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# Influence of blood metabolites and metabolic hormones on response and conception in postpartum Sahiwal cows after Ovsynch treatment*

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ABSTRACT

Present investigation was conducted to investigate the influence of blood metabolites and metabolic hormones on conception after Ovsynch treatment in postpartum Sahiwal cows. Experimental animals (12) were randomly divided in to 2 groups, of 6 animals each. Animals of group 1 were treated with traditional Ovsynch protocol beginning on day 60 postpartum (day 0 of Ovsynch protocol), while animals of group 2 were treated with a modified Ovsynch protocol, where first GnRH injection was replaced with hCG. Treated animals were inseminated at fixed time between 14 and 20 h after second GnRH injection without estrus detection. Blood samples were collected from each experimental animal on days 50, 60 and 68 postpartum; and blood glucose and serum concentrations of non-esterified fatty acid (NEFA), insulin, tri iodothyronine ($T_3$), thyroxin ($T_4$) and progesterone ($P_4$) were determined. Non-conceived animals recorded significantly higher mean serum NEFA concentration than in conceived animals on days 50, 60 and 68 postpartum. Conceived animals had significantly higher blood glucose concentration on day 68, significantly higher serum insulin concentration on days 50, 60 and 68, significantly higher serum $T_3$ concentration on days 60 and 68 and significantly higher serum $T_4$ concentration on day 60 postpartum than in non-conceived animals. From the present study, it may be concluded that decreased serum NEFA concentration and an increased level of blood glucose and serum concentrations of insulin, $T_3$ and $T_4$ probably favoured to achieve conception in Ovsynch treated postpartum Sahiwal cows.

**Key words**: Blood glucose, Conception, Insulin, Non-esterified fatty acid, Ovsynch, Postpartum Sahiwal cows, Thyroxin, Tri iodothyronine

High-producing dairy cows invariably remain acyclic for long period of time leading to financial losses to dairy farmers. Delay in onset of postpartum cyclic activity is thought to be because of suppression of ability of the hypothalamo-hypophyseal axis to execute the pulsatile release of luteinizing harmone (LH), required for fostering follicular development and ovulation. The physiological pathways by which, the hypothalamic-pituitary-ovarian axis is informed about the energy status of the animal are complex, and involve several metabolites and hormones, such as the growth hormone (GH), insulin-like growth factor-I (IGF-I) system, insulin, thyroid hormones etc. Reproductive efficiency of postpartum cows may be improved by inducing early cyclicity through Ovsynch programme, which is one of the most classical and widely known systems of cattle breeding for reproductive management (Twagiramungu *et al.* 1995, Pursley *et al.* 1997). However, many treated animals do not conceive to fixed time artificial insemination (FTAI) after Ovsynch treatment during early postpartum period, although they respond well to Ovsynch treatment. In this perspective, present experiment was conducted with the objective of investigating influence of blood metabolites, viz. blood glucose and serum NEFA and serum concentration of insulin, 3,3,5-tri-iodothyronine ($T_3$) and thyroxin ($T_4$) on conception after Ovsynch treatment and FTAI in postpartum Sahiwal cows.

## MATERIALS AND METHODS

Suckled postpartum Sahiwal cows (n, 12) maintained at Bull Mother Experimental Farm, College of Veterinary Science and Animal Husbandry, Anjora, Durg in healthy state from first to sixth lactation were utilized for present study. They were fed with green fodder, hay, straw, compound concentrate mixture and a balanced mineral mixture as per

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standard feeding schedule followed in the farm. All these animals had normal calving and subsequent normal genital health as assessed by gynaeco-clinical examination. The selection of experimental animals was strictly based on absence of postpartum metritis and endometritis in these animals. The animals were selected randomly and equally divided into 2 groups of 6 animals each, viz. Ovsynch group 1 and Ovsynch group 2. Animals of group 1 were treated with initial intramuscular injection of 10 µg buserelin acetate, a synthetic anlogue of GnRH (receptal 2.5 ml) on day 60 postpartum (day 0 of Ovsynch protocol), 8 days later 500 µg cloprostenol, a synthetic anlogue of $PGF_2\alpha$ (inj. estropur 2 ml) was injected intramuscularly. Two days later (day 10 of Ovsynch protocol) animals received second injection of 10 µg buserelin acetate (GnRH). All the cows were inseminated at fixed time (FTAI) of 14 to 20 h after second GnRH injection without estrus detection. Similar Ovsynch protocol was followed in animals of group 2 except first GnRH injection was replaced with 1500 IU of hCG (chorulon).

Blood samples were collected from each experimental animal on days –10, 0 and 8 of Ovsynch protocol for estimation of serum progesterone concentration, which was determined using radio immuno assay kits from BRIT, BARC, Mumbai (Kubasic *et al.* 1984). Animals showing serum progesterone level ≥ 1 ng/ml either on day –10 or 0 were classified as cyclic and animals with concentration of serum progesterone ≥ 1 ng/ml on days –10 and 0 were classified as acyclic (Cartmill *et al.* 2001). Animals were considered as responders to first GnRH or hCG treatment, if they recorded serum progesterone level ≥ 1 ng/ml on day 8 (day 68 postpartum) of Ovsynch protocol. Pregnancy diagnosis was carried out through examination per rectum on any day between days 50 and 60 after FTAI in animals of treatment groups.

Blood glucose was analysed immediately after collection of blood by glucometer through strip method. Serum non-esterified fatty acid (NEFA) level was determined by modified soap extraction method (Shipe *et al.* 1980). Serum concentrations of insulin, triiodothyronine ($T_3$) and thyroxin ($T_4$) were determined using radioimmunoassay kits supplied by BRIT, BARC, Mumbai. Irrespective of treatment group, animals were grouped in conceived (4) and non-conceived (8) group and differences of significance in variables were determined with the help of independent 't' test between 2 groups using SPSS computer programme version 10.0.

## RESULTS AND DISCUSSION

The cyclic status, response to treatment and conception in experimental animals data (Table 1) revealed that 4 animals each were cyclic and 2 each were acyclic before initiation of Ovsynch protocol in group 1 as well as in group 2. Three cyclic and 2 acyclic animals (83.3%, 5/6) responded to first GnRH treatment as they recorded serum $P_4$ concentration ≥ 1 ng/ml on day 8 of Ovsynch protocol in group 1. One cyclic animal did not respond to GnRH treatment as revealed by $P_4$ concentration being < 1ng/ml on day 8. Two responding cyclic animals got conceived after FTAI, whereas, 2 acyclic and 1 cyclic animal, which responded to treatment, did not conceive after FTAI. All 6 animals responded to hCG treatment in Ovsynch group 2, as they recorded serum progesterone concentration ≥1 ng/ml on day 8 of Ovsynch protocol. Two responding cyclic animals got conceived after FTAI, whereas 2 cyclic and 2 acyclic animals did not conceive after FTAI.

Blood glucose concentration, mean serum concentrations of non-esterified fatty acid (NEFA), insulin, triiodothyronine ($T_3$) and thyroxin ($T_4$) in conceived and non-conceived animals of both Ovsynch groups are presented in Table 2. There was non-significant difference in the mean blood glucose concentration between conceived and non-conceived animals on days 50 and 60 postpartum. However, conceived animals had significantly higher ($P<0.01$) blood glucose concentration than in non-conceived animals on day 68 postpartum. Non-conceived animals recorded significantly ($P<0.01$) higher mean serum NEFA concentration than in conceived animals on days 50, 60 and 68 postpartum. Conceived animals had significantly higher ($P<0.01$) serum insulin concentration than that of non-conceived animals on days 50, 60 and 68 postpartum. Similarly, conceived animals had significantly higher ($P<0.01$) serum $T_3$ concentration than that of non-conceived animals on days 60 and 68 postpartum. There was non-significant difference in mean serum $T_4$ concentration between conceived and non-conceived animals on days 50 and 68 postpartum. However, conceived animals

Table 1. Physiological status of experimental animals before initiation of treatment and response to treatments on day 8

| Groups | Cyclic status | No. of animal streated | Response to treatment | | Conception status | |
|---|---|---|---|---|---|---|
| | | | Responded (no.) | No response (no.) | Conceived (no.) | Non-conceived (no.) |
| Group 1 | Cyclic | 04 | 03 | 01 | 02 | 02 |
| GnRH treatment | Acyclic | 02 | 02 | - | - | 02 |
| Group 2 | Cyclic | 04 | 04 | - | 02 | 02 |
| HCG treatment | Acyclic | 02 | 02 | - | - | 02 |

Cyclic, $P_4 \geq 1$ ng/ml; acyclic, $P_4 \leq 1$ ng/ml.

Table 2. Mean±SE of progesterone, metabolic hormones and blood metabolites in conceived and non-conceived animals on days 50, 60 and 68 postpartum

| Parameters | Day 50 | | Day 60 | | Day 68 | |
|---|---|---|---|---|---|---|
| | Conceived | Non-conceived | Conceived | Non-conceived | Conceived | Non-conceived |
| Progesterone (ng/ml) | 0.64±0.22 | 0.47±0.04 | 2.03±0.22 | 1.32±0.26 | 4.20±0.37** | 2.14±0.23 |
| $T_3$ (ng/ml) | 1.85±0.24 | 1.85±0.19 | 2.80±0.15** | 1.70±0.16 | 3.50±0.37** | 1.68±0.26 |
| $T_4$ (ng/ml) | 108.40±2.77 | 106.41±2.54 | 114.67±2.25* | 106.42±2.26 | 118.50±0.87 | 109.37±3.01 |
| Insulin (µU/ml) | 14.04±0.19** | 11.94±0.42 | 16.35±0.35** | 13.50±0.36 | 19.12±1.08** | 14.97±0.49 |
| NEFA (mMol/L) | 0.02±0.006 | 0.15±0.02** | 0.02±0.005 | 0.14±0.02** | 0.02±0.006 | 0.17±0.02** |
| Glucose (mg/dl) | 47.20±2.82 | 49.08±1.07 | 50.50±1.93 | 49.0±1.34 | 58.50±1.32** | 50.62±1.08 |

Data with superscript ** differed significantly (P< 0.01) ; data with superscript * differed significantly (P< 0.05).

had significantly higher (P<0.05) serum $T_4$ concentration on day 60 postpartum.

The present results demonstrated that blood glucose concentration influenced the conception in animals after Ovsynch treatment. There is paucity of information in existing literature on effect of blood glucose on conception in Sahiwal cows after Ovsynch treatment. Although conceived animals had nonsignificantly lower blood glucose concentration than in non-conceived animals before the beginning of Ovsynch treatment on day 50, blood glucose level continuously increased on days 60 and 68 so much so that its level became significantly higher (P<0.01) than in non-conceived animals on day 68 postpartum. There was a close relationship between low level of glucose and the poor conception rate in the cattle (Luca *et al.* 1977). Non-returns animals showed higher mean blood glucose concentration than returns animals (Hunter 1977). Decreased level of blood glucose was associated with lower conception rate and higher number of services per conception (Pedroso *et al.* 1982). It was suggested that the lower level of blood glucose during estrus might affect the outcome of breeding in the cows (Kumar and Sharma 1991, Chandrakar *et al.* 2003). Similar observation was observed in present study. Therefore, based on present observation it may be suggested that adequate blood glucose level is required to achieve conception in Ovsynch treated animals.

Serum NEFA concentration had profound negative impact on conception in Ovsynch treated animals in the present study. It was hypothesized that the elevated plasma NEFA concentration causes lower fertility by exerting negative effect on granulosa cells and oocytes in the ovary, leading to poor CL formation with sub normal luteal functions. Furthermore, a negative relationship was demonstrated between follicular concentrations of NEFA and estradiol (Comin *et al.* 2002, Jorritsma *et al.* 2003). The present observation suggests that negative energy balance associated with higher serum NEFA concentration influenced the conception in animals after Ovsynch treatment and FTAI.

Insulin stimulates *in vitro* steroidogenesis and proliferation of bovine thecal and granulosal cell cultures (Spicer *et al.* 1993, Spicer and Stewart 1996) and thus, helps in follicular development (Beam and Butler 1999) and follicular growth rate (Lucy *et al.* 1992, Miyoshi *et al.* 1995). Insulin controls the ovulation rate by increasing the follicular development (Webb *et al.* 1994). Serum insulin concentration is positively related to CL development and progesterone concentrations (Spicer *et al.* 1990, Thatcher *et al.* 1996). Thus, serum insulin concentration has direct impact on follicular development, follicular growth rate, steroidogenesis, CL development and progesterone production. Non-conceived animals had significantly lower (P<0.01) serum insulin concentration than in conceived animals on all 3 days of observation in present study suggesting that serum insulin concentration had direct impact on conception after Ovsynch treatment in dairy cows.

Conceived animals had significantly higher (P<0.01) serum $T_3$ concentration than that of non-conceived animals on days 60 and 68 postpartum. A role of thyroid hormones was reported in regulating steroidogenesis (Spicer 2001). Low thyroid hormone concentrations were suggested to be associated with low reproductive performance in the postpartum cow (Huszenicza *et al.* 2002). Although $T_3$ and $T_4$ had little or no effect on aromatase activity per se, the stimulatory effect of $T_3$ and $T_4$ on androstenedione produc-tion could provide important estrogen precursors to granulosa cells and thus, increase estrogen production indirectly *in vivo* (Huszenicza *et al.* 2002). Based on present observation it may be suggested that serum $T_3$ concentration might have affected conception in animals after Ovsynch treatment.

An increasing trend of serum $T_4$ level was observed in both conceived and non-conceived animals with increasing postpartum interval. Present result approximates with observation of Nath *et al.* (2005) who reported similar trend of increasing serum $T_4$ level after parturition in dairy animals. Conceived animals had significantly (P<0.05) higher serum $T_4$ level than in non-conceived animals on day 60 postpartum. Thyroxin is considered as a major thyroid hormone and functions primarily as a precursor to $T_3$ (Chopra *et al.* 1978), which is responsible for the majority of the tissue effects. Therefore, based on present observation it may be suggested that serum $T_4$ concentration may influence indirectly on conception in animals after Ovsynch treatment.

Based on present study it may be concluded that Ovsynch treatment induced response in a majority of Sahiwal cows (11/12) on or after 60 days postpartum; however, animals with lower level of serum NEFA and higher levels of blood glucose, serum insulin, $T_3$ and $T_4$ could conceive after Ovsynch treatment.

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