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Progesterone profile of postpartum anestrous Ongole cows treated with heatsynch and G6G synchronization protocols

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Abstract
Postpartum lactating anestrous Ongole cows were treated with estrus and ovulation hormonal protocols Heatsynch (Group A) and G6G (Group B) and progesterone profile analysis was done following estrus induction. The mean progesterone concentration 10 days before initiation of treatment in Heatsynch treated group was 0.78±0.12 (0.3 to 1.2) ng/ml and on the treatment initiation day was 0.81±0.16 (0.7 to 1.4) ng/ml, at the time of $PGF_2\alpha$ injection on day 7 was 1.64±0.89 (1.3 to 1.9) ng/ml and on the day of estrus was 0.62±0.60 (0.4 to 1.2) ng/ml. The mean progesterone concentration 10 days before initiation of treatment in G6G treated group was 1.16±0.15 (0.6 to 2.2) ng/ml and on the treatment initiation day was 1.26±0.14 (0.5 to 2.6) ng/ml, on the day of second $PGF_2\alpha$ injection on day 17 was 1.28±0.52 (0.5 to 2.3) and on the day of estrus was 0.77±0.05 (0.5 to 1.3) ng/ml. Cows that had high concentration at -10 and on the day of intiation of treatment had conceived at induced estrus. The hormonal protocols (Heatsynch and G6G protocol) had significant influence on circulatory serum progesterone profiles.

Keywords: Anestrous, G6G, heatsynch, ongole cows, progesterone, postpartum

1. Introduction
Postpartum anestrous is the most prevalent, frustrating and challenging problem encountered in cows. Progesterone profile has become one of major endocrinological parameters and offers the possibility of determining the level of ovarian activity. Progesterone profile is useful in the detection of estrus, subestrus, anestrous, anovulation, early embryonic mortality and pregnancy diagnosis, and thereby throws some light on fertility criteria. Progesterone in cyclic animals acts as a regulator of dioestrus period because as soon as corpus luteum fails to secrete progesterone, development of follicles begins leading to pro-estrus phase.
Available reports show that estrus was manifested in a higher percentage of cows (84%) that had high progesterone concentration (>3.1 ng/ml) on the day of the last $PGF_2\alpha$ injection than the cows (56%) with low progesterone levels (Larson and Ball, 1992) [7]. Cows conceiving to artificial insemination at induced estrus had higher progesterone levels during preceeding luteal phase than those do not conceiving (Folman *et al.*, 1990) [5]. The blood gonadal steroids concentration is considered as an indicator of ovarian activity and as a marker to predict response to hormonal treatment (Webb *et al.*, 1980) [11]. Thus, estimation of hormones before treatment is helpful in studying ovarian function and response to treatment. The present study aimed at estimating the progesterone profile of postpartum anestrous Ongole cows treated with heatsynch and G6G protocols.

2. Materials and Methods
2.1 Location of the study
Animals maintained under standard feeding and managemental conditions at Cattle Project, Livestock Research Station, Lam Farm, Guntur, ($15^{0}00$ and $16^{0}10$ North latitude and $79^{0}04$ and $80^{0}02$ East Longitude), Sri Venkateswara Veterinary University, Andhra Pradesh, India, were included in this study.

2.2 Experimental animals
Ongole cows (n=16) that calved normally from December 2017 to January 2018, aged between 5 - 10 years and weighing between 350 to 500 kg, that have not been detected in estrus since 3 to 5 months were monitored for ovarian activity and estrus pattern from the

month of April 2018 to May 2018.

2.3 Experimental design

Group-A: GnRH-$PGF_2\alpha$-Estradiol benzoate (Heatsynch protocol). In this group estrus was synchronized in 8 postpartum anestrous cows by administering IM injection of GnRH analogue (Pregulate, Virbac) @ 10 µg on day 0 followed by IM injection of Cloprostenol sodium (Pragma, Intas) @ 500 µg on day 7 and IM injection of Estradiol benzoate (Pregheat, Virbac) @ 1 mg on day 8 followed by double inseminations at 48 and 60 hr post-estradiol injection.

Group-B: $PGF_2\alpha$ - GnRH - GnRH - $PGF_2\alpha$ - GnRH (G6G protocol). In this group estrus was synchronized in 8 postpartum anestrous cows by administering IM injection of Cloprostenol sodium (Pragma, Intas) @ 500 µg on day 0, followed by IM injection of GnRH analogue (Buserelin acetate, Pregulate, Virbac) @ 10 µg on day 2 and Ovsynch treatment which consists of IM injection of GnRH analogue (Buserelin acetate, Pregulate, Virbac) @ 10 µg on day 8, IM injection of Cloprostenol sodium (Pragma, Intas) @ 500 µg on day 15 i.e., 7 days later, another dose of GnRH analogue (Buserelin acetate, Pregulate, Virbac) @ 10 µg on day 17 and timed A.I (TAI) at 16-18 hr after second GnRH injection.

2.4 Blood sampling

Blood samples were collected from jugular vein with the help of serum vacutainers and disposable needles. In anestrous group A, treated with Heatsynch protocol, blood samples were collected on -10 days, day 0 (first GnRH injection), on day 7 ($PGF_2\alpha$ injection), on day 8 (first Estradiol benzoate injection) and on the estrus day. In Group B with the treatment by G6G protocol, blood samples were collected on day -10 (10 days before treatment), on the day of treatment (First PG injection), on day 2 (First GnRH injection), on day 8 (Second GnRH injection), on day 15 (Second $PGF_2\alpha$ injection), on day 17 (Third GnRH injection), on the day of induced estrus (day18). Serum was separated by centrifugation at 3000 rpm for 10 minutes. The separated serum is stored at -20° C until assay for progesterone. Luteal regression was defined to occur when plasma P_4 concentration was ≥1 ng/mL immediately before $PGF_2\alpha$ treatment and decreased to <1 ng/mL 56 hr later (at second GnRH treatment).

2.5 Progesterone (P_4) assay

The serum progesterone concentration was estimated using ELISA technique with the help of progesterone kits (CALBIOTECH Co. Ltd.). Progesterone present in the sample competes with enzyme labeled progesterone conjugate for binding with anti progesterone antibody immobilized on the micro-well surface. The amount of conjugate that binds to the microwell surface will decrease in proportion to the concentration of the progesterone in the sample. The unbound sample and conjugate were then removed by washing and the color development reagents (substrate) are added. Upon exposure to the bound enzyme, a colour change will take place. The intensity of the color reflects the amount of bound enzyme progesterone conjugate and inversely proportional to the concentration of progesterone in the sample. The resulting colour was measured at 450 nm using a spectrophotometer. The sensitivity of the test was 0.1 ng/ml. The results were presented by calculating the average absorbance value for each reference standard, control and sample.

A standard curve was plotted with the known absorbance versus the corresponding concentrations of the standards on linear log-graph paper. The sample progesterone concentrations were determined from the standard curve using the per cent absorbance of samples to be known. These results were used as supporting evidence for the ovarian activity observed per rectal examination during the study. Concentration of progesterone ≥ 1ng/ml was defined as high (H) and indicative of presence of functional corpus luteum. Concentration of progesterone < 1ng/ml was defined as low (L) which indicated absence of functional corpus luteum.

2.6 Statistical analysis

All the collected data was analyzed statistically as per the procedure described by Snedecor and Cochran (1994). In this study MTB statistical package was used for analysis of variance of different parameters.

3. Results and Discussion

Ability to induce ovulation in anestrous cows is key to the success of some of the most recently developed estrus and ovulation-synchronization protocols in cattle (Stevenson *et al.,* 2000) [9]. The level of progesterone prior to ovulation following administration of $PGF_2\alpha$ affect the fertility of cows in synchronized estrus. Serum progesterone level was estimated 10 days before (-10 d) and on the day of treatment (d 0) to classify the anestrous. The serum progesterone level on day 7 in group A, on the day of second $PGF_2\alpha$ injection in group B and at induced estrus in all the treatment groups was presented in the Table 1, 2 and Fig.1.

The mean progesterone concentration 10 days before initiation of treatment in Group A was 0.78±0.12 (0.3 to 1.2), on the day of initiation of therapy was 0.81±0.16 (0.7 to 1.4) ng/ml, on day 7 at the time of $PGF_2\alpha$ injection was 1.64±0.89 (1.3 to 1.9) and on the estrus day was 0.62±0.60 (0.4 to 1.2) ng/ml. The mean progesterone concentration on day 0 and day 7 of the treatment was significantly different with the progesterone concentration of estrus day ($P<0.05$) (Table-1, Fig.1). The mean progesterone concentration 10 days before initiation of treatment in Group B was 1.16±0.15 (0.6 to 2.2), on the treatment initiation day was 1.26±0.14 (0.5 to 2.6) ng /ml, on the day of second $PGF_2\alpha$ injection on day 17 was 1.28±0.52 (0.5 to 2.3) and on day of estrus was 0.77±0.05 (0.5 to 1.3) ng/ml (Table-2, Fig.1).

In the present study out of the 8 anestrous Ongole cows in Group A, four cows had LLHL and four cows had shown LLLH progesterone pattren on day -10, 0, 7 and the day of estrus. The cows that had LLHL had conceived at induced estrus. The first service conception rate at induced estrus and overall conception rate in cows treated with group A (Heatsynch) was 66.67 and 83.33 per cent, respectively. The serum progesterone (0.62 ± 0.60 ng/ml) level on the day of induced estrus was low. The cows that had less than 1ng/ml progesterone had failed to conceive. In the group B, 5/8, 1/8, 1/8 and 1/8 had shown HHLL, LHHL, LHHL, LHLL progesterone pattern on day-10, 0, day 15 and on the estrus day. Cows that had high concentration at -10 and on the day of intiation of treatment had conceived at induced estrus. The first service conception rate at induced estrus and overall conception rate in cows of group B (G6G protocol) was 33.33 and 66.67 per cent, respectively. The serum progesterone (0.77 ± 0.05ng/ml) level on the day of induced estrus was low. The cows that had less than 1ng/ml progesterone had failed to conceive.

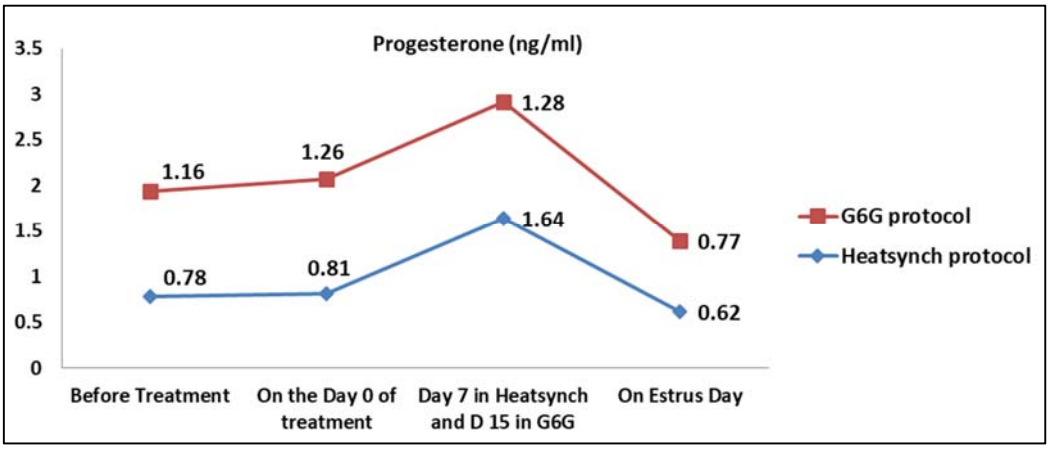

Fig 1: Mean progesterone levels before and after treatment in Heatsynch and G6G groups

Table 1: Mean levels of progesterone before and after Heatsynch treatment

Dh	Treatment			
	Group A (Heatsynch)			
	Before treatment		After treatment	
	-10 days	On the day 0 of treatment (1st GnRH inj.)	On day 7 At $PGF_2\alpha$	On estrus day
Progesterone (ng/ml)	0.78 ± 0.12^a (0.3 to 1.2)	0.81 ± 0.16^a (0.7 to 1.4)	1.64 ± 0.89^b (1.3 to 1.9)	0.62 ± 0.60^a (0.4 to 1.2)

Means bearing different superscripts within the row differ significantly (P<0.05)

Table 2: Mean levels of progesterone before and after G6G treatment

Parameters	Group B (G6G)			
	Before treatment		After treatment	
	-10 days	On the day of treatment (1st PG inj.)	On the day of treatment (2nd PG inj.)	Estrus day
Progesterone (ng/ml)	1.16 ± 0.15^a (0.6 to 2.2)	1.26 ± 0.14^a (0.5 to 2.6)	1.28 ± 0.52^a (0.5 to2.3)	0.77 ± 0.05^b (0.5 to 1.3)

Means bearing different superscripts within the row differ significantly (P<0.05)

The serum gonadal steroids concentration was considered as an indicator of ovarian activity and as a marker to predict response to hormonal treatment. The estimation of hormones to differentiate postpartum anestrous was helpful in the present study. Serum samples evaluated at 10 days interval in post partum anestrous cows revealed 0.78 to 1.64 ng/ml progesterone levels. Over all plasma progesterone levels on the day of initiating treatment were low in all groups but slightly increased on day 7 in Heatsynch/ day 15 in G6G protocol; this may be due to luteinisation of secondary follicles or ovulation of dominant follicles and formation of accessory corpora lutea under effect of GnRH (Bhoraniya *et al.,* 2012) [2]. These findings are in close agreement with Buhecha *et al.* (2016) [3], who reported serum progesterone levels as 0.81±0.22 ng/ml, while Dirandeh *et al.* (2015) [] and Heidari *et al.* (2017) [] reported high progesterone levels of 2.2±0.07 and 2.3 ng/ml, respectively. Bhoraniya *et al.* (2012) [2] reported low serum progesterone levels of 0.67±0.33 ng/ml. Bhoraniya *et al.* (2012) [2] reported that the plasma progesterone concentrations were significantly higher on day 7 as compared to corresponding values on day 0, 9/11 (AI) and on day 20 post-AI in Heatsynch protocol. Ammu *et al.* (2012) [1] and fond that plasma progesterone profile of conceived animals was more than non-conceived animals. Serum P4 concentration was significantly higher (P<0.01) in cows on 7th day post- treatment (5.34±1.74 ng/ml) compared to pre-treatment value (1.98±1.09 ng/ml) (Tripathy *et.al*, 2015) [10]. Bhoraniya *et al.* (2012) reported that the pooled mean plasma progesterone concentrations were significantly (P<0.05) higher on day 7 in Ovsynch (5.727±1.26), CIDR (4.37±0.66), Ovsynch plus CIDR (3.55±0.34), and Heatsynch (5.92±1.11) protocols as compared with their corresponding values obtained on days 0, 9/11 (AI), and on day 20 post-AI. Buhecha *et al.* (2016) [3] reported that the overall mean plasma progesterone concentrations (ng/ml) found in anestrous cows on day 0, 7, 9/10 (AI) of treatment and on day 21 post-AI were 0.85±0.21, 4.80±0.31, 0.58±0.11 and 3.97±1.14, respectively, under TriU-B protocol; 0.58±0.11, 4.55±0.28, 1.33±0.48 and 4.03±1.32 under Ovsynch protocol and 0.81±0.22, 5.11±0.75, 0.43±0.06 and 3.10±1.06 under Heatsynch protocol. Dirandeh *et al.* (2015) reported that the proportion of cows with high plasma P4 (>1 ng/mL) was greater for PG6G (77.9 and 84.2) and PG7G (81.6 and 85.0) compared with G6G (60.0 and 70.1) and G7G (58.7 and 68.7) at first GnRH ($P = 0.01$) and $PGF_2\alpha$ of Ovsynch respectively.

4. Conclusion

The influence of serum progesterone concentration on the day 0 and day 7/15 and at day of estrus on conception rate in Heatsynch and G6G protocol treatment was studied. The cows that had LLHL had conceived at induced estrus in Heatsynch group. The cows that had serum progesterone concentration of ≥ 1ng/ml on the day of second $PGF_2\alpha$ showed higher conception. Cows that had high concentration at -10 and on the day of intiation of treatment had conceived at induced estrus. The hormonal protocols (Heatsynch and G6G protocol) had significant influence on circulatory serum progesterone profiles.

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