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Assessment of conception rate and number of artificial insemination required for pregnancy in artificially induced crossbred repeat breeder animals by using reserpine and dexamethasone

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Abstract
The artificial induction of lactation were carried out in 30 animals, divided into three groups with 10 animals in each group. Group I, animals which were in first lactation served as control, not given any treatment. Group II, administered with 17β-estradiol at 0.1 mg/kg body weight and progesterone at 0.25 mg/kg body weight were administered from day 1 to 7 twice daily 12 h apart by subcutaneous route at either side neck region on alternate days. Dexamethasone (20mg/animal/day) was administered for 4 days (day 14 to 17) by intramuscular route. The treatment schedule for Group III, from day 1 to 7 were adopted same as group II and reserpine (5 mg/animal/day) were administered for 4 days from day 8 to 14 on alternate days (i.e., day 8, 10, 12 and 14) by intramuscular route. All the animals exhibited estrous between 30 to 35 from the day of milking and were inseminated when they exhibited first estrous cycle after lactation induction. The percentage of conception rate obtained was 50% (10/10), 40% (4/10), and 50% (5/10) in group I, II and III respectively. The current study in group I and III 5 out of 10 (50%) animals were confirmed pregnant. In group II conception rate was 40% where in 4 out of 10 animals were confirmed pregnant Also the number of inseminations taken for the animal to became pregnant in group I was minimum as ≤3 compared to group II and III which was ≥ 4.

Keywords: Artificial induction lactation, conception rate, artificial insemination, reserpine, dexamethasone, 17β-estradiol, progesterone

1. Introduction
It was reported that hundreds of thousands of high producing dairy cows are culled every year because they fail to become pregnant due to a variety of reproductive disorder (e.g. repeat breeding, anestrous, chronic cervicitis, pyometra, or congenital abnormalities like free martinism, underdeveloped ovaries or genitalia), anatomical defects (e.g. persistent Mullerian ducts) and hormonal dysfunction(e.g. cystic ovaries). As per statistical data about 27 to 47% dairy cows are culled due to reproductive problems reported by Allaire *et al*. (1977) [1]; Silva, *et al*. (2004) [15]; Ghulam *et al*. 2016) [6].

The repeat breeder animal is usually defined as a sub fertile 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 by Purohit (2008) [12].

The reproductive disorder in animal is troubling because it is often the higher milk producers that are at the greatest risk of failing to conceive in a timely manner, as discussed by Loeffler *et al*. (1999) [9]. In dairy herds, reproductive failure seems to be one of the major causes for involuntary culling of high milk yielding cattle, which leads to, reduce the number of milking cows in the herd and, as a consequence, affects milk production and causes economic losses to the farmers.

Erb and Martin (1980) [3] concluded that, poor reproductive performance is main cause for limiting productivity of dairy cattle and 10 to 30% of lactation may be affected by infertility and reproductive disorders.

It is well documented in literature that for profitable dairying, a cow should be maintained up to five lactations in the farm and then it should be culled which is practically not possible in India, because of socio-religious sentiments\grounds, animal cannot be disposed of. Due to this the aged unproductive cows become economic burden to the farmers and maintaining of such

unproductive animal, causing an economic losses to the farmers. Besides this frequent disease outbreak in the dairy herd and nutritional deficiencies causing drastic reduction in the milk yield leading to economic losses to the farmers. Totally an animal without fertility is a national burden.

Adopting artificial induction technology which helps to reduce economic losses to the farmers to some extent, in these unproductive animals, udder can be developed by employing combination of hormones and drugs, thus milk can be obtained even without pregnancy by Verma *et al*. (2004) [16].

Induction of lactation by hormonal treatment/other drugs can reduce the economic losses to the farmers due to infertility/sterility of the high producing dairy cows and also prevent involuntary culling. Induction of lactation by hormonal treatment of non-breeding cows has been found to be more profitable than to replacement heifers. This technology can be used as a profitable management tool to help production ability back into the herd by Magliaro *et al.* (2004) [10].

Applying artificial induction of lactation technology in repeat breeder, non-lactating and infertile animals in the dairy herd, economic benefits can be obtained through sale of milk besides establishment of normal reproductive cycles in anestrous or repeat breeder cattle and prolonging the lifespan of genetically superior cows for milk yield. This technology can reduce herd culling, economic losses and replacement costs derived from reproductive failure by Inchaisri *et al.* (2010) [8].

However, there were paucity of information regarding comparative study of artificial induction protocols and in order to continue improving the success rate, conception rate, reduced number of AI required for conception and further research in development of reliable cost efficient protocols for artificial induction is very much needed. Keeping all these facts in view the present study has been taken up to find an alternate way to reduce the economic burden of heavy maintenance costs on farmers. Hence the study has been taken with the following objectives.

A. To induce estrous cycle in an infertile (non-pregnant and non-lactating repeat breeding) dairy cattle using different artificial induction protocols.

B. To assess the relative efficacy of various methods of artificial induction.

Materials and Methods

The animals for current study were selected from various parts of Davanagere district of Karnataka state, India and the study was conducted during the period, february-2018 to march-2019, Group I (n=10) comprising of eight crossbred HF and two Jercy cattle, which were in first lactation and not given any treatment served as control, where as in Group II and III (n=10) consists of repeat breeding eight crossbred HF & two Jercy heifers each.

The study animals were selected based on the body condition scoring as per the methods described by Edmonson *et al.* (1989) [4]. The cows having body condition score of 2.5 to 3.5 were randomly selected for the study. The reproductive organs of all the animals were examined per rectally prior to the inclusion into the study to rule out the reproductive disorders, if any. Before initiation of the study, animals were evaluated for the health, by performing hematological and biochemical profile of blood on weekly basis for induction prior to initiation of the study as depicted in Table 2, 3, 4 and 5. All the animals were allowed for fifteen days acclimatization period prior to the study.

The body weight of the animals were calculated using the formula:

Body weight (in Kg) = $L \times G^2/660$

Where L (length)-the distance in inches from point of shoulder to the point of rump,

G (Girth)-is the circumference of chest in inches.

The selected animals were dewormed with fenbendazole at the dose of 5 mg/kg body weight (Panacur®, Intervet, India Pvt. Ltd, Pune, 3g/bolus) 10 days prior to initiation of experiment.

Administration of Drugs

Group I, animals which were in first lactation served as control, not given any treatment. Group II, administered with 17β-estradiol at 0.1 mg/kg body weight and progesterone at 0.25 mg/kg body weight were administered from day 1 to 7 twice daily 12 h apart by subcutaneous route at either side neck region on alternate days. Dexamethasone (20mg/animal/day) were administered for 4 days (day 14 to 17) by intramuscular route.

The treatment schedule for Group III, from day 1 to 7 was adopted as per group II. Reserpine (5 mg/kg/day) were administered for 4 days from day 8 to 16 (day 8, 10, 12 and 14) by intramuscular route as presented in Table 6.

Results and Discussion

Mean age of the group I *vs*. treatment groups

The mean age was recorded in months presented in the Table 1, were 45.80±0.57, 43.50±0.81 and 45.20±0.62, respectively for the group I, II and III. The mean age was decreased in group II as compared to group I and group III but it was non-significant (P>0.05) and it was almost identical in group III as compared to group I.

Mean Blood Hemoglobin Profile of Group I vs. Treatment Groups

The mean blood hemoglobin concentration was normal in all the groups and are presented in Table 2.

Comparison of Mean Serum Calcium Profile of Group I *vs*. Treatment Groups

The mean serum calcium concentration was normal in all the groups and are presented in Table 3.

Comparison of Mean Serum Phosphorus Profile of Group I *vs*. Treatment Groups

The mean serum phosphorus concentration was normal in all the groups and are presented in Table 4.

Comparison of Mean Serum Magnesium Profile of Group I *vs*. Treatment Groups

The mean serum magnesium concentration was normal in the groups and are presented in Table 5.

Reproductive Performance

Administration of 17β-estradiol and progesterone caused estrous like activity characterized by swollen vulva, mucus discharge and some animals mount on other animals are noticed within 2 to 3 days. It is persisted throughout the experimental period in all the treatment groups. In some animals follicles and corpus luteum were palpable on the ovary.

All the animals exhibited estrous between 30 to 35 from the day of milking and were inseminated when they exhibited first estrous cycle after lactation induction. The percentage of conception obtained was 50% (10/10), 40% (4/10), and 50% (5/10) in group I, II and III respectively and are depicted in Table 7.

The current study in group I and III 5 out of 10 (50%) animals were confirmed pregnant. In group II conception rate was 40% where in 4 out of 10 animals were confirmed pregnant. The conception rate achieved in the present study is higher in group I and III and similar in group II than reported by Priscila, *et al.* (2010) [13] and Hooda *et al.* (1997) [7], who reported 41.1% and 40% and lesser than reported by Peel *et al.* (1978) [14], Mellado *et al.* (2006) [11] and Diane *et al.* (1981) [2], who recorded 90%, 71%, 80% respectively. Also the number of inseminations taken for the animal to became pregnant in group I was minimum as ≤3 compared to group II and III which was ≥4 and are presented in Table 7. The variation in conception rate and number AI required to became pregnant in the current study may be suggestive of the variation in feeding regimens of the animals, right detection of estrus and timely insemination performed as stated by Freitas *et al.* (2010) [5].

Adverse Effects

There were few adverse effects like nymphomania and off feed during the treatment period from day 3 to 7 noticed in all groups, nonetheless, the clinical signs subsided following completion of steroid hormone treatment. Profuse salivation, lethargy and marked increase in respiratory rate were observed in reserpine treated groups. They also exhibited tendency to sit or lie down and refused to get up. While walking, twitching of the thigh and shoulder muscles were noticed for first two days of treatment.

Table 1: Comparison of mean age and body weight of Group I vs. treatment groups

	Group I	Group II	Group III
Age (in months)	45.80±0.57	43.50±081	45.20±0.62
Body weight (in kilogram)	333.50±5.53	310.50±4.24	322.50±7.15

Two way ANOVA- Bonferroni post comparison test
Values are in Mean ±S. E

Table 2: Comparison of mean blood hemoglobin profile of (g/dL) Group I *vs.* treatment groups

Day	Group I	Group II	Group III
0 day	10.80 ±0.13	10.09±0.0.31	10.34±0.31
1 week	10.32±0.18	9.54±0.44	10.20±0.30
2 week	10.25±0.20	10.32±0.34	9.45±0.28
3 week	9.85±0.27	10.06±0.21	9.93±0.19
4 week	10.26±0.24	10.45±0.36	10.57±0.17
5 week	10.11±0.27	10.24±0.29	10.08±0.30

Two way ANOVA- Bonferroni post comparison test
Values are in Mean ± S. E

Table 3: Comparison of mean Serum calcium profile of (mg/dL) Group I *vs.* treatment groups

Day	Group I	Group II	Group III
0 day	10.87 ±0.16	10.35±0.22	10.06±0.22
1 week	10.16±0.15	10.12±0.24	9.58±0.30
2 week	10.44±0.28	10.06±0.30	10.36±0.20
3 week	9.77±0.40	10.35±0.16	10.42±0.23
4 week	10.17±0.22	10.29±0.24	10.42±0.22
5 week	10.08±0.20	10.09±0.11	10.05±0.20

Two way ANOVA-Bonferroni post comparison test
Values are in Mean ± S. E

Table 4: Comparison of mean serum phosphorus profile of (mg/dL) Group I *vs.* treatment groups

Day	Group I	Group II	Group III
0 day	6.02 ±0.11	6.01±0.26	5.67±0.21
1 week	5.79±0.17	5.47±0.26	5.61±0.22
2 week	5.93±0.10	5.48±0.20	5.95±0.18
3 week	5.44±0.11	6.02±0.17	5.64±0.08
4 week	5.93±0.18	6.06±0.14	6.07±0.18
5 week	5.68±0.11	5.74±0.19	5.93±0.13

Two way ANOVA-Bonferroni post comparison test
Values are in Mean ±S.

Table 5: Comparison of mean serum magnesium profile of (mg/dL) Group I *vs.* treatment groups

Day	Group I	Group II	Group III
0 day	2.99 ±0.09	2.81±0.14	2.93±0.09
1 week	3.02±0.10	2.77±0.13	3.01±0.10
2 week	3.07±0.15	2.84±0.09	2.95±0.12
3 week	2.84±0.10	3.06±0.06	3.13±0.09
4 week	3.01±0.09	2.97±0.10	3.15±0.13
5 week	2.84±0.07	2.95±0.11	2.99±0.16

Two way ANOVA-Bonferroni post comparison test
Values are in Mean ±S.E.

Table 6: Experimental protocols

Protocol	Day 1-7	Day 8, 10, 12 & 14	Day 14-17	Day 18-20	Day 20
Group I	----------	---------	----------	----------	-------
Group II	17β-estradiol (0.1mg/kg/day) s.c. progesterone (0.25 mg/kg/day) s.c.	----------	----------	Dexamethasone (20 mg/animal) i.m.	Start milking
Group III	17β-estradiol (0.1 mg/kg/day) s.c. progesterone (0.25 mg/kg/day) s.c.	Reserpine 5mg/animal/day	----------	----------	Start milking

Table 7: Comparison of conception rate, number of AI required to became pregnant and pregnancy confirmed in Group I *vs.* treatment groups

	Group I	Group II	Group III
Conception rate	5/10 (50%)	4/10 (40%)	5/10 (50%)
Number of AI required to became pregnant	≤3	≥4	≥4
Pregnancy confirmed	5	4	5

Conclusion

It is concluded from the current study that repeat breeding animals induced hormonally and treated with dexamethasone and reserpine can help to induce estrous behavior there by animal get conceived. The conception rate obtained was more in animals induced hormonally and treated with reserpine than animals treated with dexamethasone but number of artificial insemination required by the animals to become pregnant was same in both the group.

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