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Short Communication

Effect of Different Hormonal Therapies on Day 5 of Estrus on Plasma Progesterone Profile and Conception Rates in Repeat Breeding Dairy Cows

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Abstract | The effect of treatment with a gonadotrophin-releasing hormone (GnRH) analogue-Buserelin acetate, human chorionic gonadotrophin (hCG) and exogenous hydroxy-progesterone caproate on plasma progesterone profile and conception rates was investigated in 44 repeat breeding dairy cattle. Repeat breeding dairy cows on Day 5 post artificial insemination were randomly assigned to four groups: control (no treatment, n=11), Inj GnRH analogue (buserelin acetate, 20µg, n=11), Inj hCG (3000 IU, n=11), or Inj hydroxy progesterone caproate (500 mg, n=11) administered once IM. Progesterone concentration (ng/ml) was determined in plasma of cows on Day 0, 5, 12 and 19, and pregnancy diagnosis was undertaken on Day 60 by rectal palpation. The plasma progesterone concentrations on the Day of insemination were not significantly different between the treatment groups. The plasma progesterone concentration increased significantly (P<0.01) on Day 5 in all cows in all groups. On Day 5 the differences between different treatment groups were non significant. The plasma progesterone concentrations one week after treatment i.e. on Day 12 were significantly higher compared to values on Day 5 in treatment GnRH and treatment hCG (P<0.01). In progesterone treatment and untreated control cows although the plasma progesterone concentrations increased from 2.12±0.25 ng/ml on Day 5 to 4.99±0.23 ng/ml on Day 12 in progesterone treatment and 2.02±0.08 ng/ml on Day 5 to 4.24±0.23 ng/ml on Day 12 in control cows, the differences were non significant. GnRH and hCG treatments increased the plasma progesterone and this probably supported the higher conception rates in these groups. On Day 19 the plasma progesterone concentrations were significantly higher (P<0.01) in all groups compared to progesterone profile on Day 0 and significantly higher (P<0.01) compared to concentrations on Day 5 in GnRH and hCG treatments. When compared to concentrations on Day 12 the progesterone concentration decreased significantly (P<0.01) in hCG treated cows on day 19. Transrectal palpation after treatment in treated cows revealed the formation of accessory corpus luteum in 27.27% cows in GnRH treated, 0% in progesterone treated, and 36.36% in hCG treated cows. The pregnancy rates were highest in hCG treatment (45.45%) followed by GnRH (36.36%) and progesterone (27.27%) compared to untreated control cows (18.18%). It was concluded that the hormonal therapies on Day 5 post insemination increase the plasma progesterone concentration and conception rates in repeat breeding dairy cattle with hCG treatment being the best.

Keywords | hCG, GnRH, Repeat breeding, Conception, cow, Progesterone

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INTRODUCTION

Repeat breeding is an important reproductive disorder which causes great economic losses to dairy farmers or industry (Purohit, 2008). The repeat breeder animal is usually defined as a subfertile animal which has been served three or more times with a fertile bull or inseminated with fertile semen yet fails to conceive and continually returns to

estrus in the absence of any obvious pathological disorder in the genital tract (Purohit, 2008). The cause of this type of subfertility can be divided into two major categories; fertilization failure and early embryonic deaths. Embryo mortality (EM) contributes more to pregnancy failure in cattle than fertilization failure (Diskin and Sreenan, 1980). Pregnancy failure results mainly from embryonic mortality which may or may not result in prolongation of luteal function (Diskin and Sreenan, 1980).

In dairy cows, luteal insufficiency and lower progesterone (P4) concentrations are known as a cause of embryonic mortality that reduce the pregnancy rates during early embryonic development (Shams-Esfanabadı and Shırazı, 2006). Low P4 concentrations during early pregnancy may affect embryonic development and maternal recognition of pregnancy. Lower circulating P4 concentrations from Days 3–8 post-ovulation are associated with smaller embryos on Day 16, and consequently, may result in ineffective interferon-tau secretion to block the luteolytic process and to maintain pregnancy (Mann and Lamming, 2001).

In repeat-breeder cows, the time when the embryo enters the uterus and undergoes blastocoele formation (Day 6–8) has been suggested to be a critical period during which embryonic death occurs (Shelton et al., 1990). Also, a slower than normal rise in progesterone concentration and a lower total progesterone concentration have been reported in low-fertility cows and repeat-breeder heifers in the first 6 days after estrus (Shelton et al., 1990).

Treatment with GnRH (Lewis et al., 1990) or hCG (Santos et al., 2001) a few days after AI increases P4 secretion due to the luteotrophic effect (Santos et al., 2001) and/or induction of an accessory corpus luteum (Santos et al., 2001; Hanlon et al., 2005) thus increasing progesterone concentration and potentially increasing conception. The accessory CL increases plasma progesterone concentrations and also could decrease the estrogenic environment during the period of pregnancy recognition (Schmitt et al., 1996b). Administration of 1500 IU of hCG 5 days after insemination improved first service conception rates in dairy cows (Hanlon et al., 2005).

Another approach to improve embryonic survival in repeat breeder cows is direct application of progesterone. Progesterone Releasing Intra vaginal Devices (PRID), Controlled Internal Drug Release (CIDR) and Synchro-Mate ear implants are some of the preparations which are commercially available (Shams-Esfanabadi and Shirazi, 2006). Thus, several strategies have been used to increase progesterone concentration after breeding for improved embryo survival. These strategies have been employed to test concepts about the value of P4 supplementation, but may not be practical for use in large herd operations. Despite the embryotrophic effects of increased P4 concentrations, administration of P4 during met-estrus or early diestrus may compromise the CL development (O'Hara et al., 2014), as P4 acts by inhibiting LH secretion and, consequently, luteal cell differentiation. In addition, early P4 supplementation (1–3 Days post-oestrus) may lead to earlier luteolysis and result in shortened estrous cycles in cattle (O'Hara et al., 2014). Although P4 supplementation from Days 1 to 4 post-oestrus has detrimental effects on CL function, later supplementation (Days 3–6, 4–7 or 5–8) may not have such effects (Ginther, 1970). Collectively, timing, formulation and mass of P4 supplementation are the main factors involved in the paradoxical effect of supplementary P4 on CL function and conceptus development. Injectable, single-dose long-acting P4 drugs are an alternative to P4 supplementation. Administration of GnRH, hCG or used CIDR resulted in increased pregnancy per artificial insemination in repeat breeder dairy cows (Khoramian et al., 2011).

In lieu of the problem of low luteal progesterone as a reason for embryonic deaths the present study examined the effect of administration of GnRH, hCG and progesterone injections on Day 5 of estrus on plasma progesterone and conception rates in repeat breeding dairy cows.

MATERIALS AND METHODS

Repeat breeding cows (n=44) were inseminated with fertile frozen semen and a blood sample was collected. On Day 5 post insemination cows were randomly treated with either of the following treatments in equal numbers; hCG 3000 IU IM once (n=11), GnRH 20 µg IM once (n=11), hydroxyl-progesterone acetate 500 mg IM once (n=11) and untreated control (n=11). Blood (10 ml) was collected in heparinized pre-sterilized test tubes by jugular vein puncture. Samples were centrifuged and plasma was separated and stored at -20^{0}C till further assay. Samples were collected on Day 0 (Day of insemination) and Day 5 and then at weekly intervals i.e. on Day 12 and 19. Transrectal palpation was done on the Day of insemination and at Day 5, 12 and 19 to evaluate CL development as described previously (Hanlon et al., 2005). Transrectal palpation was again performed at 60 Days post insemination in cows that did not return to estrus, to evaluate the number of cows that became pregnant.

Assay for plasma progesterone was done by solid phase enzyme immuno-assay using commercially available progesterone kits (DIALAB Wiener Neudrof- Austria). Each kit was having micro plates for 96 wells. The results were read on an Elisa reader (Lab systems, Finland).The plasma progesterone profiles in different treatments on different days of cycle were compared by ANOVA and DNMRT and results are presented as mean ± SEM.

Table 1: Comparative analysis of plasma progesterone concentrations at Day 0,5,12 and 19 of estrus in repeat breeding treated and untreated (control) cows

Group	Day 0	Day 5	Day 12	Day 19	Overall
Control	0.35±0.02^{Aa}	2.02±0.08^{Ab}	4.24±0.23^{Ac}	3.78±0.63^{Ac}	2.60±0.28^{A}
GnRH	0.36±0.03^{Aa}	2.04±0.15^{Ab}	6.90±0.15^{BCc}	6.04±0.98^{Bc}	3.84±0.48^{B}
Progesterone	0.41±0.03^{Aa}	2.12±0.25^{Ab}	4.99±0.23^{ABc}	4.26±0.58^{Ac}	2.95±0.32^{A}
hCG	0.41±0.04^{Aa}	2.06±0.13^{Ab}	8.2±0.12^{Cd}	6.36±1.27^{Bc}	4.258±0.57^{B}
Overall	0.38±0.02^{a}	2.06±0.08^{b}	6.08±0.25^{d}	5.11±0.47^{c}	3.41±0.22

GnRH: gonadotrophin-releasing hormone; hCG: human chorionic gonadotrophin

Means in a column with uncommon uppercase superscripts differ significantly between groups (P<0.01); Means in a row with uncommon lowercase superscripts differ significantly between days ineach treatment (P<0.01).

RESULTS AND DISCUSSION

The plasma progesterone concentration increased significantly (P<0.01) on Day 5 in all cows in all groups suggesting ovulation, however, the differences between different treatment groups were non significant. The plasma progesterone concentrations one week after treatment i.e. on Day 12 were significantly higher (P<0.01) compared to values on Day 5 in treatment GnRH and treatment hCG (Table 1). In progesterone treatment and untreated control cows although the plasma progesterone concentrations increased from 2.12±0.25 ng/ml on Day 5 to4.99±0.23 ng/ml on Day 12 in progesterone treatment and 2.02±0.08ng/ml on Day 5 to 4.24±0.23 ng/ml on Day 12 in control cows, the differences were non significant.

On Day 19, the plasma progesterone concentrations were significantly higher (P<0.01) in all groups compared to progesterone concentrations on Day 0 and significantly higher (P<0.01) compared to concentrations on Day 5 in GnRH and hCG treatments. When compared to concentrations on Day 12 the progesterone concentration decreased significantly (P<0.01) in hCG treated cows on Day 19.

Transrectal palpation on Day 12 after treatment in treated cows revealed the formation of accessory corpus luteum in 27.27% cows in GnRH treated, 0% in progesterone treated and 36.36% in hCG treated cows. However, at Day 19 accessory CL formed was palpable only in one cow in GnRH treated and in two cows that were treated with hCG.

The pregnancy rates were highest in hCG treated (45.45%) followed by GnRH (36.36%) and progesterone (27.27%) treated compared to untreated control cows (18.18%).

Increase in plasma progesterone on Day 12 with all the three treatments used in this study (GnRH, progesterone injections and hCG) reflects that the treatments were helpful in increasing the plasma progesterone concentrations and this increase was maintained upto Day 19 in GnRH and progesterone treatments however, with hCG treatment there was an initial high increase followed by a decrease at Day 19 although the plasma progesterone was still higher compared to control or the concentrations on Day 5. Formation of a palpable accessory CL in 27.7% of GnRH treated and 36.36% of hCG treated cows reflects that partially the rise in progesterone is due to formation of the accessory CL and the disappearance of accessory CL probably resulted in decline in the P4 in the hCG treated cows at Day 19 compared to that at Day 12.

Previous studies have shown that the administration of exogenous hormones such as hCG or GnRH on Day 5 of estrus results in formation of accessory CL that maintains high plasma progesterone (Musilova et al., 2014; Pandey et al., 2016; Hanlon et al., 2005). The accessory CL formed after induction of ovulation of the first-wave dominant follicle on Day 5 or 7 of the estrous cycle (Schmitt et al., 1996b; Santos et al., 2001; Hanlon et al., 2005) and consequent increase in plasma progesterone (P4) concentration is expected to minimize the luteolytic cascade by endometrial cells during the period of maternal recognition of pregnancy, which favors pregnancy maintenance (Mann et al., 2001).

Administration of progesterone injections would support the luteal production of progesterone and thus probably prevent the luteolytic cascade due to embryonic deaths. Kimura et al. (1987) noted that the delayed formation of CL induces estrus repetition, and suggested progesterone therapy on 4th or 5th Day post insemination. Studies have shown that administration of exogenous hydroxy progesterone injections to repeat breeder cows on Day 5-7 improves conception rates (Srivastava and Kharche, 2001; Awasthi et al., 2002).

A recent study (Jeong et al., 2016) revealed that GnRH administration after 5 days of timed AI in dairy cows resulted in the ovulation of a dominant follicle (DF) and formation of an accessory CL. Similar results were observed with the administration of hCG on Day 5 in cows (Schmitt et al., 1996a; Hanlon et al., 2005). However, ovulation did not occur in all cases after GnRH treatment because the ability of a follicle to ovulate depends on the acquisition of LH

receptors on its granulosa cells (Jeong et al., 2016).

Thus, although administration of GnRH or hCG on Day 5 improves pregnancy rates as was seen in the present study, it does not essentially result in formation of accessory CL and the higher progesterone concentrations could be due to support to the existing CL.

CONCLUSIONS

It was concluded that the hormonal therapies on Day 5 post insemination increase the plasma progesterone concentration and conception rates in repeat breeding dairy cattle with hCG treatment being the best.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

AUTHORS CONTRIBUTION

Part of MVSc research work carried out by the Surendera Kumar the guidance of Prof GN Prohit.

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