HERBAL APPROACH FOR INDUCING MULTIPLE FOLLICULAR DEVELOPMENT IN CROSSBRED COWS

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ABSTRACT

The objective of the study was to assess the efficacy of fresh indigenous herbs in inducing multiple follicular development in crossbred cows. Healthy and regularly cyclic crossbred cows (n=6) were utilized for the study. As a control study, $PGF_{2\alpha}$ (25 mg; IM) was administered to all the cows on Day 14 (Day 0 – estrum) of the cycle. In the subsequent natural cycle, fresh wet mash of *Murraya koenigii* leaves (200 – 300 g /day) and *Pedalium murex* (50 g /day) were administered daily (per os) in equally split doses at 12 h interval, for seven days starting from Day 9 of the cycle. Sap of *Aloe vera* leaves (100 g; per os; b.i.d) was administered for the first two days and $PGF_{2\alpha}$ (25 mg; IM) was administered on the penultimate day (Day 14) of herbal treatment. The ovarian status was assessed ultrasonically for the presence of large follicles ($\geq$ 9 mm) on the day of $PGF_{2\alpha}$ induced estrus in both control and herbal treated cycles. All the animals (100%) responded for the herbal treatment with $\geq$ 2 large follicles. The mean numbers of large follicles on the day of induced estrus in control and treated cycles were 1.25 $\pm$ 0.50 (1- 2) and 3.67 $\pm$ 1.37 (2 – 5) respectively with a mean diameter of 10.11 $\pm$ 0.40 mm and 9.38 $\pm$ 0.31 mm respectively. From the study it was evident that the multiple follicular development could be effectively achieved using the above combination of herbs.

Keywords : Multiple folicular development, Herbal approach, Cows.

INTRODUCTION

Multiple ovulation and embryo transfer (MOET) technology is having greater potential in accelerating genetic gain in dairy cattle. However, the induction of multiple follicular development and ovulations, the key element of MOET, which was performed by administration of gonadotrophins attribute for the high cost of embryo production and thus restricting the application of conventional embryo transfer programmes to research faculty rather than benefiting the farming community. Hence there is a need to explore scientifically an alternate agent for the gonadotrophins to reduce the cost of embryo production. Use of certain herbal preparations for fertility regulation was well documented in ancient literatures. Cost effectiveness and easy availability make herbal remedies as a viable alternative to hormones which were having limitations viz., high cost, variable results, residual effect in food animals, lack of easy availability etc. Mehrotra *et al.* (2004) and Kosif and Aktas (2009) studied the effect of certain indigenous medicinal plants and found that *Murraya koenigii* leaves, popularly known as curry leaves and *Aloe vera* gel had the potential to augment the ovarian function in terms of follicular development and steroidogenesis in rats. So the present study was aimed at applying the principle in farm animal species and designed to assess the efficacy of these herbs in inducing multiple follicular development in crossbred cows.

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MATERIALS AND METHODS

Six healthy and regularly cyclic crossbred cows maintained at Centralised Embryo Biotechnology Unit, Department of Animal Biotechnology, Tamilnadu Veterinary and Animal Sciences University, Madhavaram, Chennai –600 051, were utilized for the study. All the cows were maintained under ideal and identical stall fed conditions during the study. All the experimental cows were monitored regularly for estrus symptoms and cyclicity of the animals was confirmed by gynaecological examination at ten days interval.

Initially, as a control study, $PGF_{2\alpha}$ (Lutalyse: Dinoprost tromethamine; Pfizer; 25 mg; IM) was administered to all the cows on Day 14 (Day 0 – estrum),

after confirming the presence of prominent CL, to induce estrum. The same animals were subjected for herbal treatment from Day 9 of the subsequent natural cycle. Fresh *Murraya koenigii* leaves (200 – 300 g /day) were made into a wet mash in electrical blender and administered (per os) along with fresh *Pedalium murex* leaves (50 g /day) daily in equally split doses at 12 hrs. interval, for seven days. *Aloe vera* sap (100 g) was also administered twice daily along with the above herbs for the first two days. $PGF_{2\alpha}$ (25 mg; IM) was administered on the penultimate day (Day 14) of herbal treatment. On the day of $PGF_{2\alpha}$ induced estrus, the ovarian follicular status was assessed in both control and herbal treated cows using a real time B-mode ultrasound scanner (SONOVET 600) equipped with 7.5 MHz transrectal transducer. The number and size of large follicles ($\geq$ 9 mm) considered to be having the ovulatory capacity was recorded. Data on follicular characteristics in normal and herbal treated estrous cycles were analysed by Student's *t*-test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

All the animals (100%) subjected for the herbal treatment responded with $\geq$ 2 large follicles, with a maximum of five follicles on the day of induced estrus (Fig.). The mean numbers of large follicles in treated cycle (3.67 $\pm$ 1.37) were significantly ($P< 0.05$) more than control cycle (1.25 $\pm$ 0.5). There was no significant difference in the diameter of these follicles between the control (10.11 $\pm$ 0.40 mm) and treated cycles (9.38 $\pm$ 0.31 mm). Thus the multiple follicular development was effectively achieved using this combination of fresh indigenous herbs in crossbred cows.

Mehrotra *et al.* (2004) in an effort to study the effect of ethanolic extracts of *M. koenigii* (1000 mg/kg) on follicular development in rats, found that the mean number of large surface follicles, large preantral and antral follicles were significantly higher in treatment group as compared to control indicating favourable effect of *Murraya* on large follicle population. They suggested that *M. koenigii* induced FSH secretion thereby causing early recruitment of follicles bearing greater number of FSH receptors leading to enhanced large follicle population. Kosif and Aktas (2009) documented angiogenic and FSH like effect of *Aloe vera*. They observed vascular increase and the hyperemic form of ovary with increased follicle numbers on histological section of ovaries of female Wistar albino rats treated with *A. vera*. In the present study also, we speculate that, initiation of treatment on Day 9 of the cycle coincided with the emergence of second follicular wave (Satheshkumar *et al.*, 2008) and the recruited follicles in growing phase possessing FSH receptors would have responded for the FSH like effect of the herbs and reduced the number of atretic subordinate follicles of the wave leading to multiple follicular development during the induced estrus.

Mehrotra *et al.* (2003 and 2004) also observed higher steroidogenic activity, primarily oestradiol 17-β, in the ovaries of *M. koenigii* supplemented rats. Oestrogens are potent mitogens in granulosa cells *in vivo* (Richards, 1980), that might have stimulated granulosa cells mitosis in the developing follicles, thereby potentiating the follicular development. Moreover, curry leaves (*M. koenigii*) are very rich source of minerals, especially calcium, phosphorus, iron etc., (Gopalan *et al.*, 1996 and Shantala and Prakash, 2005) which are important in animal reproduction. The effect of enhanced recruitment and reduced atresia of follicles by FSH secretion might be further potentiated by active principles of *Pedalium murex* which was generally considered to enhance fertility of the individual (Balakrishnan *et al.*, 2009).

Further, the cost involvement for the conventional hormonal approach for inducing superovulation was approximately Rs.6000/-, whereas for the herbal approach it was only approximately Rs.400/-. The present study thus places on record a successful induction of multiple follicular development at a cheaper cost using the combination of fresh indigenous herbs in a farm animal species, which might reduce the overall cost of embryo production in MOET.

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FIG. Ultrasonograhic view and pictorial representation of right and left ovaries showing multiple follicular development in $PGF_2\alpha$ induced oestrus of herbal treated cycle