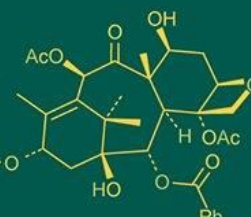
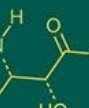
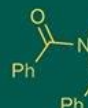


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Ramu S

MVSc Scholar, Department of
Animal Reproduction,
Gynaecology and Obstetrics,
College of Veterinary and
Animal Sciences, Kerala
Veterinary and Animal
Sciences University, Kerala,
India

Unnikrishnan MP

Associate Professor, Centre for
Pig Production and Research,
Mannuthy, Veterinary and
Animal Sciences University,
Kerala, India

Jayakumar C

Professor and Head,
Department of Animal
Reproduction Gynaecology
and Obstetrics, College of
Veterinary and Animal
Sciences, Kerala Veterinary
and Animal Sciences
University, Kerala, India

Bibin Becha B

Associate Professor and Head,
Base Farm, Kerala Veterinary
and Animal Sciences
University, Kerala, India

Karthiayini K

Professor and Head,
Department of Veterinary
Physiology, College of
Veterinary and Animal
Sciences, Kerala Veterinary
and Animal Sciences
University, Kerala, India

Corresponding Author:**Unnikrishnan MP**

Associate Professor, Centre for
Pig Production and Research,
Mannuthy, Veterinary and
Animal Sciences University,
Kerala, India

Effect of mid-luteal administration of non-steroidal aromatase inhibitor, letrozole on serum progesterone concentrations in crossbred cows exhibiting prolonged oestrus

Ramu S, Unnikrishnan MP, Jayakumar C, Bibin Becha B and Karthiayini K

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Abstract

Letrozole is a non-steroidal aromatase inhibitor which inactivates the aromatase enzyme, preventing oestrogen production. A luteotropic effect is also attributed to this drug. The study conducted to assess the effect of letrozole administration on serum progesterone profile in prolonged oestrous crossbred cows revealed that intravenous administration of 250 µg letrozole at mid luteal phase had no significant effect on subsequent serum progesterone concentration in the ensuing oestrus or mid luteal phase among prolonged oestrous cows.

Keywords: Prolonged oestrus, suprabasal progesterone, letrozole, luteotropic, corpus luteum

Introduction

Prolonged oestrus is one of the common aberrations of oestrus among crossbred cattle, recording an occurrence of up to 34.16 percent (Arun *et al.*, 2020) ^[1]. Presence of suprabasal progesterone concentration during oestrus, induced by lactational, nutritional and environmental stress is attributed as a common cause of prolonged oestrus (Singh *et al.*, 2009) ^[2]. The higher progesterone (P₄) level during oestrus reduces LH pulse frequency, due to its inhibitory effect on positive feedback of oestradiol (E₂) on the hypothalamus, leading to persistence of pre-ovulatory follicles without ovulation. These animals exhibit secondary signs of oestrus for a longer period, due to continued release of oestradiol-17β (Stock and Fortune, 1993) ^[3]. Suprabasal progesterone has adverse effect on fertility because of ovulation failure. Due to poor and unfavorable oocyte and embryo development brought on by exposure to a low LH surge and an insufficient luteal profile, conception failure occurs (Bage *et al.*, 2002) ^[4].

Management of prolonged oestrus has been traditionally attempted using prostaglandins, GnRH and steroid hormones, with variable results. Further, use of steroid hormones has been the subject of controversy in cattle industry; hence the use of non-steroidal drugs becomes the novel area of research. Letrozole, a non-steroidal aromatase inhibitor, inactivates the aromatase enzyme responsible for the synthesis of oestrogens (Cohen *et al.*, 2002) ^[5]. A luteotropic effect is also attributed to letrozole (Yapura *et al.*, 2013; Aravind *et al.*, 2025) ^[6, 7], which is due to increased LH secretion and alteration of the proportion of large and small luteal cells in the CL (Abdel *et al.*, 2020; Romerein *et al.*, 2018) ^[8, 9]. This in turn gives higher capacity for the CL to produce progesterone and luteal oxytocin and would respond better to PGF₂α due to larger number of PG receptors (Boonyaprakob *et al.*, 2003) ^[10]. Low progesterone concentration attained after luteolysis promotes LH production and early attainment of pre ovulatory follicle size (Bridges and Fortune, 2003) ^[11]. Thus, letrozole might help to correct the suprabasal P₄ levels noticed during oestrus among prolonged oestrous cows. This in turn would lead to timely ovulation of mature follicle and formation of CL that functions normally.

Hence, the present study was undertaken to evaluate how mid-luteal administration of letrozole affects subsequent serum progesterone milieu, which might provide information to

develop strategies for management of prolonged oestrus in crossbred cows using letrozole.

Materials and Methods

The study was conducted at University Livestock Farm and Fodder Research Development Scheme (ULF & FRDS), Mannuthy during the period from March 2023 to July 2023. Apparently healthy postpartum crossbred dairy cows of age 3-6 years and parity 1-3, maintained under identical conditions of feeding and management were selected for the study.

All the cows with oestrus duration longer than 24 h were considered as having prolonged oestrus (Arun *et al.*, 2020)

^[1] whereas those with duration 24 h or less were considered as normal.

Design of experiment

All the breedable cows specified in the study were thoroughly monitored for signs of oestrus. Twelve animals with prolonged oestrus formed group I and II of six cows each and another 12 normal oestrous cows were allotted to group III and IV (6 cows each). Blood samples were collected from all the cows on the days of oestrus (Day 0, 1 and 2) for serum P₄ estimation. Blood samples were again collected before treatment on 10th day of cycle for hormone estimation. Cows were subjected to the following treatment as mentioned in the Table below.

Table 1: Treatment given to the cows under study

Group (n=6)	Treatment
Group I (Cows with prolonged oestrus)	10ml of phosphate buffered saline (PBS) given I/V on day 10 of oestrous cycle
Group II (Cows with prolonged oestrus)	Letrozole 250 µg dissolved in 10ml of PBS given I/V on day 10 of oestrous cycle
Group III (Cows with normal oestrus)	10ml of PBS given I/V on day 10 of oestrous cycle
Group IV (Cows with normal oestrus)	Letrozole 250 µg dissolved in 10ml of PBS given I/V on day 10 of oestrous cycle

As in the previous cycle, blood collection was again done in the ensuing cycle (2nd cycle) during oestrus and on 10th day of second cycle.

Blood collection and serum progesterone analysis

A quantity of 5 ml whole blood from each cow was collected by jugular puncture using a sterile 18 gauge disposable hypodermic needle. It was transferred to a 15 ml sterile test tube and these test tubes were kept in slanting position for 15 min to allow the whole blood to clot. It was placed in a refrigerator at 4 °C overnight for clot retraction. After overnight storage in refrigerator, samples were centrifuged at 3000 rpm for 10 min and clear supernatant

sera were collected into storage vials. The vials were labelled and stored at -20 °C till assay. Serum P₄ concentration in all samples were measured using quantitative ELISA kits (Omega Diagnostics, Scotland, UK), following manufacturer's instructions. The data obtained were tabulated and analysed by One-Way ANOVA using SPSS statistics, version 24.0. software programme.

Results and Discussions

Mean serum P₄ concentration (ng/mL) during different days of oestrus and on 10th day of cycle among the four groups of cows under study in two consecutive cycles is presented in Table 2.

Table 2: Serum progesterone concentration (Mean ± SE) during oestrus and dioestrus among crossbred cows under study, before and after treatment

Day of oestrous cycle	Sample collection	Progesterone concentration (ng/mL)			
		Group I	Group II	Group III	Group IV
Day 0	BT	0.63 ^a , x±0.02	0.66 ^a , x±0.02	0.25 ^b , x±0.04	0.25 ^b , x±0.03
	AT	0.64 ^a , x±0.02	0.66 ^a , x±0.03	0.27 ^b , x±0.02	0.27 ^b , x±0.03
Day 1	BT	0.55 ^a , x±0.02	0.60 ^a , x±0.02	No oestrus	No oestrus
	AT	0.56 ^a , x±0.01	0.59 ^a , x±0.02	No oestrus	No oestrus
Day 2	BT	No oestrus	0.54 ^a , x±0.02	No oestrus	No oestrus
	AT	0.49 ^a ±0.01	0.55 ^a , x±0.03	No oestrus	No oestrus
Day 10	BT	1.94 ^a , x±0.05	1.98 ^a , x±0.11	3.48 ^b , x±0.19	3.81 ^b , x±0.16
	AT	1.95 ^a , x±0.08	2.02 ^a , x±0.12	3.66 ^b , y±0.18	4.05 ^b , y±0.14

a, b-means with different superscript within a row differ significantly at 5% level

x, y-means with different superscripts within column on any particular day differ significantly at 5% level

BT-before treatment, AT-after treatment

In both the prolonged oestrous groups (I and II), no significant change ($p \geq 0.05$) in serum P₄ levels before and after treatment, either on the days of oestrus or during dioestrus (day 10) was noticed. Yapura *et al.* (2009) ^[12] also reported no significant difference in plasma P₄ levels on any of the days of cycle between letrozole treated and control animals, after intravenous administration of letrozole on days 5 to 7 of cycle. Yapura *et al.* (2013) ^[6] reported that persistent release of letrozole in the body has an impact on its hormonal profiles. Single intravenous administration of letrozole during mid-cycle in the present study might be insufficient to bring about luteotropic effect and consequent changes in hormone profile. Hence, a persistent letrozole

releasing system might be required to evoke the expected response in hormonal milieu of prolonged oestrous cows.

In both the cycles, mean serum P₄ concentration during oestrus was significantly higher ($p \leq 0.05$) in prolonged oestrous groups (I and II) than normal animals (III and IV). In both the cycles, mean serum P₄ concentration on day 10 was significantly lower in prolonged oestrous cows than normal oestrous groups. Similar pattern of difference in serum P₄ concentration between normal and prolonged oestrous cows was reported by Niyas (2017) ^[13] and (Arun *et al.*, 2020) ^[1]. Existence of suprabasal P₄ concentration during oestrus (Singh *et al.*, 2009) ^[2] and luteal deficiency during dioestrus (Chander *et al.*, 2002) ^[14] was reported

earlier. Chander *et al.* (2002) ^[14] reported value of 3.12 ± 0.51 ng/mL, as the normal serum P₄ concentration during mid-luteal phase.

Suprabasal P₄ concentration during oestrus could be due to improper luteolysis of CL from the previous cycle, because of decreased PGF₂ α synthesis or from extra-gonadal source like adrenal, when the cows are exposed to stress (Duchens *et al.*, 1995) ^[15]. Presence of luteal tissue at oestrus might have a significant impact on subsequent luteal phase activity, causing luteal insufficiency during dioestrus (Singh *et al.*, 2012) ^[16]. Milk yield also contributes to disturbed luteal profile, as a strong unfavourable genetic relationship exists between milk production and fertility (Royal *et al.*, 2000) ^[17].

Though there existed no significant difference in serum P₄ levels between cycles on the day of oestrus (day 0) in either of the normal oestrous groups (III and IV), there existed significant difference ($p \leq 0.05$) on day 10 serum P₄ levels in both the groups between cycles, with higher values during 2nd cycle. Since the hike is not limited to letrozole treated group alone, such changes cannot be attributed to the effect of the drug. Significant variations in serum P₄ levels between cycles among normal oestrous cows is reported earlier also (Arun, 2019) ^[18].

Conclusion

Single intravenous administration of 250 μ g letrozole at mid luteal phase was not beneficial to correct the defective serum P₄ milieu among prolonged oestrous cows. Existence of suprabasal P₄ concentration during oestrus and luteal deficiency during dioestrus among prolonged oestrous cows was reinforced in the present study. Letrozole administration at different dose level and route of administration, with suitable vehicle for slow release of the drug might be required to achieve a desired result among prolonged oestrous cows.

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