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Oestrus induction and fertility response in post-partum anoestrus dairy cows treated with homeopathic medicine

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ABSTRACT

The study was carried out to evaluate the effect of two homeopathic medicines to induce oestrus in 36 postpartum anoestrus crossbred Jersey dairy cows. Group 1 cows were fed a mineral mixture, and group 2 and 3 were medicated with *Pulsatilla* and *Sepia* drenched at the dose rate of 20 mL twice daily for 8 days, respectively. The mean plasma progesterone level (ng/mL) estimated using a radio immunoassay kit was significantly higher in group 2, followed by group 3 before and after medication on day 28 and 60, post artificial insemination (AI). Out of 12 induced for oestrus, 10 cows (83.33 %) and 9 cows (75.0 %) in group 2 and 3 were confirmed pregnant by rectal palpation on day 60 post AI, respectively. Both the homeopathic medicines showed 100% oestrus induction with a mean interval of 25.17 to 25.50 days, and *Pulsatilla* medicated cows showed improved fertility response than *Sepia*. The significantly higher level of progesterone after the withdrawal of homeopathic medication might be due to the release of circulating hormone, which blocks estradiol negative feedback. The subsequent increase reduces uterine PGF2 alpha secretion for a better conception rate.

Keywords: Fertility response, Oestrus induction, Post-Partum anoestrus, Progesterone, *Pulsatilla*, *Sepia*

Anoestrus is characterised by the absence of expected oestrus behaviour at a particular time, observed either as postpartum or post-service anoestrus in cows wherein the cows did not exhibit oestrus signs for more than 30-120 days. Postpartum anoestrus is a kind of infertility often encountered in dairy cows, manifested as a prolonged calving interval. To re-establish ovarian cyclicity, follicular dominance and its fate are the main key factor because initiation of follicular growth is not usually affected in the postpartum period, as evident by functional corpus luteum (Hafez and Hafez 2000). Though many physiological and pathological factors impart postpartum anoestrus, exogenous progestins are commonly used in the synchronisation protocol for noncyclical anoestrus postpartum cows wherein a desirable follicle is produced (Hammam *et al*. 2013), but it fails to ovulate being its long-acting nature (Manisha *et al*. 2021) and shown to elicit an increase in luteinizing hormone (LH) pulse frequency (Xu 2000).

Homeopathic medicine provides potential treatments for various conditions related to infection (Almeida *et al*. 2008) and infertility (Lobreiro 2007 and Aziz *et al*. 2012) in veterinary science. The results of hormonal therapy are unsatisfactory, variable and not economical and hence homeopathic remedy has been increasing at a steady pace in the management of anoestrus cows as an effective, economic, and alternative approach (Domenick and John 2017).

Hence, the present study was designed to evaluate the efficacy of two homeopathic medicines, viz. *Pulsatilla and Sepia*, to induce oestrus in postpartum anoestrus crossbred Jersey dairy cows.

MATERIALS AND METHODS

Selection of cows: The present study was conducted in 36 postpartum anoestrus crossbred Jersey cows (4-7 years of age) in and around Salem district. Healthy lactating cows from 1st to 3rd calving not exhibiting oestrus signs for more than 3-5 months of the postpartum period, owned by private farmers and maintained by traditional husbandry practices were selected.

Experimental design: The selected experimental postpartum anoestrus cows were randomly divided into three groups with 12 per group after a thorough gynaecoclinical examination and were dewormed with Albendazole (7.5 mg/kg body weight). Group 1 (n=12) (Placebo) cows were fed 100 g mineral mixture daily once for 8 days and considered as a control. Group 2 and group 3 cows were medicated with homeopathic medicine, viz. *Pulsatilla 30c and sepia 30c* (SBL Pvt Ltd.) as a drench at the dose rate of 20 mL twice daily for 8 days, respectively. Oestrus detection (mounting behaviour) was carried out

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twice daily. The day following the day of oestrus (0 day) was considered day 1 of the cycle. Fixed-time artificial insemination was done in cows twice at an interval of 12 h after induction of oestrus. The cows were monitored for return to oestrus (28th day post AI), and pregnancy confirmation was performed by rectal palpation 60 days after insemination.

Sample collection: Blood samples of about 5 mL were collected in EDTA K3vials from jugular venipuncture of selected animals during the early morning before (0 day) and after (9 day) medication, at the time of artificial insemination (AI) and 28th and 60th day post insemination of the experiment. The clear plasma obtained was kept in a dry sterile cryovial and stored in a deep freezer at −20°C until further use.

Hormonal analysis: Plasma progestrone concentration (ng/mL) was estimated in duplicate in all the groups for the above specified periods using a commercial radioimmunoassay kit (Immunotech, Czech Republic).

Statistical analysis: Data generated were subjected to statistical analysis using ANOVA with repeated measures to compare the differences between the groups and within the group on different days (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

The plasma progesterone concentrations (ng/mL) before and after medication, at the time of AI, oestrus interval and percent oestrus induction in experimental groups are presented in Table 1.

The progesterone value (on day 0) before medication among the treatment groups did not show any significant difference. Similarly, at the time of AI also, no significant difference was observed. Group 2 cows showed significantly higher (P≤0.05) progesterone value after medication (day 9), followed by group 3 compared to group 1. Between group 2 and 3 there was no significant difference on day 9 of medication. However, a non-significant progesterone value was observed on the day of AI in all the groups.

The progesterone values between different days of sampling differed significantly among the treatment groups. All the groups had a significantly higher (P≤0.05) progesterone concentration after medication (day 9) as compared to before medication (day 0) and on AI day. On the contrary, all groups recorded a significantly lower (P≤0.05) progesterone concentration at the time of AI.

Compared with control, group 2 and group 3 showed a significant variation (p≤0.05) among the different days of sampling with a higher (P≤0.05) progesterone concentration after medication (day 9), than before medication (day 0) and on AI day. On the contrary, progesterone concentration did not differ significantly (P≤0.05) within the days of sampling (day 9) for both G2 and G3 experimental groups.

Both the homeopathic medicine showed 100% oestrus induction compared to the control. All treated animals showed oestrus signs, i.e. *Pulsatilla* medicated cows showed at an interval of 25.17±0.110 days while *sepia* medicated cows showed at an interval of 25.50±0.221 days after initiation of treatment, while in control, 6 cows exhibited oestrus signs at an interval of 31.66±0.304 days. Between G2 and G3 experimental groups, there was no significant difference in the interval of oestrus exhibition as compared to the control.

In group 1, control cows, among the six cows exhibited oestrus signs, 3 cows induced to oestrus on day 23, 2 cows on day 24, and one cow on day 25 of mineral mixture supplementation. In group 2, and group 3, 10 cows and 8 cows were induced to oestrus on day 17, whereas two cows in group 2 and group 3 were induced to oestrus on day 18 after initiation of medication. The remaining two cows in group 3 were induced to oestrus on day 19 after medication.

Blood progesterone level rose rapidly after *Pulsatilla* and *Sepia* medication and declined rapidly, causing ovulatory oestrus. The present results of the abrupt decrease in progesterone levels on the day of oestrus are in accordance with the findings of Jena *et al*. (2016).

This finding corroborates with the observation made by earlier workers (Wheatona and Lamb 2007, Mishra *et al*. 2010), who revealed that treatment of anoestrus HF cows with progesterone therapies, diminishes the supportive effect of circulating estradiol-17 β, increased secretion of LH to stimulate preovulatory follicular development.

The significantly higher (p≤0.05) level of progesterone after medication (day 9) might be due to the sustained release of circulating hormone, which resulted in down-regulation of hypothalamic estradiol-17 β receptors and blocked its negative feedback. The greater follicular fluid and circulating concentration of estradiol increases the pulsatile release of LH and increase LH receptors in granulosa and theca cells in pre ovulatory follicles (Xu *et al*. 2000).

The plasma progesterone concentrations on day 28 and 60 of post-insemination, the number of cows returned and

Table 1. Mean±SEm values of progesterone level before, after homoeopathic medication and at the time of artificial insemination, oestrus interval and percent oestrus induction in postpartum anoestrus Jersey crossbred cows

Experimental group	Progesterone level (ng/mL)			Oestrus induction		
(n=12)	Before (day 0) medication	After (day 9) medication	At time of AI	No of cows	Per cent	Oestrus interval (days)
Control (G1)	1.570±0.08^{b}	1.890± 0.31^{cA}	0.894±0.34^{a}	6	50.00	31.66± 0.30^{B}
Pulsatilla (G2)	1.620±0.47	5.427±1.11^{cB}	1.021±0.81^{a}	12	100.00	25.17±0.11^{A}
Sepia (G3)	1.554±0.77	5.151±0.92^{cB}	0.961± 0.65^{a}	12	100.00	25.50± 0.22^{A}

Values bearing different superscripts in a row and column differs significantly (p≤0.05).

Table 2. Mean±SEm values of progesterone level and fertility response in cows induced to oestrus

Group	Day 28 post AI			Day 60 post AI				Fertility response (%)
	Progesterone level (ng/mL)	Oestrus exhibition No of cows		Rectal palpation No of cows		Progesterone level (ng/mL)		
		Return to oestrus	Non-return to oestrus	Pregnant	Non-pregnant	Pregnant	Non-pregnant	
Control Placebo (G1)	2.140± 0.745^{bA}	4	2	2	4	2.984±1.55^{cA}	1.539±0.14^{aA}	2/6=33.33
Pulsatilla (G2)	10.510± 1.090^{bC}	2	10	10	2	10.762±2.11^{cC}	2.091±0.51^{aB}	10/12=83.33
Sepia (G3)	9.321±1.55^{bB}	2	10	9	3 (2+1*)	9.841±0.14^{cB}	1.896±0.44^{aB}	9/12=75.00

*The progesterone level (3.041 ng/mL) in one non-pregnant cow exhibited oestrus on day 35 post AI. Values bearing different superscripts in a row and column differ significantly (p≤0.05).

non-return to oestrus, diagnosis of pregnancy by rectal palpation (day 60 post AI), and fertility response based on oestrus induction are presented in Table 2.

In the present study, plasma progesterone concentration exceeding 2.5 ng/mL was taken as a criterion for diagnosing pregnant cows. The accuracy of the pregnancy diagnosis was confirmed by rectal palpation on the day 60 after insemination. Based on this, "non-pregnant" cows were identified correctly in the present experimental groups.

The homeopathic medicine, *Pulsatilla* and *Sepia,* sufficiently increased the progesterone level, i.e. group 2 followed by group 3 and maintained a higher progesterone concentration in blood (>2.5 ng/mL) on day 28 and 60 post AI than group 1. Between the experiments, the non-pregnant cows of both the homeopathic medicated groups showed significantly higher progesterone levels (<2.5 ng/mL) than the control.

Earlier reports by Robertson and Sarda (1971) confirmed pregnancy 19 days after insemination using plasma progesterone values at fixed times after breeding. In the same way, Wishart *et al*. (1975) found that pregnancy diagnosis by high progesterone concentration in the blood on day 20, which was 77% agreement with rectal palpation, and non-pregnancy was diagnosed with 97 to 100% accuracy with low progesterone concentrations.

In Group 2, out of 12 induced to oestrus, the mean progesterone level observed in 10 pregnant cows was 10.762±2.11 ng/mL and the per cent conception rate was 83.33% which was confirmed pregnant by rectal palpation on day 60 post AI, whereas the level of progesterone level in the two non-pregnant cows was 2.091±0.51 ng/mL.

The present observation agrees with the serum progesterone level (<0.5 ng/mL) in anoestrus cattle (Singh *et al*. 1998). Similarly, Henricks *et al*. (1972) also observed increased plasma progesterone levels from 1.2 ng/mL on day 3; 8.2 ng/mL on day 12 after mating and 9.9 ng/mL, on day 39 of pregnancy in postpartum anoestrus cows. Similarly, Ergene 2012 reported progesterone concentrations in the GnRH-treated group on day 21 were 15.93±5.63 ng/mL in pregnant cows, 3.00±1.08 ng/mL in non-pregnant cows while 7.44±1.15 ng/mL in the pregnant control group. On the contrary, Mishra *et al*. (2010) and Mohapatra *et al*. (2012) also observed the progesterone concentration in postpartum anoestrus cows ranges from 2.5 to 3.6 ng/mL of pregnant cows.

In light of published literature and the results of the present study concurred with the findings of Macmillan *et al*. (1991), who hypothesized that increasing peripheral progesterone concentrations during the dioestrus after insemination might improve embryo development and suppress luteolysis, resulting in reduced embryonic loss and higher progesterone concentration associated with improved embryo development.

In group 3, the progesterone concentration on day 60 post AI was 9.841±0.14. Among the 10 induced cows, one cow returned to oestrus with the progesterone level of 3.540 ng/mL, which was considered pregnant based on the progesterone level (>2.5 ng/mL) on day 28 post AI. However, it exhibited oestrus signs a week later at an abnormal interval of day 35 post AI, which was confirmed non-pregnant (false positive) by rectal palpation also.

This might be due to a prolonged cycle or due to early embryonic mortality (Shemesh *et al*. 1973). Luteal insufficiency and lower progesterone concentrations during early embryonic development cause embryonic mortality and reduced pregnancy rates in dairy cows (Mann 2002). Delay in the normal rise in progesterone concentrations between day 4 and 5 post-ovulation and low systemic progesterone concentrations during the subsequent dioestrus period resulted in reduced pregnancy rates and conception rates (Larson *et al*. 2007). The above findings concur with the results of the present experiment.

The resumption of cyclicity in six animals of the control G1 group might be due to the supplementation of mineral mixture which would have normalised the cycle. Among the six induced to oestrus, two cows were confirmed pregnant by rectal palpation on day 60 and recorded the progesterone concentration of 2.984±0.55 ng/mL. Progesterone concentration remained almost unaltered in the remaining six non-cyclical control anoestrus cows, which indicated the absence of corpus luteum in the ovaries of anoestrus cows. These findings corroborated with Kumar *et al*. (2011).

Available literature reports suggested that endogenous progesterone hormone concentrations were higher for all pregnant cows in homeopathic treatment groups than for the non-pregnant group (Rajkumar *et al*. 2006, Kalampokas *et al*. 2014 and Kumar *et al*. 2020). 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. After day 12, the progesterone level declined in the non-pregnant cows and gradually increased (13.9 ng/mL) in the pregnant cows (Henricks *et al*. 1972).

The subsequent increase in the level of progesterone during the sampling period on day 28 and 60 post AI in this experiment was an indication of the presence of corpus luteum (Kumar *et al*. 2004), which in turn reduced uterine PGF2 alpha secretion for better conception rate in dairy cows (Kawate *et al*. 2004).

The homoeopathic medicated *Pulsatilla* and *Sepia* groups successfully induced the resumption of cyclicity and augmented fertility response in anoestrus cows by increasing and maintaining the plasma progesterone level sufficiently (Table 2). This was in agreement with Chandel *et al*. (2009), who reported significantly higher oestrus induction response (71.42%) and conception rate (78.95%) than control (20%) using homeopathic combination "Hit-O-Gen" two tablets twice a day in anoestrus buffaloes.

In the oestrus induction protocols, progesterone was the prime hormone to potentiate the action of estrogen, and hypothetically greater blood progesterone concentration preceding insemination during the luteal phase increases conception rate and during the late luteal phase reduces the uterine secretion of prostaglandin F2α (PGF2α) in response to oxytocin (Jena *et al*. 2016). The study revealed, improved fertility response in postpartum anoestrus dairy cows with higher plasma progesterone concentration.

REFERENCES

Almeida L, Campos M C, Herrera H M, Bonamin L V and Fonseca A H. 2008. Effects of homeopathy in mice experimentally infected with *Trypanosoma cruzi*. *Homeopathy* **97**(2): 65–69.

Aziz D, Schnurrbusch U and Enbergs H. 2012. Effects of two homeopathic complexes on bovine sperm mitochondrial activity. *Homeopathy* **101**(2): 99–102.

Chandel B S, Dadawala A I, Chauhan H C, Parsani H R and Pankajkumar. 2009. Efficacy of Hit-O-Gen, A homeopathic preparation for management of anoestrus in buffaloes. *Veterinary World* **2**(7): 280.

Domenick J M, John D L. 2017. Homeopathic treatment of infertility: A medical and bioethical perspective. *International Journal of Complementary and Alternative Medicine* **5**(5): 11–12.

Ergene O. 2012. Progesterone concentrations and pregnancy rates of repeat breeder cows following post insemination PRID and GnRH treatments. *Turkish Journal of Veterinary Animal Science* **36**(3): 283–88.

Hammam A M, Abdel-Gawad E M M, Ali A H and Amer H A. 2013. Effect of CIDR-ovsynch regimen as a pharmacological agent on fertility, blood and hormonal patterns of cross breed cows. *Global Journal of Pharmacology* **7**(4): 383–89.

Hafez B and Hafez E S E. 2000. *Reproduction in Farm Animals*. 7th Ed. pp. 41-42. Blackwell Willey, Maryland.

Henricks D M, Dickey J F, Hill J R and Johnston W E. 1972. Plasma estrogen and progesterone levels after mating, and during late pregnancy and postpartum in cows. *Endocrinology* **90**: 1336–42.

Jena D, Das S , Patra B K , Biswal S S, Mohanty D N and Samal P. 2016. Certain hormonal profiles of post-partum anoestrus jersey crossbred cows treated with controlled internal drug release and ovsynch protocol. *Veterinary World* **9**: 1102–06.

Kalampokas T, Botis S, Kedikgianni-Antoniou A, Papamethodiou D, Kivellos S, Papadimitriou V, Salvanos G, Paparistidis N, Gavaris I, Sofoudis C and Kalampokas E. 2014. Homeopathy for infertility treatment: A case series. *Clinical and Experimental Obstetrics and Gynaecology* **41**(2): 158–59.

Kawate N , Itami T, Choushi T, Saitoh T, Wada T, Matsuoka K, Uenaka K, Tanaka N, Yamanak A, Sakase M, Tamada H, Inaba T and Sawada T. 2004. Improved conception in timed-artificial insemination using a progesterone-releasing intravaginal device and ovsynch protocol in postpartum suckled Japanese black beef cows. *Theriogenology* **61**: 399–406.

Kumar H, Srivastava S K, Yadav M C and Varshney J P. 2004. Management of post-partum anestrus in dairy animals with a homeopathic combination remedy. *Indian Journal of Animal Science* **74**: 739–40.

Kumar R, Butani M G, Dhami A J, Kavani F S, Shah R G and Killedar A. 2011. Management of anoestrus and suboestrus cows using hormonal and non hormonal drugs. *Indian Journal of Animal Reproduction* **32**(1): 24–27.

Kumar J, Srivastava S and Kumar R. 2020. Effect of herbal, homeopathic and hormonal drug on haematology, ovarian cyclicity and conception rate in postpartum anoestrus cows. *Indian Journal of Veterinary Science and Biotechnology* **16**(1):17–21.

Larson F S, Butler R W and Currie B W. 2007. Pregnancy rates in lactating dairy cattle following supplementation of progesterone after artificial insemination. *Animal Reproductive Science* **102**: 172–79.

Lobreiro J. 2007. Homeopathic treatment for infertility in a prize Nelore bull. *Homeopathy* **96**(1): 49–51.

Macmillan K L, Taufa V K, Day A M and Peterson A J. 1991. Effects of supplemental progesterone on pregnancy rates in cattle. *Journal of Reproduction and Fertility* **43** (Suppl.): 304.

Mann G E. 2002. Corpus luteum function and early embryonic death in the bovine. *XXII World Buiatrics Congress*. pp. 300-06. 18-23rd August 2002, Hannover, Germany.

Manisha S, Nadeem S, Mohanty T K, Bhakat M, Dewry R K, Yadav D K, Gupta V K and Nath S. 2021. The induction of cyclicity in postpartum anoestrus buffaloes: A review. *Journal of Experimental Zoology* **24**: 989–97.

Mishra G K, Derashri H J, Sarvaiya N P and Kavani F S. 2010. Effect of different therapies in early postpartum period on fertility of HF cows. *Indian Journal of Animal Reproduction* **31**(1): 48–51.

Mohapatra J, Das S, Sahoo G R, Patra B K and Dash S K. 2012. Hormonal profile of anoestrus cows of industrial areas of odisha. *Proceedings of XXVII Annual Convention and national symposium of ISSAR*. pp.21-23. 27-29th September 2011 CAU, Selesih, Aizawl, Mizoram.

Rajkumar R, Srivastava S K, Yadav M C, Varshney V P, Varshney J P and Kumar H. 2006. Effect of a homeopathic complex on oestrus induction and hormonal profile in anoestrus cows. *Homeopathy* **95**(3): 131–35.

Robertson H A and Sarda I R. 1971. A very early pregnancy test for mammals: Its application to the cow, ewe and sow. *Journal of Endocrinology* **49**(3): 407–19.

Snedecor G W and Cohoran W G. 1989. *Statistical Methods*. 8th

Ed. Iowa State University Press, Iowa.

Singh U, Khurana N K and Inderjeet. 1998. Plasma progesterone profiles and fertility status of anooestrus Zebu cattle related with norgestomet-estradiol-eCG regimen. *Theriogenology* **50**: 1191–99.

Wheatona J E and Lamb G C. 2007. Induction of cyclicity in postpartum anestrous beef cows using progesterone, GnRH and estradiol cypionate (ECP). *Animal Reproduction Science*. **102**: 208–16.

Wishart D F, Head V A and Horth C E. 1975. Early pregnancy diagnosis in cattle. *Veterinary Record* **96**: 34.

Xu Z Z, Verkerk G A, Mee F, Morgan S R, Clark B A, Burke C R and Burton L. 2000. Progesterone and follicular changes in postpartum noncyclic dairy cows after treatment with progesterone and estradiol or with progesterone, GnRH, PGF2α, and estradiol. *Theriogenology* **54**: 273–82.