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Estrus Induction Response and Fertility Performance in Delayed Pubertal Heifers Treated with *Aegle marmelos* and *Murraya koenigii*

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ABSTRACT

The present study was designed to examine the estrus induction response and fertility performance in delayed pubertal heifers treated with two medicinal plants *Aegle marmelos* in combination with *Murraya koenigii*. A total of 11 heifers were treated with the plants leaf powder for 9 days per orally after determining the dose by the extrapolation from effective dose of 50% ethanolic extract in rats (1000 mg/ kg b. wt.) with optimum ovarian response. Extrapolated extract dose was subsequently converted to powder dose based on per cent yield. Six heifers were kept as untreated control. Six out of 11 (54.1%) heifers in treatment group and 1 out of 6 (16.7%) in control group exhibited standing estrus. All these estrus heifers were inseminated and confirmed as pregnant and calved in due course. Though the proportion of heifers that showed estrus, became pregnant and calved in the end, did not achieve statistical significance (P>0.05) between the groups, nevertheless, the number and proportion of such heifers was higher in the treatment group than that of control. In conclusion, the present study indicates that *Aegle marmelos* in combination with *Murraya koenigii* have the potential to induce estrus in delayed pubertal heifers with appreciable fertility performance.

Keywords: Heifers, delayed puberty, medicinal plants, estrus, fertility

Reproductive disorders will continue to remain the major bottleneck in the coming decades in exploiting the fullest production potential of livestock. Among the reproductive disorders, delayed puberty or pubertal anoestrus is the most important and challenging problem associated with dairy heifer reproduction (Iyer, 1978; Shrivastava and Kadu, 1992; Chaudhari *et al.* 2012). It lowers the reproductive rate of heifers by delaying the entry in to the breeding herds, decreases the number of total calves and milk yield per cow produced in life time and also culling of valuable germ plasm resulting in significant economic loss to the small and marginal farmers as well as dairy industry as a whole (Gupta *et al.* 2010; Chaudhari *et al.* 2012). Delayed puberty is a problem of both farm-bred as well as heifers under rural condition. The condition remains as frustrating challenge to the field veterinarians, researchers and planners.

To date several attempts have been made to reduce the extent of loss due to anoestrus in heifers by various hormonal and non-hormonal treatments (Khade *et al.* 2011; Shukla *et al.* 2011; Lone *et al.* 2012). But due to high cost involvement, inconsistent results and poor availability in rural pockets, most of the synthetic drugs and exogenous hormones became unacceptable to the poor dairy farmers. Nowadays, various medicinal plants have brought future promise to treat various reproductive disorders in livestock as being cheaper, efficacious and safer alternatives to costlier hormones (Kadu *et al.* 2001; Mehrotra, 2011). Ethno veterinary practices (EVPs) have significant contribution in maintenance of animal health and regarded as sustainable veterinary medicine in the new era (Lin *et al.* 2003). The WHO has recognized the value and imperative need for adopting traditional herbal practices in global health care and recommended all

member countries to actively promote native medicines of their country as well to initiate steps to conserve and/ or cultivate medicinal plants, so that genuine raw materials become readily available to a large section of the population (c.f. Mehrotra, 2002). Hence, there is urgent need of the hour to identify suitable EVPs, validate scientifically and recommend to the farmers to combat many age old reproductive ailments of livestock (Mehrotra, 2002).

Aegle marmelos and *Murraya koenigii,* are the two commonly known medicinal plants have been used independently for augmentation of reproduction in laboratory animals (Mehrotra *et al.* 2003; Jondhale *et al.* 2009 a, b; Nandini *et al.* 2010) and livestock (Hegde *et al.* 2002; Mehrotra *et al.* 2005; Satheshkumar and Punniamurthy, 2009). Recently, it has been shown that combination of both the plants can induce estrus, ovulation and restore appreciable fertility in anoestrus goats under farm (Dutt *et al.* 2010) and post-partum anestrus buffaloes under field condition (Dutta *et al.* 2011). Considering the above facts, the present study was designed to examine the effect of *A*egle *marmelos* and *Murraya koenigii* in combination on the estrus induction response and fertility performance in delayed pubertal heifers under farm condition.

MATERIALS AND METHODS

Location

The present study was conducted at the Division of Animal Reproduction and Institute Livestock Farm, Livestock Production and Management Section of Indian Veterinary Research Institute located at an altitude of 564 ft MSL, at latitude of 28º North and 79° East Average rainfall is 266.4 mm. The experiment was done in the last week of March to first week of April, 2013.

Experimental animals

Seventeen apparently healthy pubertal heifers of Vrindavani (n=8) and Tharparkar (n=9) of above 18 months of age (range 19-35 months) and above 200 kg body weight (range 200- 315 kg) were selected for the study based on the availability. All the heifers were maintained at the Institute Farm under Livestock Production and Management Section of the Indian Veterinary Research Institute and were confirmed anoestrus based on the history of non-exhibition of estrus for last two cycle length till the assignment of the treatment and trans-rectal clinical examination revealing true anoestrus characterized by absence of any palpable structures on the ovarian surface along with flaccid uterine horns.

Management and nutrition

The experimental heifers were maintained under standard farm management practices with routine veterinary care in a loose housing system. Green fodder (berseem, oat, cowpea, maize and jowar), depending on seasonal availability, and water were provided ad libitum. An additional concentrate mixture at the rate 2 kg/heifer/day was given during the period of the study.

Treatment grouping

The heifers were divided in to two groups, G-I and G-II. In G-I, 11 heifers of Vrindavani (n=5) and Tharparkar (n=6) breeds were fed with ground leaf powder of *Aegle marmelos* and *Murraya koenigii*. In G-2, 6 heifers of Vrindavani and Tharparkar breeds with equal (3+3) in number were kept as untreated control.

Collection of leaves and preparation of dose

Fresh and mature green leaves of *Aegle marmelos* and *Murraya koenigii* were collected from their natural habitat located in and around IVRI campus. The green leaves were shade dried at room temperature. Following de-leafing from the stems, dried leaves were stored in plastic bag separately until grinding. Finally, they were pulverized into powder from the local market and stored in closed plastic bags at room temperature till further packing after individual dose calculation. The dose for heifer was calculated by extrapolating the 50% ethanolic extract dose of *Aegle marmelos* (Jondhale 2007) and *Murraya koenigii* (Mehrotra 2002) with optimum ovarian response i.e. 1000 mg /kg b. wt in rats to cattle by dose equivalent system using Km factor as described by Van Miert (1986). Subsequently, for ease of use to the large animals, the calculated extract dose was converted into powder form based on per cent yield using the formula as described previously from our laboratory (Dutt *et al.* 2010; 2011). Thus, the leaf powder dose for *Aegle* and

Murraya was worked out separately according to the body weight of the animals and divided by two, to yield the half of the dose and the final dose was worked out by mixing both the calculated doses.

Herb treatment

The calculated powder dose was mixed in concentrate mixture (2 kg/ heifer) and administered to all the heifers in G- I PO give full form for 9 days or till onset of estrus whichever was earlier. Heifers in G- II were provided only concentrate mixture at the rate 2 kg/ heifer and remained as untreated control.

Estrus detection and breeding: Estrus detection in experimental heifers was carried out twice (morning-evening) daily by parading teaser bull since the initiation of the treatment. Heifers exhibiting standing estrus were inseminated twice as AM-PM schedule with frozen thawed semen of proven bull.

Pregnancy diagnosis

Following artificial insemination, all the inseminated heifers were subjected for estrus detection in AM-PM schedule by parading teaser bull as a routine practice of the farm. Per-rectal clinical examination was performed after 60 days following A.I. in order to diagnose the pregnancy.

Statistical analyses

Response of the heifers to medicinal herb treatment was cross-tabulated in two ways: responder (estrus) versus non-responder and cyclic vs acyclic. The latter classification was made on the fact that some of the non-responders were in silent estrus as evidenced by presence of CL 10 days after the expected date of estrus. The resultant 2 × 2 contingency table was analyzed by Chi-square test corrected for Yate's continuity as some cell frequencies were <5. Significance was set at 5% and 1%.

RESULTS AND DISCUSSION

In the present study, the potential and future promise of two medicinal plants namely *Aegle marmelos* and *Murraya koenigii* to induce cyclicity, behavioural estrus and augmentation of fertility in delayed pubertal heifers have been demonstrated. The estrus induction response and fertility performance obtained in delayed pubertal heifers following the treatment with *Aegle marmelos* and *Murraya koenigii* is presented in Table 1.

Estrus response

Overall, the number of heifers (10/11) that showed resumption of cyclicity [combining the heifers showing standing estrus (6/11) and silent estrus (4/11)] was significantly higher ($P<0.01$) in treatment group than that of control group. But the number of heifers that showed standing estrus did not differ ($P>0.05$) significantly between the groups. However, there was a tendency of more number of heifers exhibiting standing estrus in treatment group than the control heifers. Four (36.3%) out of 11 heifers in the treatment group showed silent estrus in this study as evident by the luteal structure in the ovarian surface while per-rectal examination after the end of the treatment (Table 1). In the present study, 54.5% treated heifers exhibited behavioral signs of estrus including the cardinal sign i.e. standing estrus. Compared to our earlier (66.7-71.4%) findings in delayed pubertal heifers (Das *et al.* 2012a, c) and postpartum anestrus (60.0%) buffaloes (Dutt *et al.* 2011), estrus response observed in the present study was relatively lower after using the similar treatment. The reason behind the decline in the behavioural response of estrus is unclear. Further, the estrus response in terms of proportion of heifers showing standing estrus was lower in this study than the response (85.7%) found in delayed pubertal Shahiwal heifers when treated with the similar herbs under validation trial (Kumar *et al.* 2012a).

Previously, it was reported that anestrus animals show incidence of silent estrus when treated with *Aegle marmelos* and *Murraiya koenigii* leaf powder (Das *et al.* 2012a, c, d; Kumar *et al.* 2012a). The incidence was reported to vary between 12.5% and 16.7% in heifers (Das *et al.* 2012a, c; Kumar *et al.* 2012a) and 25% in buffalo heifers (Das *et al.* 2012d). In the present study, the incidence of silent estrus was much higher (36.3%), than we found in earlier occasions. The reason for such variation in the incidence of silent estrus is unclear to us at present. This indicate that beside the standing estrus, the combined treatment can also induce cyclicity to about an additional 36% heifers which appeared to acyclic before the treatment.

The estrus induction response of this study was, however, higher than that previously reported either with *A.*

marmelos (Kumar 2008) or with *M. koenigii* (Umashankar *et al.* 2006) alone in anoestrus buffaloes. Higher estrus response after using both the plants together might be due to synergistic actions of the component parts of the plants and supports the previous views of Dutt *et al.* 2010; 2011.

Previously, it has been reported that ethanolic extracts of Murraya *koenigii* (Mehrotra *et al.* 2003; Nandini *et al.* 2010) and *Aegle marmelos* (Jondhale *et al.* 2009a) advances puberty in immature female rats. The possible effect is attributed to either the presence of phytoestrogens and high mineral content in the leaf of the plants or stimulating effect on the endogenous steroidogenic activity under the hypothalamic effect (Mehrotra *et al.* 2003, 2004; Jondhale *et al.* 2009a; Nandini *et al.* 2010).

Further, when both the plant leaves were combined it induced the estrus in anestrus goats (Dutt *et al.* 2010), postpartum buffalo (Dutt *et al.* 2011) and delayed pubertal heifers (Kumar *et al.* 2012a). The effect in the above studies has been attributed to either the increase in the number of medium and large size follicles as well as estradiol production in anoestrus goats (Dutt *et al.* 2010) or due to the increased follicular growth at the terminal stage of development (Kumar *et al.* 2012b). The beneficial effects of the above said plants on increased follicular turnover, promotion of terminal follicular growth and increased estradiol production (Dutt *et al.* 2010: Kumar *et al.* 2012b) probably caused exhibition of estrus in delayed pubertal heifers in this study.

Supplementation of *Aegle marmelos* in combination with *Murraya koenigii* induced estrus and fertility in more than 50 per cent of the treated delayed pubertal heifers in this study. The present finding strengthens our preliminary observation on delayed pubertal heifers reported recently (Das *et al.* 2012a, c) and also the previous reports from our group in anestrus goats (Dutt *et al.* 2010) and post-partum buffaloes (Dutt *et al.* 2011). Our present finding together with our previous reports indicate that medicinal plants namely *Aegle marmelos* and *Murraya koenigii* have the potential for the induction of estrus in anestrus cattle, buffalo and goats.

Latent period (Onset of treatment to estrus interval): The mean interval between the initiation of treatment and the onset of standing estrus in the heifers was 6.00±1.69 days (ranged between 1 to 12 days) in treatment group. The only heifer that showed standing estrus in control group was within 3 days in respect to first day of treatment in treatment group (Table 1).

Aegle marmelos in combination with *Murraya koenigii* is reported to induce estrus by 5.33 d and 9.33 d after the end of treatment in anoestrus goats (Dutt *et al.* 2010) and buffaloes (Dutt *et al.* 2011), respectively. Similar treatment is reported to induce estrus at an interval of 22.8 d and 8.8 d in delayed pubertal heifers under farm (Das *et al.* 2012a) and field (Das *et al.* 2012b) condition, respectively. Compared to our previous reports, the mean interval between the commencements of the treatment to the onset of standing estrus was the shortest in the present study. The variation in the mean interval to onset of estrus within the delayed pubertal heifers among the allied studies is, however, unclear to us. Therefore, it can be speculated that individual animal variation may possibly the reason for the difference in the outcome of various studies.

Conception and Calving rate

Six (54.6%) out of 11 heifers in treatment group and 1 (16.7%) out of 6 in control group were found pregnant in

Table 1: Estrus induction response and fertility in delayed pubertal heifers fed with *Aegle marmelos* and *Murraya koenigii* in combination under farm condition

Treatment (s)	No. of heifers treated	No. (%) showed cyclicity	No. (%) showed standing estrus	Interval to estrus X± SEM (days)	No. (%) pregnant	No. services/ conception	No. (%) Heifers calved
1. AME+MUR	11	10 (90.9*)	6 (54.1)	6.00±1.69	6 (54.1)	1.83 (11/6)	6 (54.1)
2. Control	6	1 (16.7)	1(16.7)	3.00	1(16.7)	1.0 (1/1)	1(16.7)

AME= *Aegle marmelos*; MUR=*Murraya koenigii* *P<0.01

the present study. The number of insemination (s) required per conception was 1.83 (11/6) and 1 (1/1) in treatment and control groups, respectively. All the heifers that showed standing estrus and were inseminated, they became pregnant and finally calved. However, no significant (P>0.05) difference was seen in either in conception rate or calving rate between herbs treated and control groups (Table 1).

Under field condition, *Aegle marmelos* and *Murraya koenigii* in combination is reported to yield about 40% and 42.9% pregnancy rate (on treated basis) in anoestrus buffaloes (Dutt *et al.* 2011) and heifers (Das *et al.* 2012b). Further, under farm condition, the pregnancy rate is reported to be higher ranging between 50% (Das *et al.* 2012c; Kumar *et al.* 2012a) to 71.4% (Das *et al.* 2012a) in anoestrus heifers. With the similar treatment, the pregnancy rate obtained in the present study was within the range as we reported previously (Das *et al.* 2012a, c; Kumar et al, 2012a). Result pertaining to the pregnancy rate obtained in this study thus supports our previous findings (Das *et al.* 2012a, c; Kumar *et al.* 2012a) stating the unraveling potential for the use of the leaf powder of the above said plants in combination for fertility augmentation in anoestrus heifers.

In the present study all the pregnant heifers calved. We do not have any comparable literature on the calving rate in the anoestrus heifers or anoestrus animals in general after treatment with both the plants together. But the calving rate over 50% after the treatment indicates the potential effect of both the plants on fertility augmentation in delayed pubertal heifers.

CONCLUSION

The present study suggests the beneficial effect of *Aegle marmelos* and *Murraya koenigii* plant leaves for estrus induction and fertility augmentation in delayed pubertal heifers as in anestrus goats and buffaloes reported earlier. There is a need to conduct more validation trials using the treatment protocols in a larger population of heifers. Further, exploration of the major constituents that trigger the effect and the possible mechanism that is involved in this cascade of events leading to induction of cyclicity and estrus exhibition also warrant future investigation. The potential use of the plants as sources of antioxidants for promoting luteal function following breeding, in fertility augmentation programme may another area under future investigation.

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