

ORIGINAL PAPER

Effect of a Homeopathic complex on oestrus induction and hormonal profile in anoestrus cows

R Rajkumar*, SK Srivastava, MC Yadav, VP Varshney, JP Varshney and H Kumar

Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

This study was undertaken to evaluate the efficacy of a homeopathic complex in the management of true anoestrus in crossbred cows. Six anoestrus cows were treated with a homeopathic complex (*Calcarea phosphorica 30c, Aletris farinosa 30c, Pulsatilla 30c, Aurum muriaticum natronatum 30c, Sepia 30c and Phosphorus 30c* in equal proportion, 15 pills twice daily orally for 10 days). Six animals acted as control without any treatment.

Treatment was 100% effective in inducing oestrus in anoestrus cows with mean interval of 27.5 ± 5.3 days. All animals conceived and overall conception rate was 54.5% with 1.83 services per conception. In the homeopathic complex treated group, increased serum oestradiol concentration (20.88 ± 5.60 to 27.80 ± 7.28 pg/ml) was observed compared to the pretreatment (11.71 ± 2.06 pg/ml) and control value (10.43 ± 1.77 to 13.94 ± 3.14 pg/ml). The homeopathic complex medicine may be effective and economical in the treatment of true anoestrus condition in cows. *Homeopathy* (2006) 95, 131–135.

Keywords: anoestrus; crossbred cows; homeopathic complex

Introduction

The success of dairy cattle and buffalo husbandry depends on optimal reproductive rhythm of every female in the herd. It is estimated that around 18–40% cattle are culled per annum due to infertility or sterility in India.¹ Anoestrus is the most common single cause of infertility in dairy cattle and is associated with large economic losses mainly because of prolonged calving interval, reduced calf crop and shorter productive life. In India, the incidence of anoestrus has been reported to vary from 12.3% to 64.8%.^{2,3} The occurrence of prolonged period of ovarian quiescence and anovulation are mainly due to lowered plasma luteinising hormone (LH) level. Factors that suppress LH pulse frequency include energy deficiency, stress factors,

endogenous opioid peptide, suckling and lowered insulin concentration.⁴

Many hormonal preparations including GnRH, eCG, progestogen alone or in combination have been tried to restore the ovarian cyclicity and to induce oestrus in anoestrus animals, but the results are generally unsatisfactory.⁵ The major constraints to the use of hormonal preparations are variable results and high cost. Therefore, the search for cost-effective alternative approaches is being pursued throughout the world.⁶ Homeopathy has gained the reputation of an effective alternative therapy in veterinary practice in Europe. Homeopathic drugs such as *Sepia* have been evaluated in the management of anoestrus cows.⁷ Homeopathic combination remedies are also increasingly popular.⁸ Recently, encouraging results with a homeopathic combination remedy in management of anoestrus have been reported in dairy cows and buffalo.⁹ However, these studies were preliminary and not conducted systematically, hence the present experiment was performed.

*Correspondence: R. Rajkumar, Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar 243122, Bareilly, Uttar Pradesh, India.
E-mail: rajrkumar@rediffmail.com
Received 21 February 2005; revised 19 July 2005; accepted 22 March 2006

Materials and methods

Treatment

A homeopathic complex medicine marketed as Healwell VT-4 (Sintex International Ltd, Kalol, North Gujarat, India) comprising *Calcarea phosphorica* 30c, *Aletris farinosa* 30c, *Pulsatilla* 30c, *Aurum muriaticum natronatum* 30c, *Sepia* 30c and *Phosphorus* 30c in equal proportion was evaluated. Fifteen pills of Healwell VT-4 were given, twice daily orally, over 10 days.

Animals

This study was conducted on 12 crossbred anoestrus dairy cows (Hariana × Exotic) maintained at the Livestock Production and Management farm, Indian Veterinary Research Institute, Izatnagar, Bareilly (UP) from September 2003 to May 2004. All the experimental animals were between the ages of 4 to 7 years, weighing 300–400 kg. They were in first to third lactation with a history of previous normal calving. The cows were selected for the experiment on the basis of history of having acyclic condition for more than 60 days postpartum during the current lactation and examination of genital organs. Animals with small, smooth and inactive ovaries (without any palpable structures like corpus luteum, follicle) and flaccid and atonic uterine horns, on rectal examination performed twice at 10 day interval after 60 days postpartum, were selected for study. Blood sample was collected at the time of second rectal examination for progesterone estimation to confirm ovarian inactivity (below 0.5 ng/ml indicates absence of corpus luteum in the ovaries). Treatment was started on the day of second rectal examination. The experimental animals were divided symmetrically into two groups consisting of two animals in first lactation, three animals in second lactation and one animal in third lactation in both groups (Table 1). The animals were maintained in one-four covered shed, under loose housing system (common housing system).

Experimental design

Cows in the treatment group were given orally 15 pills of VT-4 twice daily for 10 days, while cows in the control group were given blank sugar pills, 15 pills twice daily for 10 days by the same veterinarian.

Nutritional and husbandry conditions of both groups remained identical throughout the experiment.

Oestradiol and progesterone estimation

Blood samples (10 ml) were collected by jugular venepuncture on day 0, 4, 8, 12, 16, 20 at onset of oestrus or on 50th day after initiation of treatment. The serum was separated and stored at -20°C until further use. Oestradiol and progesterone concentrations in serum was measured by a gamma counter calibrated for ^{125}I using RIA Kits (ICN pharmaceuticals, Inc Diagnostic division, Costa Mesa, CA). The sensitivity of the assay for oestradiol and progesterone were 0.474 pg/ml and 0.095 ng/ml, respectively. The intra and inter-assay coefficients of variation were <15%. The cross-reactivity of oestradiol antiserum with other hormones was oestrone, 6.20%; oestriol, 1.45%; and ethinyl oestradiol, aldosterone, androstenedione, cholesterol, cortisol, 11-deoxy cortisol <0.01%. The cross-reactivity of progesterone antiserum with other hormones was corticosterone, 0.3%; 20 α -hydroxy progesterone, 0.1%; 20 β -dihydroxy progesterone, 0.1% and aldosterone, androstenedione, cortisol, 17 β estradiol, cortisone <0.01%.

Serum calcium was estimated using calcium kits (Glaxo India Ltd Worli, Mumbai, India) based on the O-Cresolphthalein Complexone method. Phosphorus levels were estimated using phosphorus kits supplied by Monozyme India Limited, Secunderabad, India.

Oestrus and pregnancy detection

Oestrus was detected by parading vasectomized bull between 07:00–09:00 and 16:00–18:00 h daily from the start of treatment as well as on the basis of heat signs such as mucus discharge from genitalia, swelling of vulva and mounting of fellow cows. Animals showing signs of oestrus were examined per rectum and oestrus confirmed by uterine tonicity, cervical relaxation and expulsion of mucus from genitalia at the time of rectal examination. Vulval mucus membrane was also examined for congestion during oestrus.

Cows showing oestrus, were inseminated by the same veterinarian twice at 10–12 h interval using 0.25 ml frozen semen straws from a single fertile bull and pregnancy was confirmed (per rectum) after 50–60 days. Treatment response was defined as oestrus

Table 1 Demographics of experimental animals

	Treatment group						Control group					
Animal no	1	2	3	4	5	6	1	2	3	4	5	6
Age (months)	48	61	38	51	54	36	58	34	50	49	39	51
Average milk yield/day (kg)*	9.23	8.04	9.24	6.12	7.14	10.02	7.25	9.24	8.11	9.27	9.63	6.48
SPC**	3	1	3	2	2	2	1	3	3	2	1	2
Peripartur disorders	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

* Average milk yield per day (total milk yield/no. of days in lactation) of current lactation.

** Service per conception of previous pregnancy.

symptoms; and (uterine tonicity, cervical relaxation and expulsion of mucus from genitalia on rectal examination within 50 days after initiation of treatment. The overall conception rate was defined as the number of inseminations which resulted in a pregnancy not less than 42 days later. The data were analysed using student 't' test to compare the differences between the groups and paired 't' test to compare the differences within the group on different days as per Snedecor and Cochran (1989) manually.¹⁰

Results

All animals treated with the homeopathic complex showed signs of oestrus at an average interval of 27.50 ± 5.33 days after initiation of treatment (Table 2). Ovulatory oestrus was confirmed by elevated levels of progesterone and presence of corpus luteum (CL) on 10th day post-oestrus. Out of six treated animals, only one conceived (16.66%) at first AI. The remaining five animals exhibited subsequent oestrus after an average interval of 20.80 ± 0.37 days. All these five animals conceived at second AI. The overall conception rate in treated animals was 54.5% with 1.83 service per

conception. None of the six control animals expressed oestrus symptoms until at least 50 days post-treatment and therefore considered not responded.

The mean concentration of oestradiol in the treated group was significantly higher ($P < 0.05$) on days 12 and 20 compared to day 0 (Table 3). The mean concentration also differed significantly ($P < 0.05$) on day 20 compared to control animals on the same day. However, the mean oestradiol concentration did not differ significantly on any day compared to day 0 in control animals. The mean concentration of progesterone (Table 3) did not differ significantly between days or groups. The mean serum calcium concentration in the treatment and control groups was 1.98 ± 0.07 and 1.86 ± 0.11 mmol/l before treatment and 2.23 ± 0.10 and 1.92 ± 0.08 mmol/l after the treatment respectively (Table 4). The concentration was significantly higher ($P < 0.05$) after treatment compared to control animals and pretreatment value. The mean serum phosphorus concentration in the treatment and control groups was 1.41 ± 0.04 and 1.41 ± 0.06 nmol/l before treatment and 1.74 ± 0.06 and 1.45 ± 0.06 nmol/l after the treatment, respectively (Table 4). In the treated group, concentration differed significantly ($P < 0.01$) from pretreatment value and control.

Table 2 Effect of homeopathic combination remedy on oestrus induction and conception in anoestrus crossbred cows (findings upto 50 days from initiation of treatment)

Experimental group	Animals in oestrus	Interval from treatment to onset of oestrus (days)	Service per conception	Overall conception rate
Treatment (n = 6)	6 (100.00%)	27.50 ± 5.33	1.83	54.54%
Control (n = 6)	None	—	—	—

Table 3 Mean serum estradiol (pg/ml) and progesterone (ng/ml) concentration in anoestrus cows treated with homeopathic combination remedy

Experimental group		Days of blood collection						
		0	4	8	12	16	20	50
Oestrogen	Treatment	11.71 ± 2.06^C	20.88 ± 5.60	21.52 ± 6.40	27.80 ± 7.28^A	22.01 ± 5.61	24.02 ± 4.89^{Aa}	—
	Control	10.43 ± 1.77	11.87 ± 2.63^C	11.09 ± 2.58^C	13.94 ± 3.14^C	12.48 ± 2.05^C	12.59 ± 1.65^C	11.47 ± 1.60
Progesterone	Treatment	0.413 ± 0.05	0.429 ± 0.07	0.429 ± 0.06	0.591 ± 0.13	1.160 ± 0.58	2.142 ± 0.92	—
	Control	0.399 ± 0.02	0.382 ± 0.06	0.417 ± 0.03	0.445 ± 0.02	0.446 ± 0.02	0.480 ± 0.03	0.445 ± 0.02

Values with superscripts A within same row differ significantly at $P < 0.05$ values with superscript C.

Values with superscripts a within same column differ significantly at $P < 0.05$ from values with superscript c.

Table 4 Mean serum calcium (mmol/l) and phosphorus (nmol/l) concentration in anoestrus cows treated with homeopathic combination remedy

Experimental group	Calcium (mmol/l)		Phosphorus (nmol/l)	
	Before treatment	After treatment	Before treatment	After treatment
Treatment	1.98 ± 0.07^a	2.23 ± 0.10^b	1.41 ± 0.04^A	1.74 ± 0.06^B
Control	1.86 ± 0.11^a	1.92 ± 0.08^a	1.41 ± 0.06^A	1.45 ± 0.06^A

Values within same row and column with different superscripts (A,B) differ significantly ($P < 0.01$).

Values within same row and column with different superscripts (a,b) differ significantly ($P < 0.05$).

Discussion

The results show an encouraging effect of homeopathic combination remedy in the management of anoestrus in cows. The result on oestrus induction activity in the present study was higher than in a preliminary trial of the same homeocomplex conducted by Kumar *et al*⁹ who treated 25 anoestrus cows and 6 anoestrus buffaloes with same dosage schedule and observed oestrus in 68% cows and 50% buffaloes. The average oestrus induction interval (14.4 days in cows; 16.0 days in buffaloes) in their study was shorter than our observations. In our study, cows were matched with no significant difference in their milk yields between groups (Table 1). After initiation of treatment, animals were not examined per rectum until expression of signs of oestrus. Hence any impact of rectal palpation on oestrus induction was equalized.

There was gradual increase in oestradiol concentration with treatment (Table 3). This might be due to *Pulsatilla* and *Aletris farinosa* present in complex, as these have emmenagogue and oestrogenic activity in the human female.^{11,12} *Aurum muriaticum natronatum* is also thought to have a stimulatory effect on female genital organs.¹³ The increase in oestradiol concentration in our study might be due to stimulatory effect of *Pulsatilla* and *Aletris farinosa* present in the complex on ovarian follicular growth and development as a positive relationship between plasma oestradiol concentration and ovarian follicular size during oestrus cycle has been reported in normal cyclic cows.¹⁴ In an earlier study, oestradiol concentrations were reported from 5–15 pg/ml during the follicular phase of oestrus cycle which is lower than our experiment.¹⁵ The pattern of oestradiol secretion in the present study reflects treatment induced follicular growth. In the control group, there was no significant rise in oestradiol concentration during sampling period and on day 50 (Table 3). This suggest that there was no major follicular activity in the control animals.

The level of serum progesterone in anoestrus cattle is less than 0.5 ng/ml.¹⁶ Following ovulation, the corpus luteum develops and plasma progesterone rises to a plateau of 6–10 ng/ml at 7–18 days in cyclic cows.¹⁷ The increase in level of progesterone on days 16 and 20 in this experiment was an indication of presence of corpus luteum and resumption of cyclicity in two animals of the treated group during the sampling period (one animal on 9th day and another on 12th day after initiation of treatment). Progesterone concentration remained almost unaltered in the control anoestrus cow. This indicates the absence of corpus luteum in the ovaries of control animals.

There was also significant increase in serum calcium and phosphorus concentration in cows after treatment as compared to pretreatment and control value (Table 4). This might be due to *Calcarea phosphorica* and *Phosphorus* present in the homeocomplex. Anoestrus, suboestrus or irregular oestrus occurs in animals as a

consequence of phosphorus deficiency. Marginal deficiency of phosphorus is sufficient to cause disturbance in the pituitary–ovarian axis without manifestation of deficiency symptoms and might cause infertility.¹⁸ Low levels of calcium and phosphorus have been reported in anoestrus cows as compared to normal cyclical cows.¹⁹ Mean concentration of serum calcium and phosphorus has been reported to vary from 8.98 ± 0.22 to 10.50 ± 0.44 and 4.98 ± 0.06 to 5.513 ± 0.26 mg%, respectively in normal cyclic Indian crossbred cows.^{19,20} Any effect of phosphorus deficiency may be mediated through impairment of phosphate-dependent biochemical reactions.²¹ Thus it appears that the homeopathic combination remedy induced oestrus through stimulating follicular growth and development, reflected by increased oestradiol level and this effect was enhanced by increasing level of phosphorus and calcium.

The cost of 15 pills given twice daily was approximately US \$0.02 (Rs 1/-). Hence total average cost of therapy in the management of anoestrus was highly economical; US \$0.21.

These findings suggest that the homeocomplex (VT-4) might offer an effective, safe and economical method for treatment of anoestrus condition in cows. However, for a conclusive remark regarding its wide application, further elucidation in a large scale trial is warranted.

Acknowledgement

We acknowledge Dr Shivang Swaminarayan, Head, Health care division, Sintex International Ltd, Kalol, Gujarat (India) for his keen interest and supplying homeo-complex (VT-4). Authors are also thankful to Director, IVRI for providing necessary facilities for conducting trial on Institute farm animals.

Statement of Conflict of Interest

This study was not sponsored by Sintex International, but the homeopathic complex (VT-4) was supplied by Sintex International Ltd, Kalol.

References

- 1 Kaikini AS. Reproductive disorders of Livestock. in: *Handbook of Animal Husbandry*. ICAR Publication, 2002. p. 692–718.
- 2 Naidu KV, Rao AR. Incidence of infertility among crossbred cattle of Andhra Pradesh. *Indian J Anim Sci* 1981; **51**: 829–831.
- 3 Luktuke SN, Sharma C. Studies on the incidence of true anoestrus in rural cattle and buffaloes. *Indian Vet J* 1978; **55**: 940–942.
- 4 Grunert E. Zur ovaridystrophia beim Rind. *Collegium Veterinarium* 1981; **73**: 77.
- 5 Odd KG. A review of synchronization of estrus in postpartum cattle. *J Anim Sci* 1990; **70**: 817–830.

- 6 Kaikini AS. Field problems of infertility in cattle and buffaloes. *Indian J Anim Reprod* 1989; **10**: 79-84.
- 7 Williamson AV, Mackie WL, Craford WJ, et al. A study using sepia 200C given prophylactically postpartum to prevent anoestrus problems in the dairy cow. *Br Homeopathic J* 1991; **80**: 149.
- 8 Reilly D. Is homeopathy a placebo response? Controlled trials of homeopathic potency with pollen in hay fever as model. *Lancet* 1986; **2**: 881.
- 9 Kumar H, Srivastava SK, Yadav MC, et al. Management of postpartum anoestrus in dairy animals with a homeopathic combination remedy. *Indian J Anim Sci* 2004; **74**: 739-740.
- 10 Snedecor GW, Cochran WG. *Statistical Methods*, 6th edn. USA: The Iowa State University Press, 1989.
- 11 Boericke W. *Pocket Manual of Homeopathic Materia Medica*, 9th edn. NewDelhi: Indian Books and Periodicals Publishers, 2001.
- 12 Duke A, Jo Bogenschutz-Godwin M, du Cellier J, et al. *Hand Book of Medicinal Herbs*, 2nd edn. Boca Raton, FL: CRC Press, 2002 p. 43-297.
- 13 Madrewar BP. Diseases of the female genital reproductive tract, infertility. in: *Therapeutics of Veterinary Homeopathy*. NewDelhi: B. Jain Publishers (P) Ltd, 1995. p. 127-152.
- 14 Staigmiller RB, England BG, Webb R, et al. Estrogen secretion and gonadotropin binding by individual follicles during estrous cycle. *J Anim Sci* 1982; **55**: 1473-1482.
- 15 Nachreiner RF. Diagnostic endocrinology. in: Morrow DA, editor. *Current Therapy in Theriogenology 2*. Philadelphia: W.B. Saunders Company, 1986. p. 17-25.
- 16 Singh U, Khurana NK, Inderjeet. Plasma progesterone profiles and fertility status of anoestrus zebu cattle related with norgestomet-estradiol-eCG regimen. *Theriogenology* 1998; **50**: 1191-1199.
- 17 Knickerbocker JJ, Drost M, Thatcher WW. Endocrine changes during the initiation of puberty, the estrous cycle, pregnancy and parturition in cattle. in: Morrow DA, editor. *Current Therapy in Theriogenology 2*. Philadelphia: W.B Saunders Company, 1986. p. 117-125.
- 18 Blood DC, Radostits OM, Henderson JA, et al. Diseases caused by nutritional deficiencies. in: *Veterinary Medicine*. 6th edn. Oxford: ELBS, 1983. p. 1015-1079.
- 19 Kumar S, Sharma MC, Dwivedi SK. Calcium, phosphorus and serum electrolyte changes in anoestrus and repeat breeding cows and heifers. *Cherion* 1986; **17**: 9-12.
- 20 Das S, Basu S, Sarkar AK, et al. Comparative study of certain serum macro and micro minerals in anoestrus and normal cyclical rural crossbred cows. *Indian J Anim Health* 2002; **41**: 99-102.
- 21 Parkinson TJ. Infertility in the cow. in: Noakes DE, Parkinson TJ, England GCW, editors. *Arthur's Veterinary Reproduction and Obstetrics*. 8th edn. Philadelphia: W.B. Saunders Company, 2001. p. 383-473.