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COMPARATIVE ASSESSMENT OF VARIOUS PARAMETERS IN LACTATION INDUCED REPEAT BREEDING CATTLE VS. NATURAL LACTATING NULLIPAROUS CATTLE, USING RESERPINE

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ABSTRACT

The pharmacological induction of lactation was 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. 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. Reserpine (5mg/animal/day) were administered for 4 days (on day 8, 10, 12 and 14) by intramuscular route. The treatment schedule for Group III, from day 1 to 7 was adopted as per group II. Reserpine (5 mg/animal/day) and

dexamethasone (20mg/animal) were administered for 4 days from day 14 to 17 by intramuscular route. The relative efficacy of lactation induction protocol employed in group II and III was 100 per cent. In all the animals milking was initiated on day 20 and milking was carried out twice a day in the morning and evening with 12 h interval between the milking. The first day milk was watery, yellowish to white in color. The milk secretions in all the animals become normal within 3 to 7 days. The mean daily milk yield was varied between the groups and the mean peak daily milk yield of 4.29±0.08 and 4.81±0.15 liters was observed on day 90 and 75 in group II and III respectively. The mean total milk yield for the period of 90 days of group I, II and III group were 357.83±4.05, 224.±4.30 and 326.48±5.04

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liters respectively. The mean total milk yield for 90 days of lactation in the group III was highly significant (P<0.001) than group II.

KEYWORDS: Lactation induction, dexamethasone, 17β-estradiol, progesterone, reserpine.

INTRODUCTION

India, one of the leading producer of milk in the world, producing 146.3 million tones with per capita availability of 322 gm/day in 2014-2015 (DAHD and F). Since milk is a major animal product being broadly consumed by human communities irrespective of age and religion, there is a need to enhance the production limits in order to satisfy the ever growing demand for milk and its byproducts by the consumers.

The main factors which cause decline in milk production and productivity of the dairy cattle are frequent disease outbreak in the herd, nutritional deficiency and repeat breeding. Among which repeat breeding is a major reproductive disorder, which causes economic losses to dairy farmers. The repeat breeder animal is 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 (Purohit, 2008).

It was reported that hundreds of dairy cows are culled every year because they fail to become pregnant due to a variety of reproductive problems (e.g. repeat breeding, anestrus, 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 (Allaire *et al.*, 1977; Silva *et al.*, 2004; Ghulam *et al.*, 2016).

The reproductive disorder are observed often in the higher milk producers that are at the greatest risk of failing to conceive in a timely manner (Loeffler *et al.*, 1999). In dairy herds, reproductive failure seems to be one of the major causes for involuntary culling of high milk yielding cattle, reduce the number of milking cows in the herd and affects milk production and causes economic losses to the farmers.

Erb and Martin (1980) 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 socioreligious sentiments\grounds, animal cannot be disposed off. Due to this the aged unproductive cows become economic burden 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 milk production is a national burden (Mohan *et al.*, 2010).

All above problems may be resolved by adopting artificial induction of lactation 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 (Verma *et al.*, 2004).

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 (Magliaro *et al.*, 2004).

Adding replacement heifers to the milking string is one of the largest costs of dairy farming.

It requires long duration and high rearing cost to bring a single heifer into production (Heinrichs, 1996).

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 (Inchaisri *et al.*, 2010).

The milk obtained following artificial induction lactation procedure did not differ from that after natural lactations following pregnancy and that histological sections of mammary glands in the induced cows did not show abnormalities (Narendran *et al.*, 1979).

Keeping all these above facts in view, the present study was taken up to find an alternate way to reduce the economic burden on farmers. Hence it is proposed to induce lactation in dry or unproductive animals.

MATERIALS AND METHODS

The animals for current study were selected from various parts of Davanagere district of Karnataka state, India. The study was conducted during the period february-2018 to march 2019. Group I (n=10) comprising of eight crossbred HF and two Jersey cattle, which were in first lactation served as control, where as in Group II and III (n=10) consists of repeat breeding eight crossbred HF and two Jersey cattle each.

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.

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

Body weight (in Kg) =
$$L \times G2 / 660$$

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 ten days prior to initiation of experiment. All the animals were allowed for fifteen days acclimatization period prior to the study.

Administration of drugs

Group I, animals which were in first lactation served as control, not given any treatment and given *add libitum* of food and water. 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. Reserpine (5mg/animal/day) were administered for 4 days (on day 8, 10, 12 and 14) by intramuscular route. The treatment schedule for Group III, from day 1 to 7 was adopted as per group II. Reserpine (5 mg/animal/ day) and dexamethasone (20mg/animal) were administered for 4 days from day 14 to 17 by intramuscular route.

Collection of milk sample

Milk samples were collected from all induced animals regularly for 90 days with the first samples were collected on the first day of milking i.e. day 20 of experiment from all

treatment groups. Milk samples were also collected from normally parturated animals regularly from the day of parturition i.e. (control group) on day 1 of parturition to day 90.

The data generated for different parameters were tabulated group wise and expressed as mean \pm S.E. Two way analysis of variance with Bonferroni post-test for milk yield and one way analysis of variance with Turkey's Multiple comparison test by using Graph Pad Prism trial version 5.00, Graph Pad, Software, San Diego, California, USA.

RESULTS AND DISCUSION

Selection of animals for the study

The study was carried out in 20 non-lactating and non-pregnant cross bred cattle with the history of repeat breeding for 10-20 months, apparently healthy and which were selected based on following criteria.

Before conducting the study 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. The mean age and body weight of the animals also taken into consideration. There was non significant (P>0.05) in mean age and body weight among control ant treatment groups presented in Table 1.

The selection of animals for study were done based on the body condition scoring as per the method described by Edmonson *et al.*, (1989). The cows having body condition score of 2.5 to 3.5 were randomly selected for the study. Before initiation of the study health of the animals were also evaluated based on blood hematological and biochemical profile on weekly basis. The treatment group of animals were found normal in blood hematology and biochemical profile when compared with the control group were selected for the study and are depicted in Table 2 and 3.

Lactation Induction

Success rate

The relative efficacy of various treatment protocols employed in the present study, and are depicted in Table 4. It can be observed that the protocol employed in group II and III were 100 *per cent* efficacious.

In group II and III out of 10 animals in each group all were responded for the induction therapy resulting 100 per cent success rate with \geq 2.5liters/day in group II \geq 3.5liters/day of

milk in group III respectively. The success rate in the present study was in accordance with Mohan, (2007) and Mellado *et al.*, (2006) who reported similar success rate of 100 *per cent*. While, the success rate in the present study was higher than the Smith and Schanbacher, (1973), Collier *et al.*,(1975), Chakriyarat *et al.*, (1978) and Tracy, (2002) who reported the success rate of 60, 69, 82 and 92 *per cent* respectively. Increased success rate in the present study might be due to dose, duration and the type of hormones used for the induction of lactation.

Milk yield

The mammary gland of lactation induced animals by using steroid hormone showed marked enlargement in size of udder by day 7, contained secretion from day 9 to 14. The mammary gland became fully distended with fluids and the teats were elongated, full and turbid in group II and III on days 19 and 16 respectively and are depicted in Figure 1 and 2. Dribbling of milk from teats was noticed on day 18 and 19 in eight and ten animals respectively in groups II and III. In all the animals milking was initiated on day 20 and milking was carried out twice a day in the morning and evening with 12 h interval between the milking. The first day milk was watery, yellowish to white in color. The milk secretions in all the animals become normal within 3 to 7 days.

The mean daily milk yield was varied between the groups and the mean peak daily milk yield of 4.29 ± 0.08 and 4.81 ± 0.15 liters (presented in Table 5) was observed on day 90 and 75 in group II and III respectively. The attaining daily peak milk yield in group II was delayed compared to group III. The time taken to reach peak mean daily milk yield in the current study is similar in accordance with Hooda *et al.*, (1997) and (Ramgattie *et al.*, 2014), who were reported time taken to reach peak mean daily milk yield varied between 12-14 and 12.7 \pm 1.8 weeks. The time taken to reach peak daily milk yield in the present study is higher than the (Smith and Schanbacher, (1973) and Tervit *et al.*, (1980) who were reported, time taken to reach peak mean daily milk yields from 7 to 8 weeks on onset of lactation. The time taken to reach peak daily milk yield in the present study is lower than reported by Priscila, *et al.*, (2010) who reported delayed daily peak milk production of 15 liters occurred from 104 to 145 days of induced lactation. The delayed peak daily milk yield in induced cows might be due to the less amount of secretory tissue present at the starting of lactation as compared with non induced cows, since the mammary development occurs over three week period in the induced lactation and during the last month of gestation (Knight and Wilde, 1993). The

variation in time taken to attain peak daily milk yield is suggestive of variation in feeding regimens of the animals, genetic character and health condition.

The mean total milk yield for the period of 90 days of group I, II and III were 357.83±4.05, 224.±4.30 and 326.48±5.04 liters respectively were recorded. In all the group of animals, a gradual increase in milk yield was observed and the yield continued to increase up to day 90. The mean total milk yield for day 90 was extrapolated to 305 days (Rice *et al.*, 1970) and the extrapolated mean total milk yield for 305 days in group I, II and III were 1224.92±14.78, 813.88±17.73 and 1097.60±17.20 liters respectively. The mean total milk yield production was varied between the groups and mean total milk yield for period of 45, 90, 180, 270 and 305 days were presented in Table 6.

The animals in group III which were treated with combination of dexamethazone with reserpine the response to the treatment occurred 2 days earlier, the daily milk yield increased more rapidly and reached peak daily milk yield more rapidly than group II. Consequently, 90 day mean total milk yield and daily peak milk yield were higher in group III compared to group II.

The mean total milk yield for 305 days of lactation in the group III, which received combination of dexamethazone with reserpine was highly significant (P<0.001) than group II which received reserpine alone. Further in group III the total milk yield for 305 days was significantly decreased (P>0.001) than group I. The mean total milk yield for 305 days in group I and III was significantly higher (P<0.001) than the group II, which was treated with reserpine alone.

The quantity of milk obtained in the current study is similar in accordance with the reported results of Mohan *et al.*, (2009), whose study was also conducted in same latitude and longitude. However, higher mean milk yield was obtained by Tracy (2002), Freitas *et al.*, (2010) and Ramgattie *et al.*, (2014). The increased in milk yield obtained by the aforementioned authors could be due to breed, managemental practices, superior genetic potential for milk yield (Ramgattie *et al.*, 2014). Further, Tracy, (2002) had administered recombinant bovine somatotropin once every two weeks on the day of milk letdown throughout the period of her experiment (21 weeks) which resulted in higher average daily milk yield.

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

| | Control (n=10) | Group II (n=10) | Group III (n=10) |
|---------------------------|----------------|--------------------|---------------------|
| Age (in months) | 45.80±0.57 | 45.20±0.62 | 46.40±0.49 |
| Body weight (in kilogram) | 333.50±5.53 | 322.50±7.15 | 303.50±3.50 |

Two way ANOVA-Bonferroni post comparison test

Values are in Mean \pm S. E

Table 2: Comparison of mean blood hemoglobin and serum calcium profile of control *vs.* treatment groups.

| | Hemoglobin (g/dL) | | | Serum Calcium (mg/dL) | | |
|--------|-------------------|------------|-----------------|-----------------------|------------|-----------------|
| Day | Control | Group II | Group III | Control | Group II | Group III |
| | (n=10) | (n=10) | (n=10) | (n=10) | (n=10) | (n=10) |
| 0 day | 10.80 ± 0.13 | 10.34±0.31 | 10.42±0.25 | 10.87 ± 0.16 | 10.06±0.22 | 10.52±0.33 |
| 1 week | 10.32±0.18 | 10.20±0.30 | 9.44±0.30 | 10.16±0.15 | 9.58±0.30 | 10.10±0.23 |
| 2 week | 10.25±0.20 | 9.45±0.28 | 9.88 ± 0.28 | 10.44±0.28 | 10.36±0.20 | 10.50±0.21 |
| 3 week | 9.85±0.27 | 9.93±0.19 | 10.31±0.20 | 9.77±0.40 | 10.42±0.23 | 9.82 ± 0.36 |
| 4 week | 10.26±0.24 | 10.57±0.17 | 10.08±0.29 | 10.17±0.22 | 10.42±0.22 | 10.10±0.15 |
| 5 week | 10.11±0.27 | 10.08±0.30 | 10.23±0.30 | 10.08±0.20 | 10.05±0.20 | 10.43±0.29 |

Two way ANOVA-Bonferroni post comparison test

Values are in Mean \pm S. E

Table 3: Comparison of mean serum magnesium profile of (mg/dL) control vs. treatment groups.

| Serum Magnesium (mg/dL) | | | Serum Phosphorus (mg/dL) | | | |
|-------------------------|-----------------|-----------|--------------------------|-----------------|---------------|-----------|
| Day | Control | Group II | Group III | Control | Group II | Group III |
| | (n=10) | (n=10) | (n=10) | (n=10) | (n=10) | (n=10) |
| 0 day | 2.99 ± 0.09 | 2.93±0.09 | 2.92 ± 0.11 | 6.02 ± 0.11 | 5.67±0.21 | 5.89±0.21 |
| 1 week | 3.02±0.10 | 3.01±0.10 | 2.88 ± 0.08 | 5.79±0.17 | 5.61±0.22 | 5.66±0.19 |
| 2 week | 3.07±0.15 | 2.95±0.12 | 3.14±0.10 | 5.93±0.10 | 5.95±0.18 | 5.71±0.10 |
| 3 week | 2.84 ± 0.10 | 3.13±0.09 | 3.02±0.11 | 5.44±0.11 | 5.64 ± 0.08 | 5.47±0.21 |
| 4 week | 3.01±0.09 | 3.15±0.13 | 3.05±0.10 | 5.93±0.18 | 6.07±0.18 | 5.72±0.11 |
| 5 week | 2.84 ± 0.07 | 2.99±0.16 | 2.96±0.12 | 5.68±0.11 | 5.93±0.13 | 5.98±0.08 |

Two way ANOVA-Bonferroni post comparison test

Values are in Mean \pm S. E

Table 4: Relative efficacy of various treatment protocols in induction of lactation.

| | Group II (n=10) | Group III (n=10) |
|---------------------------------|-----------------|------------------|
| Number of animals responded | 10(100) | 10(100) |
| Number of animals not responded | 0(0) | 0(0) |
| Total animals | 10 | 10 |

Note: Values in the parenthesis indicates percentage

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Table 5: Comparison of mean daily milk yield of control vs. treatment groups.

| Day | Control | Group II | Group III | |
|--------|-----------------|--------------|--------------|--|
| Day | (n=9) | (n=10) | (n=10) | |
| 1 day | 1.04 ± 0.07 | 0.18±0.01*** | 0.35±0.03*** | |
| 3 day | 1.47±0.03 | 0.43±0.03*** | 0.73±0.03*** | |
| 6 day | 1.59±0.06 | 0.72±0.03*** | 1.03±0.04** | |
| 10 day | 1.89±0.09 | 0.95±0.02*** | 1.29±0.05** | |
| 12 day | 2.24±0.07 | 1.04±0.04*** | 1.48±0.07*** | |
| 15 day | 2.55±0.09 | 1.15±0.04*** | 1.81±0.05*** | |
| 20 day | 2.96±0.13 | 1.25±0.06*** | 2.17±0.11*** | |
| 30 day | 3.74±0.0.17 | 1.66±0.05*** | 3.31±0.14 | |
| 45 day | 4.73±0.15 | 1.92±0.13*** | 3.71±0.12*** | |
| 60 day | 4.62±0.14 | 2.82±0.08*** | 4.32±0.10 | |
| 75 day | 4.47±0.15 | 4.61±0.05*** | 4.81±0.15 | |
| 90 day | 4.65±0.09 | 4.29±0.08 | 4.76±0.17 | |

Two way ANOVA-Bonferroni post comparison test

Values are in Mean \pm S. E

Mean values bearing different superscripts within a column are significantly different.

*P < 0.05

**P< 0.01

***P< 0.001

Table 6: Comparison of mean total milk yield of control vs. treatment groups.

| Day | Control | Group II | Group III | |
|----------|---------------|-----------------|------------------|--|
| Day | (n=10) | (n=10) | (n=10) | |
| 45 days | 151.43 ±3.34 | 63.37±2.28*** | 118.12±3.11 | |
| 90 days | 357.83±4.05 | 224.37±4.30*** | 326.48±5.04 | |
| 180 days | 715.66±8.11 | 448.60±8.53*** | 652.96±10.09** | |
| 270 days | 1073.48±12.15 | 679.17±14.55*** | 979.44±15.13*** | |
| 305 days | 1224.92±14.78 | 813.88±17.73*** | 1097.60±17.20*** | |

Two way ANOVA-Bonferroni post comparison test

Values are in Mean \pm S. E

Mean values bearing different superscripts within a column are significantly different.

**P< 0.01

***P< 0.001



Figure 1: Udder of the cattle before starting of the lactation induction treatment

Figure 2: Development of udder in cattle after 7^{th} day of lactation induction treatment

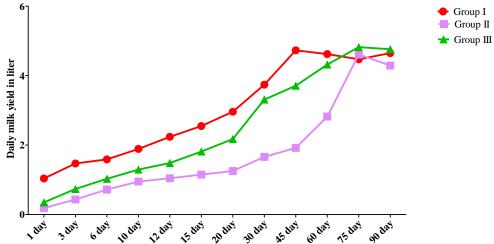


Figure 3: Comparison of mean daily milk yield at different $\,$ intervals, group I vs. Group II and III.

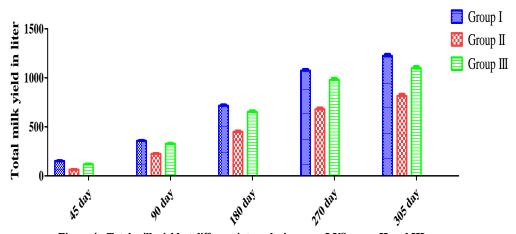


Figure 4: Total milk yield at different intervals in group I VS group II and III

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