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The evaluation of ethno-veterinary practice (EVP) in managing infertility in cattle

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Abstract

The study was conducted to assess the effectiveness of EVP in managing infertility in cattle. A total of 351 cows belong to HF and Jersey hybrids between 2 to 7 years of age were included in this study with the history of infertility. Each animal in the study group was given sequential administration of *Raphanus sativus*, *Aloe vera*, *Moringa oleifera*, *Cissuss quadrangularis* and *Murraya koenigii* and *curcuma longa* for a period of 20 days. Thirty one cows were treated once, 95 of them were treated 2 times and 225 of them are treated 3 times. Research findings indicate that 323 (92.0%) out of 351 cows were conceived after providing treatment with EVP. The conception rate was 35.5% for single treatment, 95.79% for 2 treatments and 98.22% for 3 repeated EVP therapies. The average cost of treatment of infertility with EVP is Rs. 343 while the conventional treatment cost is Rs.3544. There is huge economic loss to farmers due to infertility and therefore, there are immense opportunities for transferring this technology among dairy farmers.

Keywords: Infertility, repeat breeding, metritis, endometritis Ethno-veterinary practice, herbal formulations, dairy farmers

Introduction

An animal with a healthy reproductive function normally calve every 12-14 months. Infertility is a short-term disturbance in reproductive function leading to failure of the animal getting pregnant. It may be due to diseases of genital organs, infectious diseases, physiological causes like absence of heat, repeat breeding, silent heat, cystic ovary, anatomical causes and faulty artificial insemination (AI) technique. Cows with high milk production in the dairy production systems has also suffered a decline in cow fertility (Walsh *et al.*, 2011) [1].

The postpartum infection of uterus with bacteria like *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, *Prevotella melaninogenica* and *Proteus* species is common (Sheldon *et al.*, 2006) [2]. These infections are associated with increased endometrial inflammation and purulent vaginal mucus (Sharma *et al.*, 2017) [3]. About 20% of these cows are unable to overcome the infections and develop metritis and persistence of pathogenic bacteria results in clinical endometritis. Many of these cows that are treated for clinical endometritis have low conception rates and remain infertile (Sheldon *et al.*, 2009, Sheldon *et al.*, 2009a, Sharma *et al.*, 2017) [4, 5, 3]. Cows with clinical endometritis were likely to have delayed resumption of ovarian cyclicity and likely to have prolonged postpartum luteal phases (Opsomer *et al.*, 2000) [6]. Reduced immune system of lactating dairy cows are more susceptible to invading pathogens causing mastitis (Ingvarsen *et al.*, 2003, Sordillo and Aitken, 2009) [7, 8]. Cows with clinical mastitis in the early days after calving had delayed onset of oestrous behaviour compared to healthy ones (Huszenicza *et al.*, 2005) [9] and indicate that clinical mastitis in the early postpartum period had a profound effect on reproductive success. The inability to easily detect oestrus further hinders insemination at the correct time. The recovery from uterine inflammation and infection after parturition are critical for the uterus to provide a favourable environment for establishment and maintenance of pregnancy.

Repeat breeding is the failure of the cow without any detectable abnormalities to conceive from three or more regularly spaced inseminations (Gustafsson and Emanuelson, 2002) [10]. It is a serious problem in dairy industry leading to increased inseminations, delay in conception, increased calving intervals, culling of the animal and economic loss (Perumal *et al.*, 2013, Satheskumar *et al.*, 2021) [11, 12]. Full signalling of oestrous behaviour is vital for proper timing of AI and good reproductive performance. Repeat breeding may be due to nutritional deficiency, ovulatory disturbances, luteal inadequacy and sub-clinical uterine infections.

Murraya koenigii and *Aegle marmelos* have been reported to be efficacious for the treatment of anoestrus, repeat breeding and endometritis (Dutt *et al.*, 2011, Dutt *et al.*, 2018, Baitule *et al.*, 2016) [13-15]. Sequential administration of *Raphanus sativus*, *Aloe vera*, *Moringa oleifera*, *Cissus quadrangularis* and *Murraya koenigii* for a period of 20 days have enhanced the follicular development, induced oestrus and improved conception rate in anoestrus cows (Satheshkumar *et al.*, 2021) [12]. Supplementation of *A. marmelos* and *M. koenigii* in combination, as well as *M. koenigii* alone, were effective in fertility improvement in delayed pubertal buffalo heifers by increasing ovulation and conception rate (Kujur *et al.*, 2022) [16]. The combination of both *Murraya* and *Aegle* showed higher results in oestrus induction and also enhanced biochemical profile which is crucial for reducing the fertility in cattle. It was hypothesized that the combination of these herbs can potentiate the ovarian and uterine health status and thereby improve the conception rate among repeat breeders. Hence the present study was undertaken to evaluate effect of sequential administration of *R. sativus*, *A. vera*, *M. oleifera*, *C. quadrangularis*, *Curcuma*

longa and *M. koenigii* on infertility, conception rate and the cost effectiveness of EVP.

Materials and Methods

A total of 351 Holstein-Friesian crossbred and Jersey crossbred heifers and cows between 2 to 7 years of age (Heifers: 52; 1st-parity: 136; 2nd parity: 114; 3rd parity: 38 and 4th parity: 7) with the history of infertility from Kottayam, Ernakulum, Alappuzha, Trissur and Palakkad districts of Kerala state, India were utilised for the study. They were inseminated 3 to 15 times and many of them also have been received intrauterine (i.u.) infusion but did not conceive. One fifty one of these cows including 2 heifers had reddish smelly secretion indicating infection in the uterus. Two hundred of them did not have prominent oestrus signs. These animals were undergone rectal examination and confirmed that they did not have apparent reproductive tract deformities. These cows were fed with of the ethno-veterinary herbal preparations as follows. The name of the plants, parts used and the ratio of the ingredients are given in Table 1.

Table 1: The ingredients, parts used and the ratio of the ingredients

S. No.	Local name	Botanical name	Parts used	Quantity
1.	Aloe	<i>Aloe vera</i> (L.) Burm.f.	Leaf	One leaf (about 100 g)
2.	Cumin, Jeera	<i>Cuminum cyminum</i> L.	Seeds	10 g
3.	Drum stick plant	<i>Moringa oleifera</i> Lam	Leaves	4 handful (about 100 g)
4.	Veldt grape Devil's backbone	<i>Cissus quadrangularis</i> L.	Stem	4 handful (about 100 g)
5.	Curry leaves	<i>Murraya koenigii</i> (L.) Sprengel	Leaves	4 handful (about 100 g)
6.	Turmeric	<i>Curcuma longa</i> L.	Rhizome	10 grams
7.	Jaggery			Sufficient (about 100 g)
8.	Salt			

Methods of preparation and treatment

Administration of herbal combination was started in following order on first or second day of the observed oestrus (Day 0).

- Day 1 to Day 3:** One radish (about 100 g) each in the morning and evening was administered orally after applying a paste of cumin and salt over it.
- Day 4 to Day 7:** *A. vera* leaf (100g after removing the thorns) along with Jaggery (about 100 g) was administered orally for the next four days.
- Day 8 to Day 11:** Animals were fed with *M. oleifera* leaves (100 g) once daily.
- Day 12 to Day 15:** Four handfuls of *C. quadrangularis* along with jaggery were administered once daily.
- Day 16 to Day 20:** Paste of four handful of curry leaves (100 g) and turmeric (10 g) was administered orally once daily.
- If the cow is not shown signs of oestrus, repeat this treatment once again in the next cycles

Thirty one cows were treated once, 95 of them were treated 2 times and 225 of them are treated 3 times (Table 2). Interval between each treatment is between 21 to 22 days. The signs of oestrus were explored as visual observation of presence of clear mucus vaginal discharge, restlessness, licking or sniffing of the perineal region, bellowing, butting and mounting other cows (or attempt) and of standing heat. Once cows showed good oestrus signs after EVP treatment, they were inseminated twice at 24 houses interval. Only semen from NDDB (5 star bulls) were used to keep the quality. The

pregnancy diagnosis was confirmed by trans-rectal palpation through rectal examination after 3 months of AI.

Result and discussion

Out of 351 cows selected with variable infertility status for the study. One fifty of them had infectious uterus indicated because of the smelly secretion. Metritis is a severe inflammatory response that occurs within all the layers of the uterus including the endometrium, submucosa, myometrium and perimetrium. Endometritis is defined as inflammation of mucus membrane of uterus and presence of mucopurulent to purulent discharge in uterus after three weeks of parturition or later. Endometritis continues to be a major cause of poor fertility and delayed conceptions (Couto *et al.*, 2013) [17]. Sixty one of the infected cows recovered in 30 days with one course of EVP treatment, eighty six took 60 days to restore normal vaginal discharge with 2 to 3 course of treatment and 4 of them took 80 days to become normal. Two hundred did not show any significant oestrus signs. Oestrus signalling is restored between 20-40 days with one course of EVP treatment in sixty of them. One hundred and forty of them needed two to three repeated treatment with EVM and 60 - 90 days to display good signs of oestrus (Table 3). A total of 323 (92.0%) out of 351 cows were conceived after providing treatment with EVP (Table 3). The conception rate was 35.5% for single treatment, 95.79% after 2 treatments and 98.22% after 3 repeated EVP treatments (Table 2). The average cost of treatment of infertility in cows with EVP is Rs. 343 while the conventional treatment cost is Rs.3544.

Table 2: Shows number of repeated EVP treatment number of days took for oestrus signaling and per cent of PD positive cows

Number of time(s) EVP Treatment	Number of cows treated	Number of days took for Oestrus Signalling	PD positive	% of cow P D positive
1	31	20-40	11	35.5
2	95	60	91	95.79
3	225	80-90	221	98.22

Table 3. Showing the age of the cows, number of AI before EVP treatment, Number of days took for oestrus Signalling after EVP treatment and the pregnancy diagnosis

No. of times AI conducted Before EVP	No. of cow(s) AI conducted after EVP treatment	No. of days for oestrus Signalling	No. of times EVP Provided	No. of cows	No. PD Positive	No. PD Negative
3	22	20	1	3	1	2
		30	1	5	5	0
		40	1	1	1	0
		60	2	6	6	0
		90	3	7	7	0
4	110	20	1	1	0	1
		30	1	3	2	1
		40	1	1	0	1
		60	2	41	40	1
		90	3	64	64	0
5	64	21	1	3	1	2
		40	1	1	1	0
		60	2	19	17	2
		90	3	41	39	2
6	83	30	1	1	0	1
		60	2	14	14	0
		90	3	68	67	1
7	22	21	1	1	0	1
		31	1	1	0	1
		60	2	3	3	0
		90	3	17	16	1
8	20	60	2	3	2	1
		90	3	17	17	0
		21	1	3	0	3
9	9	60	2	2	2	0
		90	3	4	4	0
		20	1	2	0	2
10	14	28	1	1	0	1
		60	2	4	4	0
		90	3	7	7	0
12	1	21	1	1	0	1
14	3	26	2	1	0	1
		60	2	2	2	0
15	3	21	1	1	0	1
		35	1	1	0	1
		60	2	1	1	0
	351			351	323	28

Animals treated with *Cuminum cyminum* and *Raphanus sativus* for subclinical endometritis shown 100% recovery (Elamaram *et al.* 2018) [18]. *A. vera* and *R. sativus* help in cleansing the uterine environment (Dutta *et al.* 2022) [19]. However, it is less effective in high degree of clinical endometritis (33.3%). *Raphanus sativus* contain ‘raphanin’ which has already been reported for its strong activity against *Escherichia coli*, *Pseudomonas pyocyaneus*, *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococci* and *Pneumococci* (Elamaram *et al.* 2018, Pérez Gutiérrez and Perez, 2004) [18, 20]. *Cuminum cyminum* is antimicrobial, insecticidal, anti-inflammatory, analgesic, antioxidant, (Surjushe *et al.* 2008) [21]. The use of EVM formulation against smelly reddish coloured secretion has rational pharmacological basis. *A. vera* and *R. sativus* help in

cleansing the uterine environment (Elamaram *et al.* 2018) [18]. It was reported that the overall 57.1% conception rate among the post-partum anoestrus cows (n=14) treated with same EVP protocol (Satheshkumar, *et. al* 2021) [12]. “Herbal combo treatment” (HCT) induce oestrus and enhance fertility among PPA cows. Steroidogenic activity, rich nutrient source and anti-inflammatory properties of HCT would have potentiated the follicular development and thereby induced oestrus and improved conception rate in anoestrus cows (Satheshkumar *et al.*, 2021) [12]. *Aloe vera* is reported to have anti-microbial and anti-inflammatory activities and also may help in cleansing the uterine environment (Dutta *et al.* 2022, Surjushe *et al.* 2008, Bettaieb *et al.* 2011) [19, 21, 22]. The combination of *Moringa oleifera leaves*, *Cissus quadrangularis stem* and *Murraya*

koenigii leaves improve the follicular maturation and ovulating capacity (Elamaran *et al.*, 2018) [18]. *Aloe vera*, *Murraya koenigii*, *Agele marmelos* and other number of plants are listed to induce follicle growth and oestrus in cows (Chandrasekhar and Purohit, 2020) [23]. Oral administration of *Raphanus sativus* for first 5 days, *Aloe vera* for next four days, *Moringa oleifera* for next four days, *Cissus quadrangularis* for next four days and *Azadirachta indica* leaves with *curcuma longa* for last four days for augmenting fertility in heifers (Sagadevan *et al.* 2021) [24]. *M. oleifera* (*Moringa*) leaves are rich in minerals and vitamins and also a source of insulin like proteins (Gopalakrishnan *et al.*, 2016) [25]. The dietary *Moringa oleifera* leaves improved reproductive performances of mice. *Moringa oleifera* leaves may serve as a functional feed additive for improving animal reproductive performance (Bin Zeng *et al.*, 201) [26]. The lactagogue, made of phytosterols, acts as a precursor for hormones required for reproductive growth. *Moringa oleifera* is rich in phytosterols like stigmaterol, sitosterol and kampesterol which are precursors for hormones. These compounds increase the oestrogen production, which in turn stimulates the proliferation of the mammary gland ducts to produce milk. *C. quadrangularis* phytoestrogen rich fraction has the capacity to increase the serum oestrogen which play a fundamental role in the physiology of the reproductive system (Franck *et al.* 2013) [27], blood calcium level and vitamin D3 (Seema, 2015) [28]. *C. quadrangularis* extract has an oestrogenic property and oestrogen plays a major role in the regulation and development of the female reproductive system (Rex and Lokesh, 2020) [29]. Evidence from the previous studies suggested that *C. quadrangularis* has antimicrobial, anti-diabetic, anti-inflammatory, anti-obesity, anti-oxidant, bone turnover, cardiovascular and hepatoprotective activities (Zaki *et al.* 2020, Seema 2015) [30, 28].

Conclusion

Infertility is a serious problem in dairy industry leading to substantial economic loss. Sequential feeding of *Raphanus sativus* (radish) root, *Aloe vera*, (Aloe) leaf, *Moringa oleifera* (drum stick) leaves, *Cissus quadrangularis* (Veldt grape/ Parandai) stem and *Murraya koenigii* (curry leaves) along with *Curcuma longa* (turmeric) rhizome for a period of 20 days is a cost effective and efficacious alternative for managing repeat breeding in cattle.

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