The Indian Journal of Animal Reproduction; 26(1): 20-23; June 2005

Effect of certain indigenous medicinal plants on ovarian hormone profile and reproductive performance in anestrus cattle

S. MEHROTRA[1†], S.K. SINGH[2], UMASHANKAR[3] AND S.K. AGARWAL[4]

Division of Animal Reproduction
Indian Veterinary Research Institute, Izatnagar - 243 122, (U.P.)

Received : January 2, 2004
Accepted : August 7, 2004

ABSTRACT

Considering the limitations of hormones, the plant remedies mentioned in ancient and mediaeval texts/traditional practice need to be explored scientifically to evolve suitable alternatives in correcting reproductive dysfunction. In such an attempt the effects of medicinal plants viz. *Murraya koenigii* (Curry leaf plant) and *Urtica dioica* (Bichchu grass) were observed on serum estrogen progesterone concentrations and induction of estrus and conception in anestrus cattle. Twenty crossbred anestrus cattle were divided in three groups of 7 (*Murraya*), 7 (*Urtica*) and 6 (control). Animals in two treatment groups were assigned with oral administration of plant powder for nine days (day 0-8) based on extrapolated doses from rats. Serum sampling was carried out on day -8, -4, 0, 4, 8, 12 and 16, and estrogen, progesterone concentrations were determined using ELISA. Onset of estrus and pregnancy establishment was recorded. Results dipicted significantly rising estrogen levels in *Murraya* treated group without altering progesterone concentrations and appreciable induction of fertility, in the same group.

Key words : Medicinal plants, anestrus, cattle, hormone

Uptill now, considerable attention has been focussed on reproductive endocrinology as a mean to identify specific problems and to adopt therapeutic measures by using exogenous hormones for augmenting fertility in farm animals (Hukeri, 1995), but hormones with constraints viz. inconsistent results, high cost, non availability of commercial preparations with ease, lack of quick assay facilities, adverse effects, and residual nature, needs to be substituted by evolving efficacious, cheap and safer plant based remedies from our rich natural resources. The need to systematically explore the traditional knowledge, in the interest of suffering livestock has been emphasized by Singh and Kohli (1955) and Raviprakash and Sabir (1983). In such an attempt, the medicinal plants viz. *Murraya koenigii* (Curry leaf plant) and *Urtica dioica* (Bichchu grass) traditionally believed for their influence on reproduction, were chosen to examine their effects on ovarian hormone profiles (Estrogen and progesterone) and reproductive performance in anestrus cattle after finding encouraging results in rat (Mehrotra *et al.*, 2003).

[1]Senior Scientist
[2]Scientist,
[3,4]Principal Scientist

[†]Corresponding author

MATERIALS AND METHODS

Twenty anestrus crossbred (Holstein Friesian x Hariana) animals maintained under isomanagerial conditions were taken for study. The animals were diagnosed anestrus on the basis of anamnesis and twice clinico-gynaecological examination at ten days interval, finding smooth inactive ovaries, flaccid horns and closed cervix.

Animals were randomly divided into three groups viz. I *Murraya* (n=7), II *Urtica* (n=7) and III control (n=6). Group I and II were allocated with oral administration of plant powders mixed with concentrate and jaggery daily for nine days (day 0 to 8) whereas control received only concentrate and jaggery. The powder was formed after shade drying and grinding of freshly collected leaves. The experimental animals were dosed after extrapolation of doses described by Van Miert *et al.* (1986), from effective ethanolic extract dose level of rat i.e. 1000 mg kg^{-1} (Mehrotra *et al.*, 2003). This dose extrapolation from rat to cattle yielded extract dose for cattle but it was considered worth while to conduct the clinical investigation directly on whole leaf powder which becomes more useful for field practice. Therefore this extract dose was further converted to powder dose from the percent yield of extract using the formula :

Dosage of powder =

Dosage of extract x 100 / percent yield of extract

Blood collection in each group (n=5) through jugular veinipuncture was carried out on days -8, -4, 0, 4, 8, 12 and 16 followed by serum seperation and its storage at -20°C. Estrogen and progesterone concentrations were determined in serum using enzyme immunoassay (EIA) through pathozyme estrogen and progesterone kits (Omega Diagnostics Limited, Scotland, United Kingdom).

The estrus was detected using vasectomized bull followed by observing behavioural signs. Animals exhibited estrus were inseminated with good quality semen and pregnancy was confirmed per rectally at 45-60 day of insemination.

Statistical analysis : F values were calculated from one way analysis of variance (ANOVA) and the means for different experimental groups were compared by Duncan's multiple range test. Within group hormonal concentrations were compared using paired 't' test (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Serum estrogen concentrations are dipicted in Table 1. It is evident that the concentrations on day 4, 8, 12 and 16 were significantly higher (P<0.05) in group I (77.0±6.04, 87.0±4.35, 102.0±6.04 and 120.0±5.70) compared to control values (60.0±4.18, 63.0±6.63, 59.0±6.78 and 69.0±9.27). Concentations on day 12 and 16 were significantly higher (P<0.01, P<0.05) than day 0 value (66.0±7.31). The values of group II did not differ signficiantly from control. Perusal of Table 2 reveals that serum progesterone levels did not differ signfiicantly between groups or within group on different experimental days.

Anestrus profiles : The mean values of estrogen and progesterone were ranging between 57.0±7.68 to 73.0±7.51 pg/ml and 0.522±0.08 to 0.718±0.06 ng/ml respectively on day -8, -4 and 0 in anestrus cattle without showing any significant difference. Lower estrogen levels were reported by Prasad *et al.* (1989), Bernes *et al.* (1980), Humphrey *et al.* (1983) and Rawlings *et al.* (1980) as 3.50, 4.3, 7.3 and 0 to 20 pg/ml in anestrus cattle. The difference might be attributed to breed and climatic variation as well as depth of anestrus. However, the progesterone concentrations in present study are comparable to findings of Takkar and Singh (1980), Narayana *et al.* (1984) and Agarwal *et al.* (2001) who reported 1.0, 0.427 to 0.685 and 0.496 to 0.620 ng/ml progesterone, respectively in cows having smooth ovaries. Boyd (1977) opined that levels of plasma progesterone remain less than 0.5 ng/ml in anestrus cattle.

Table 1. Effect on serum estrogen concentrations (pg/ml) in anestrus cattle (Mean±SE)

Experimental group (n=5)	Days of blood collection -8	-4	0	4	8	12	16
I (Mur)	59.0±5.78	69.0±5.09	66.0±7.31	77.0±6.04^{a}	87.0±4.35^{a}	102.0±6.04^{a}**	120.0±5.70^{a}**
II (Urt)	57.0±7.68	60.0±4.47	58.0±4.63	54.0±4.30^{b}	65.0±4.18^{b}	63.0±6.04^{b}	70.0±3.53^{b}
III (Control)	61.0±4.30	73.0±7.51	69.0±5.78	60.0±4.18^{b}	63.0±6.63^{b}	59.0±6.78^{b}	69.0±9.27^{b}

Means with different superscripts differ significantly (P<0.05) in a column.
*Mean differ significantly (P<0.05) from day 0 value of same row.
**Mean differ significantly (P<0.01) from day 0 value of same row.

Table 2. Effect on serum progesterone concentrations (ng/ml) in anestrus cattle

Experimental group (n=5)	Days of blood collection -8	-4	0	4	8	12	16
I (Mur)	0.616±0.06	0.634±0.07	0.522±0.08	0.756±0.02*	0.822±0.03^{a}*	0.730±0.06	0.704±0.05
II (Urt)	0.624±0.09	0.536±0.05	0.550±0.08	0.622±0.03	0.600±0.03^{b}	0.704±0.03	0.638±0.07
III (Control)	0.682±0.05	0.564±0.09	0.718±0.06	0.724±0.04	0.682±0.03^{b}	0.638±0.07	0.712±0.03

Means with different superscripts differ significantly (P<0.05) in a column.
*Mean differ significantly (P<0.05) from day 0 value of same row.

Table 3. Effect on reproductive performance of anestrus cattle

Experimental group	No. of animals	No. expressed estrus	Time taken for onset of estrus after treatment (days)	Duration of estrus (h)	No. of animals confirmed pregnant out of expressed estrus
I (Mur)	7	4 (57.14%)	8.25±1.31	19.0±1.19	3 (75%)
II (Urt)	7	2 (28.57%)	11.5±2.5	20.0±4.01	1 (50%)
III (Control)	6	1 (14.28%)	12.0	20.0	1 (100%)

Effect of treatment : Significantly higher serum estrogen concentrations in group I animals on day 4, 8, 12 and 16 compared to control and on day 12 and 16 compared to day 0 value, indicated the enhancement of follicular development. The anestrus condition is reported to occur due to failure of ovulation rather than failure of development of dominant follicle (Roche and Boland, 1991). The dominant follicle undergoes atresia due to its inability to produce sufficient concentrations of estradiol to induce preovulatory gonadotrophin surge and thereby ovualtion (Roche and Diskin, 2000) and cow remains anestrus without showing signs of heat. McNatty *et al.* (1984) reported that most small (>2 mm) ovarian follicles of cattle acquire the ability to respond to LH and secrete androstenidione but only a relatively small number of larger (>5 mm) follicles have granulosa cells capable of metabolizing androstenedione to estradiol-17ß. In present study rising estrogen levels in group I animals might be a reflection of development of follicles in larger size (>5 mm) avoiding atresia, which seems possible either through stimulation of central mechanism for endogenous gonadotrophin release or mimicking effect of plant active principle(s) with possible local role in rescuing atresia.

Perusal of Table 3 dipicts appreciable induction of fertility in group I compared to control, in terms of percent onset of estrus and establishment of pregnancy out of expressed estrus (57.14 and 75) though statistical significance could not be obtained, which might be due to lesser number of animals. Time taken for onset of estrus and duration of estrus did not show any considerable difference between gorups. The results of the present study revealed that plant *Murraya koenigii* has promising response in fertility regulation, however, it is suggested that further studies involving greater number of animals with elucidation of precise mechanisms of action are warranted.

ACKNOWLEDGEMENT

Authors are thankful to Head, Animal Reproduction Division and Director, IVRI for providing necessary facilities for carrying out the present investigation.

REFERENCES

Hukeri, W.B. (1995). Modern trends in gynaecological therapy for augmenting bovine fertility. Indian J. Anim. Reprod., 16: 1-4.

Singh, A. and Kohli, J.D. (1955). A plea for research into indigenous drugs employed in veterinary practice. Vet. J., 32: 271-280.

Raviprakash, V. and Sabir, M. (1983). Scope of herbal medicines in veterinary practice. Vet. Res. J., 6: 1-9.

Mehrotra, S., Umashankar, Jawaherlal, Majumdar, A.C. and Agarwal, S.K. (2003). Effect of indigenous medicinal plants on onset of puberty in immature female rats. Indian J. Anim. Reprod., 24: 131-133.

Van Miert, A.S.J.P.A.M. (1986). The use of animals of drugs dicensed for human use only. In: *Comparative Veterinary Pharmacology, Toxicology and Therapy*. Eds. A.S.J.P.A.M. Van Miert Bogaert, M.G. and Debackere, M. Proc. III Congress of European Assoc. Vet. Pharmacol. and Toxicol., Belgium, MTP Press Limited, Lancaster, 489-500.

Snedecor, G.W. and Cochran, W.G. (1989). *Statistical Methods*. 8th edn., Affiliated East West Press, New Delhi.

Prasad, C.S., Sarma, P.V., Reddy, A., Obi and Cinnaiya, G.P. (1989). Trace elements and ovarian hormonal levels during different reproductive conditions in crossbred cattle. Indian J. Dairy Sci., 42: 489-492.

Bernes, M.A., Kagmer, G.N., Bierley, S.T., Richardson, M.F. and Dickey, J.F. (1980). FSH and estradiol-17ß in dairy cows treated with progesterone releasing intravaginal device(PRID). J. Dairy Sci., 63: 161-165.

Humphrey, W.D., Kaltenbach, C.C., Dunn, T.G., Koritnik, D.R. and Niswender, G.D. (1983). Characterization of hormonal patterns in beef cows during post-partum anoestrus. J. Anim. Sci., 56: 445-453.

Rawlings, N.C., Weir, L., Todd, B., Manns, J. and Hyland, J.H. (1980). Some endocrine changes associated with the post-partum period of the suckling beef cows. J. Reprod. Fert., **60**: 301-308.

Takkar, O.P. and Singh, Meher (1980). Progesterone levels vis-a-vis anoestrus in buffaloes concurrent with those during various stages of oestrous cycle. Abstr., 2nd All-India Symposium on Animal Reproduction, Bangalore, India.

Narayana, K., Honappa, T.G., Krishnamurthy, K. and Kumar, V.V. (1984). Serum progesterone profile in anoestrus buffaloes and cattle. Indian J. Anim. Reprod., **5**: 31.

Agarwal, S.K., Shanker, U., Kumar, S. and Mohan, G. (2001). Ovarian cyclicity and progesterone profile in post-partum anestrus cattle, using a synthetic progestogen, nongestomet regimen. Indian J. Anim. Sci., **71**: 1120-1123.

Boyd, H. (1977). Anoestrus in cattle. Vet. Rec., **100**: 150-153.

McNatty, K.P., Heath, D.A., Handerson, K.M., Lun, S., Hurst, P.R., Ellis, L.M., Montgomery, GW., Morrison, L. and Thurli, D.C. (1984). Some aspects of thecal and granulosa cell function during follicular development in the bovine ovary. J. Reprod. Fert., **72**: 39-53.

Roche, J.F. and Boland, M.P. (1991). Turnover of dominant follicles in cattle of different reproductive stages. Theriogenology, **35**: 81-90.

Roche, J.F. and Diskin, M.D. (2000). Resumption of reproductive activity in the early post-partum period of cows. In: Proc. of Conference on fertility in the high producing dairy cows, Galway, Ireland, Sept. 1999.