Haematological Profile of Postpartum Anestrous Ongole Cows Treated with Hormonal Protocols

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A B S T R A C T

Haematological profiles play a key role in diagnosis of array of reproductive disorders. Postpartum lactating anestrous Ongole cows were treated with estrus and ovulation hormonal protocols Heatsynch (Group A) and G6G (Group B) and haematological analysis was done following estrus induction. The mean of haemoglobin level was 9.94±0.47, 10.51±0.27 and 10.23±0.68, 10.93±0.45 g/dl on day of selection (0 day), on day of induced estrus in Heatsynch and G6G groups, respectively. The mean PCV was 28.97±1.04, 33.92±1.31 and 30.24±1.74, 34.49±0.99 per cent on day of selection (0 day), on day of induced estrus in Heatsynch and G6G groups, respectively. The mean level of PCV was statistically significant (P<0.05) on the day 0 and on the day of induced estrus in both the treatment groups where as the mean level of haemoglobin was non-significantly different (P>0.05) between conceived and non-conceived cows, in both the treatment groups.

Keywords
G6G, Haemoglobin, Heatsynch, Ongole cows, PCV, Postpartum

Introduction

The State of Andhra Pradesh situated on the eastern coast of peninsular India is known for the world famous Ongole breed of cattle. However certain reproductive impediments like long calving interval, short estrus duration, incidence of estrus at night and postpartum anestrous are limiting the economic use of this Ongole cattle breed (Malik et al., 2011). Clinical surveys in India have revealed the incidence of postpartum anestrous due to inactive ovaries in cattle that greatly affects economy of farming community. It is one of the most common reproductive disorders encountered in livestock farms leading to prolonged inter-calving period and reduced milk production. Haematological profiles play a key role in diagnosis of array of reproductive disorders. Low haemoglobin level can influence the tissue oxygenation of reproductive tract and cyclicity (Kumar et al., 1991).
Estrous synchronization is the hormonal regulation of estrus cycle at a time in a group of animals with timed artificial insemination. Heatsynch protocol employs an injection of gonadotropic releasing hormone (GnRH) combined with single administration of prostaglandins (PGF$_2\alpha$) and an injection of estradiol benzoate (Bhoraniya et al., 2012). G6G protocol consists of pre-synchronisation with prostaglandins (PGF$_2\alpha$) and an injection of gonadotropic releasing hormone (GnRH) followed by Ovsynch protocol (Heidari et al., 2017). Lower level of Haemoglobin and Packed cell volume is an indicative of anaemia in anestrous cow. Thus, estimation of haematological profiles before and after treatment is helpful in studying ovarian function and response to treatment. The present study aimed at estimating the haematological parameters of postpartum anestrous Ongole cows treated with heatsynch and G6G protocols.

**Materials and Methods**

**Location of the study**

Animals maintained under standard feeding and managerial conditions at Cattle Project, Livestock Research Station, Lam Farm, Guntur, (15°00 and 16°10 North latitude and 79°04 and 80°02 East Longitude), Sri Venkateswara Veterinary University, Andhra Pradesh, India, were included in this study.

**Experimental animals**

Ongole cows (n=24) 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.

**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.

**Group-C**

In this group 8 untreated cows were kept as control animals (Control group).

**Blood sampling and Haematological analysis**

For haematological analysis, blood was collected aseptically from jugular vein of each
animal with 18 gauge needle on day 0 - just before treatment, on day 7/9 - at the time of PGF$_2\alpha$ administration, on day of induced estrus/FTAI and on day 21 post-AI. Blood was collected into screw capped sterilized anticoagulant vials containing EDTA @ 2mg/ml of blood. Haemoglobin was estimated by using Sahli’s haemoglobinometer method and expressed in g/dl while packed cell volume (PCV) was observed by using microhaematocrit scale and expressed in per cent.

**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.

**Results and Discussion**

Postpartum lactating anestrous Ongole cows were treated using Heatsynch (Group A) and G6G (Group B) protocols and haematological parameters i.e. Haemoglobin and PCV were estimated. In the present study, the mean of haemoglobin level was 9.94±0.47, 10.51±0.27 and 10.23±0.68, 10.93±0.45 g/dl on day of selection (0 day), on day of induced estrus in Heatsynch and G6G groups, respectively (Table 1 and 2; Fig. 1). Similar view was expressed by Kalsotra et al. (2016) that the haemoglobin value was lower on day 0 than on the day of estrus. Mane et al. (2018) reported highly significant variations in haemoglobin concentration (11.78±0.21 vs 12.39±0.19 g/dl) found before and after treatment of anestrous bovines. Babar (1998) reported lower Hb concentration in anestrous cows. Sharad and Atul (2010) and Ali and Shukla (2012) reported significant variation in anestrous and regular cyclic bovines.

Pariza et al. (2013) reported that the mean haemoglobin percentage (Hb%) in anestrous cows as 10.2±1.5 g/dl which was lower than the control group (13.1±2.6 g/dl). Prasad et al. (1984) stated that haemoglobin concentration was slightly lower (10.61 g/dl) at estrus as compared to (10.95 g/dl) during anestrous in crossbred cows. Ali et al. (1991) reported mean haemoglobin concentration as 10.92±0.34 g/dl in cyclic and 7.92±0.25 g/dl in anestrous heifers. In contrast, Khan et al. (2016) reported that haemoglobin values did not show any significant change in between breeding and anestrous state.

The low level of haemoglobin in anestrous cows indicates long term protein deficiency and influences tissue oxygenation of reproductive tract which in turn affects the cyclicity (Ramakrishna, 1997). According to Calvo et al. (1989) haemoglobin decreased significantly during first half of gestation time, with the lowest values in the second month. Haemoglobin values decrease during pregnancy not only due to the mobilization of the mother’s haemoglobin into foetal circulation, but also due to dilution of blood which occurs as a consequence of plasma volume increase (Singh et al., 1991). Lack of sufficient quantities of Hb in blood is responsible for reduced oxygen transport to the vital tissues, causes reduced oxidation of nutrients, which in turn affects the whole cellular metabolism in gonadal cells which is metabolically more active (Swenson and Reece, 1993).

In the present study, the mean PCV was 28.97±1.04, 33.92±1.31 and 30.24±1.74, 34.49±0.99 per cent on day of selection (0 day), on day of induced estrus in Heatsynch and G6G groups, respectively. The mean PCV varied significantly (P<0.05) between the day of treatment and on the day of estrus in both the groups (Table 1 and 3; Fig. 2).
Table 1: The haematological parameters in postpartum anestrous cows

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Day 0</th>
<th>Day of induced estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heatsynch (Group A)</strong></td>
<td>Haemoglobin (g/dl)</td>
<td>9.94±0.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PCV (%)</td>
<td>28.97±1.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>G6G (Group B)</strong></td>
<td>Haemoglobin (g/dl)</td>
<td>10.23±0.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PCV (%)</td>
<td>30.24±1.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

Table 2: Haemoglobin concentrations (g/dl) in anestrous postpartum lactating Ongole cows on different days of treatment /AI under two estrus induction protocols

<table>
<thead>
<tr>
<th>Group/Estrus induction protocol</th>
<th>Status</th>
<th>No</th>
<th>Days from treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heatsynch (Group A)</strong></td>
<td>Conceived</td>
<td>6</td>
<td>10.08±0.62</td>
</tr>
<tr>
<td></td>
<td>Non-conceived</td>
<td>2</td>
<td>9.54±0.33</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>8</td>
<td>9.94±0.47</td>
</tr>
<tr>
<td><strong>G6G (Group B)</strong></td>
<td>Conceived</td>
<td>7</td>
<td>10.48±0.72</td>
</tr>
<tr>
<td></td>
<td>Non-conceived</td>
<td>1</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>8</td>
<td>10.23±0.68</td>
</tr>
<tr>
<td><strong>Control (Group C)</strong></td>
<td>Conceived</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Non-conceived</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3: Packed cell volume levels (%) in anestrous lactating Ongole cows on different days of treatment /AI under two estrus induction protocols

<table>
<thead>
<tr>
<th>Group/Estrus induction protocol</th>
<th>Status</th>
<th>No</th>
<th>Days from treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heatsynch (Group A)</strong></td>
<td>Conceived</td>
<td>6</td>
<td>29.01±1.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Non-conceived</td>
<td>2</td>
<td>28.90±2.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>8</td>
<td>28.97±1.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>G6G (Group B)</strong></td>
<td>Conceived</td>
<td>7</td>
<td>30.62±1.96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Non-conceived</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>8</td>
<td>30.24±1.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Control (Group C)</strong></td>
<td>Conceived</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Non-conceived</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>

Means bearing different superscripts within the row (a,b) column (p,q) differ significantly (P< 0.05) between conceived vs non conceived cows. D-0 = Day of starting the treatment, D-7/15 = after Administration of PG (day 7 in Heatsynch; day 15 in G6G), D- Estrus ( Day 10 in Heatsynch /day 18 in G6G ) D-21 = Day 21 post-AI
Similar view was expressed by Kalsotra et al. (2016) that the PCV value was lower on day 0 than on the day of estrus. The mean Packed cell volume (PCV %) in anestrous cows was 31.3±5.2% which was lower than the control group i.e., 43.2±7.9 % as reported by Pariza et al. (2013). Ahmad et al. (2003) reported non-significant lower PCV values in non-cyclic cows than cyclic cows. Ashwani et al. (2015) reported that the values of PCV were lower on the day of treatment and increased significantly (P<0.05) on the day of estrus in heatsynch protocol. A decreased PCV generally means red blood cell loss from any
variety of reasons like cell destruction, blood loss, and failure of bone marrow production. An increased PCV generally indicates dehydration or an abnormal increase in red blood cell production (Kalsotra et al., 2016).

In conclusion, lower level of Haemoglobin and Packed cell volume is an indicative of anaemia in anestrous cows. Though the importance of haemoglobin level has not been directly implicated in the reproductive disorders, yet decrease in Hb value is indicative of certain systemic disorders which could indirectly affect the functional activity of reproductive organs. In the present study, both the Haemoglobin and packed cell volume percentage were lower in anestrous cows on the day of treatment and increased on the day of estrus in both heatsynch and G6G protocols.

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