with respect to conception rates were obtained by Hailu *et al.* (2015) (32%); Mehrotra *et al.* (2015) (20%) and Parikh *et al.* (2018) (25%) than results of present study in cows.

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