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Effect of multiple insulin injections on estrus expression in cross bred cows

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Abstract
The present study was conducted on 12 cross bred cows divided into two groups (n=6 each with 6) Group I and Group II. Both groups on day 10 post estrum received an injection of PGF2α. In addition group I received insulin @ 0.25 IU/Kg B.Wt, SC every 24 hours until estrus expression while cows in group II received every 12 hours on the day of induced estrus (at 0 hr, 12 hr and 24 hr). The results showed a 100% estrus induction rate in both groups. The mean time taken for estrus induction was 71.00 ± 4.97 hrs in Group I and 65.33 ± 5.17 hrs in Group II, with no statistically significant difference between the groups ($P > .05$). The duration of estrus was found to be 25 ± 0.85 hrs in Group I and 23.83 ± 1.32 hrs in Group II ($P > 0.05$). The mean estrus intensity score was 16.16 ± 1.10 in group I and 14.00 ± 1.18 in group II, respectively ($P > .05$). Among the total number of cows in the present study, most of the cows expressed intense (58.33%) to normal estrus (41.66%). A higher proportion of cows exhibited intense estrus (66.66%) in group I compared to group II (50.00%). The present study could be concluded that the administration of multiple insulin injections have a positive impact on parameters of estrus expression. However, the influence may be subtle or variable and influenced by a range of factors.

Keywords: PGF2α, insulin, estrus characters, crossbred cows

1. Introduction
Reproductive management of infertile animals had been identified as a key area for enhancing the animal production. Metabolic hormones and growth factors like insulin, somatotrophin, and Insulin-like growth factor (IGF) are receiving significant attention in regulating ovarian functions. Application of metabolic hormones like insulin to regulate reproductive functions in livestock is a recent advancement. Administration of exogenous insulin in cattle raises intrafollicular and peripheral IGF-I levels (Simpson *et al,* 1994) [1]. Elevated plasma IGF-I concentrations during follicular phase following insulin administration may affect follicle development and the function of corpus luteum (Leeuwenberg *et al.,* 1996) [2]. Insulin is affordable, readily available and appears to be useful to improve the reproductive performance in cattle and buffaloes. Continued exploration of nuanced effects of insulin administration on follicular development and overall reproductive performance will pave the way for targeted interventions aimed at optimizing fertility and addressing reproductive challenges in cows. Hence, the present investigation has been undertaken to evaluate the effect of multiple insulin injections on estrous expression in cross bred cows.

2. Materials and Methods
For the present study 12 apparently healthy cows, calved at least once through normal delivery and free from uterine infections were enrolled. Cross bred cows weighing approximately 250 to 350 kg and 2.5-3.5 body score condition were selected. The cows were divided into two groups *viz*., Group I and Group II. On day 10 post estrus all the cows in both the groups received an injection of PGF2α. In addition cows in group I received insulin @ 0.25 IU/Kg B.Wt, SC every 24 hours until estrus expression while cows in group II received insulin @ 0.25 IU/Kg B.Wt, SC every 12 hours on the day of induced estrus (at 0 hr, 12 hr and 24 hr). The animals were observed twice daily for behaviuoral signs of estrus like bellowing,

discharge through vulva, vulval oedema and hyperemia of vestibular mucosa after PGF2α injection. The estrus induction rate was calculated from the number of animals responded to PGF2α injection expressed in percentage. The estrus induction interval was calculated from the time of administration of PGF2α to the time of the first expression of estrus signs and it is calculated in hours. Duration of estrus was recorded in hours as the interval between the onset and cessation of estrus signs. The intensity of the estrus was measured according to the score card designed by Rao and Rao (1981) [3] with marginal modifications (Table .1). The intensity of estrus was classified as weak, normal and intense estrus.

Table 1: Score card for assessment of intensity of estrus in animals

Estrus Symptoms	Points allotted
Bellowing	2
Homosexuality	4
Excitement (or) restlessness	2
Estrual mucus discharge	4
Edema, congestion and wetness of vulva	3
Raising of tail in response to placing of palm on the rump region	3
Micturition	1
Off fed	1
TOTAL	20

Weak estrum - < 7 points.
Normal estrum – 8 -14 points
Intense Estrum - > 14 points

3. Results and Discussion

In this study, all the animals in both the groups exhibited estrus signs accounting to a 100 per cent estrus induction rate (Table 2). This aligns with previous studies by Selvaraju *et al.* (2002) [4] and Mani *et al.* (2021) [5], who also reported 100% estrus induction rates following PGF2α administration. The mean estrus induction interval following PGF2α injection in both the groups was 71.00 ± 4.97 hours in group I and 65.33 ± 5.17 hours in group II. The difference was not statistically significant ($P > .05$). The results of this study are comparable to those reported by other researchers. Mujic *et al.* (2012) [6] reported that the mean interval of estrus induction was 3.92 ± 0.49 to 4.69 ± 0.68 days from treatment with single PGF2α injection. Selvaraju *et al.* (2002) [4] reported a longer time for estrus onset in insulin-treated cows (84.5 ± 6.6 hours) compared to control cows (72.3 ± 5.9 hours). Conversely, Shukla *et al*. (2005) [7] found a shorter time for estrus onset in cows treated with insulin and GnRH combined. The mean duration of estrus in group I and group II were 25 ± 0.85 hours and 23.83 ± 1.32 hours, respectively. The observed difference was not significant statistically ($P > .05$). These findings were consistent with the results reported by Velladurai *et al.* (2015) [8], Nayana *et al.* (2020) [9], and Ratnaparkhi *et al.* (2019) [10], who documented similar durations of induced estrum in cows, with mean durations of 28.50 ± 0.56 hours, 28.13 ± 1.06 hours, 29.38 ± 0.74 hours, and 23.80 ± 0.55 hours, respectively. In group I, 66.66% and 33.33% cows exhibited intense and normal estrus, respectively. While in group II cows intense and normal estrus was expressed 50.00 per cent each. There was no significant difference ($P > .05$) in the instensity of estrus between the groups. The mean estrus intensity score was 16.16 ± 1.10 in group I and 14.00 ± 1.18 in group II with no statistically significant difference ($P > .05$) between the groups. The mean estrus intensity scores indicate that both groups exhibited relatively high levels of estrus behavior, though the difference between the groups was not significant. The results in the present study showed higher percentage of animals with intense estrus contrary to earlier studies (Velladurai *et al.*, 2015 and Ratnaparkhi *et al*., 2019) [8, 10]. However, Mani *et al.* (2021) [5] reported that 87.50 per centage cows exhibited very good estrus after double PGF2α injection. Role of insulin in promoting follicular growth and steroidogenesis through the IGF system suggested a mechanism by which it might affect estrus behavior (Lucy, 2000 and Butler, 2003) [11, 12].

Table 2: Characteristics of induced estrus in crossbred cows

Groups	Number of animals (n)	Estrus induction rate (%)	Estrus induction interval (hours) (Mean±SE)	Duration of estrus (hours) (Mean ±SE)	Estrus intensity score (Mean ±SE)	Estrus intensity (% of animals)	
						Intense	Normal
Group I	6	100	71.00 ± 4.97	25 ± 0.85	16.16 ± 1.10	66.66	33.33
Group II	6	100	65.33 ± 5.17	23.83 ± 1.32	14.00 ± 1.18	50.00	50.00

4. Conclusion

Critical analysis of results indicated that administration of multiple insulin injections have a positive impact on parameters of estrus expression. However, the influence may be subtle or variable and influenced by a range of factors.

5. Conflict of Interest

Not available

6. Financial Support

Not available

7. References

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