CIRCULATING OVARIAN STEROIDS IN RELATION TO SUPEROVULATORY RESPONSE AND EMBRYO RECOVERY IN SAHIWAL COWS AND HEIFERS*

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ABSTRACT

Relationship between concentrations of circulating ovarian steroids and superovulation (sov.) response and embryo recovery was studied in 12 Sahiwal cattle (6 cows; 6 heifers) treated with FSH-P and $PGF_{2\alpha}$ given i.m. 48 hr after first FSH injection. On day 7 of first AI, donors were flushed and recovered embryos/ova were graded. Blood plasma samples were analysed for estrogen and progesterone concentrations using RIA. Among 6 cows (Group A), only 3 (50%) responded to sov treatment with 17 CL (5.67/donor) and total 10 embryos were recovered. Mean recovery of total and viable embryos for responded donors and overall were found to be 3.33 and 1.00 and 1.67 and 0.50 embryos/flush, respectively. All heifers (Group B) responded to sov. treatment with 50 CL (8.33/ donor) and total 37 embryos (18 VE; 16 DE, 2 UFO and 1 zona) were recovered. Mean recovery of total and viable embryos was found to be 6.17 and 3.00 embryos/flush, respectively. Heifers yielded comparatively higher number of mean ovulations (8.33) and embryos, both total (6.17) and viable (3.00), than those of cows (3.17, 1.67 and 0.50, respectively). No significant variation was found in plasma progesterone concentrations among both groups at all sample collection stages. The mean concentrations of plasma estrogen varied significantly between groups. It was found to be significantly ($t=3.293$, $p<0.05$) higher at all stages for heifers than cows. Mean progesterone and estrogen concentrations at synchronized estrus (0.42+0.17 ng/ml and 18.50+7.55 pg/ml, respectively) and at $PGF_{2\alpha}$ injection during FSH treatment (1.85+0.76 ng/ml and 12.67+5.17 pg/ml, respectively) in heifers were found to be correlated significantly with 8.33 ovulations/donor ($R^2=0.902$, $p<0.031$; $R^2=0.831$, $p<0.069$, respectively). Estrogen concentrations remained highest at superovulatory estrus in cows (39.33+16.06) and at synchronized estrus following $PGF_{2\alpha}$ II injection in heifers (18.50+7.55 pg/ml). Mean progesterone concentration declined significantly between $PGF_{2\alpha}$ injection during FSH treatment and on the day of superovulatory estrus in heifers (2.11+0.86 to 0.25+0.10 ng/ml) and cows (1.85+0.76 to 0.73+0.30 ng/ml). The mean estrogen concentrations were significantly higher at all stages in heifers than cows and mean concentrations of progesterone and estrogen at synchronized estrus and at the time of $PGF_{2\alpha}$ injection during FSH treatment in heifers were correlated significantly with ovulation rate. It was concluded that better sov. response and embryo recovery in heifers were related with the better profile of ovarian steroids.

Key words: Ovarian steroids, Superovulation response, Embryo recovery, Cows.

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INTRODUCTION

Animal bio-technologies such as the embryo transfer (ET) have a vital role to play in livestock breeding and improvement. ET technique is an effective tool to exploit the female genetics in terms of number of offspring produced. The maximum utilization of female

genetics has been possible with the advent of this technique. However, it has always been difficult to predict the superovulatory (sov.) response and embryo recovery in donors. Many scientists have tried to establish relationship of various reproductive hormones with sov.response and embryo recovery. Chagas e Silva *et al.* (2002) found significantly higher plasma progesterone concentration on the day of embryo collection (Day 7 of sov. estrus) in dairy heifers than in dairy cows. Joe Arosh *et al.* (2001) reported a positive significant correlation between concentrations of circulating ovarian steroids at the time of initiating sov. treatment and at sov estrus and the number of ovulations in crossbred cows. Contrary to this, Agarwal *et al.* (1992) reported that there was no correlation between concentrations of circulating ovarian steroids at the time of initiating sov treatment and subsequent ovulation rate in Hariana crossbred cows. They however found that concentration of estrogen at sov estrus was positively correlated with the subsequent ovulation rate. Present study was undertaken to find out the relationship between concentrations of circulating ovarian steroids and sov response and embryo recovery in Sahiwal cows and heifers.

MATERIALS AND METHODS

A total of 12 pure Sahiwal cattle comprising 6 cows (Group A) and 6 heifers (Group B) were selected. Estrus synchronization of selected donors was initiated by injecting two doses of 5 ml Iliren (Intervet International, GmbH, Germany) by i.m. route at 11 days interval. The synchronized estrus observed after second dose of Illiren was considered as Day 0 for initiating sov treatment at Day 9-11 of synchronized estrus. The donors were superovulated using standard protocol of Folltropin-V (Bioniche Animal Health, Canada), with a reduced dose to half as suggested by Patel *et al.* (2004) for crossbred cattle. Multiple injections of FSH in constant dose regimen of 1.25 ml (25 mg NIH FSH-P1) were administered by i.m. route in the morning and evening (at 12 hourly interval) over four days starting between Day 9 and 11 post estrus. A single dose of 5 ml Iliren ($PGF_{2\alpha}$) was injected 48 hr after the first FSH injection, to bring the donors in heat. Donors evinced estrus 36 to 48 hr after the prostaglandin injection, detected by parading vasectomised teaser bull and visually by trained persons twice daily. The donors were bred by AI thrice, first AI after 48 hr of Illiren injection followed by two more AIs at 12 hr intervals.

Donors were examined per rectal on Day 7 of first AI to ascertain sov response. Donors having > 2 CL were considered as responded to FSH treatment, whereas presence of less than two CL in a donor was considered as non-responded donor. All the donors were subjected to uterine flushing for embryo recovery by non-surgical technique (Siddiqui *et al.*, 1999) with flush media containing Dulbecco's Phosphate Buffer Saline (D-PBS, IMV, France) supplemented with 0.1% Bovine Serum Albumin fraction V (BSA, SIGMA, USA) using an 18 gauze embryo flushing catheter (Minitub, Germany). The recovered media was searched for ova/ embryos under stereo zoom microscope at low (15x) magnification and recovered ova/embryos were graded as viable embryos (VE), degenerated embryos (DE) and unfertilized ova (UFO).

A total of 8 jugular blood samples were collected from each donor using heparinized vacutainers, I: at estrous synchronization of donors just before giving $PGF_{2\alpha}$ I injection, II: at the time of induced estrus following $PGF_{2\alpha}$ I injection, III: at estrus synchronization of donors just before giving $PGF_{2\alpha}$ II injection, IV: at the time of synchronized estrus following $PGF_{2\alpha}$ II injection, V: on the day of starting sov. treatment (day 9 to 11 of estrus), VI: at the time of $PGF_{2\alpha}$ injection during sov treatment (day 3 of treatment), VII: at sov estrus / first insemination of donors and VIII: on the day of flushing (embryo collection from donors). The plasma was separated out by centrifuging the blood for five minutes at 3000 RMP and stored at -20° C in deep freezer till analysed for estrogen and progesterone levels using standard Radio Immuno Assay (RIA) technique (Kubasik *et al.*, 1984). Plasma samples were analysed using Estradiol C.T. RIA kits (Lot No. 20B) and Progesterone C.T. RIA kits (Lot No. 3017B) procured from M/s Immunotech (A Beckman Coulter Company), France.

RESULTS AND DISCUSSION

The mean blood plasma progesterone and estrogen concentrations per ml were 1.67+0.68 ng and 12.67+ 5.17 pg, respectively, at stage I. The concentrations of both the hormones changed to 0.31+0.12 ng/ml and 16.67+6.80 pg/ml, respectively, 72 hours after $PGF_{2\alpha}$ I injection when donors were supposed to be in estrus (stage II). At the time of $PGF_{2\alpha}$ II injection (stage III), concentrations of progesterone and estrogen were found to be 2.14+0.87 ng/ml and 19.00+7.76 pg/ml, respectively. Progesterone concentration was found to be higher in most of the donors which were expected to have CL at this stage.

Concentrations of progesterone and estrogen at stage IV (synchronized estrus) were 0.41+0.17 ng/ml and 20.17+8.23 pg/ml, respectively. At this stage, the progesterone level went down and estrogen level went up due to absence of CL and presence of Graafian follicle on ovaries. At the time of injecting first FSH dose (stage V), concentrations of progesterone and estrogen were 2.17+0.88 ng/ml and 22.67+9.25 pg/ml, respectively. At this point, progesterone level went up as all donors had functional CL which was confirmed by rectal palpation before initiation of FSH treatment, whereas higher level of estrogen at this stage might be the result of numerous growing follicles. At stage VI, concentrations of progesterone and estrogen were found to be 2.11+0.86 ng/ml and 30.17+12.32 pg/ml, respectively. At this point, estrogen level was found to be increased substantially suggestive of positive response to FSH treatment, due to the presence of numerous follicles on ovaries, whereas progesterone level remained constant as functional CL was present on either ovaries of each donor. The concentrations of progesterone and estrogen at first AI (stage VII) were observed to be 0.25+0.10 ng/ml and 39.33+16.06 pg/ml, respectively. At this stage, progesterone level was the lowest of all stages due to absence of CL, as such, estrogen level was found to be the highest due to multiple follicles on ovaries. On the day of embryo collection (stage VIII), concentration of circulating ovarian steroids remained to be 3.28+1.38 ng/ml for progesterone and 28.50+11.64 pg/ml for estrogen. Progesterone level was the highest due to multiple CL on the ovaries, whereas higher level of estrogen at this stage might be the result of unovulated follicles.

Out of six donors of Group A, only 3 (50 %) responded to FSH treatment. The mean ovulation rate was found to be 5.67 ovulations/donor (total 17 ovulations). In case of three non-responded donors, 2 ovulations took place with an average of 0.67 ovulations/donor. Overall number of ovulations was 19 with an average of 3.17/donor inclusive of responded and non-responded donors. At the time of rectal palpation, two unovulated follicles were also found on the ovary of one of the donors. Embryo recovery revealed that three donors, which responded to sov treatment, gave total 10 embryos. Out of 10, only 3 embryos (30%) were viable at the rate of 1 VE/flush and remaining 7 embryos (70%) were degenerated. Sahiwal cows (responded as well as non-responded) gave only 0.5 VE/flush against 3.17 ovulations/donor.

The mean progesterone and estrogen concentrations were found to be 0.86+0.35 ng and 11.17+4.56 pg, respectively, at $PGF_{2\alpha}$ I injection (stage I). The concentrations of both hormones changed to 0.21+0.09 ng/ml and 10.00+4.08 pg/ml, respectively, at expected estrus (stage II). At $PGF_{2\alpha}$ II injection (stage III), concentrations of progesterone and estrogen were found to be 2.41+0.98 ng/ml and 13.67+5.58 pg/ml, respectively. Progesterone concentration was found to be higher in most of the donors which were supposed to have CL at this stage. Progesterone and estrogen levels at synchronized estrus (stage IV) were 0.42+0.17 ng/ml and 18.50+7.55 pg/ml, respectively. At this point, progesterone level went down and estrogen level went up due to absence of CL and presence of Graafian follicle on ovaries. At stage V, concentrations of progesterone and estrogen were 1.17+0.48 ng/ml and 10.00+4.08 pg/ml, respectively. Here progesterone level went up as all the donors had functional CL. At stage VI, progesterone and estrogen concentrations were 1.85+0.76 ng/ml and 12.67+5.17 pg/ml, respectively,

showing an increase in estrogen level suggestive of positive response to FSH treatment. Similarly, progesterone level also increased as functional CL was present on either ovaries of each donor. At the time of first AI (stage VII), concentrations of progesterone and estrogen were 0.73+0.30 ng/ml and 18.33+7.48 pg/ml, respectively, showing a decline in progesterone level due to absence of functional CL. Accordingly, estrogen level was found to be increased due to the presence of multiple follicles on the ovaries. On the day of embryo collection (stage VIII), concentration of circulating ovarian steroids remained to be 3.46+ 1.41 ng/ml progesterone and 13.67+5.58 pg/ml estrogen, respectively. Progesterone concentration was the highest of all the stages due to presence of multiple CL on the ovaries.

In Group B (Sahiwal heifers), all (100%) donors responded to FSH treatment with two or more CL palpated prior to embryo recovery. The mean ovulation rate was found to be 8.33 ovulations/donor (total 50 ovulations). Embryo recovery revealed that all the donors gave total 37 embryos. Out of 37, only 18 embryos (48.65%) were viable at the rate of 6.17 viable embryos (VE) per flush and remaining 19 embryos (51.35%) were non-viable comprising 16 degenerated embryos, 2 unfertilised ova and 1 empty zona pellucida.

No significant variation was found in concentrations of progesterone in both groups of donors at various stages of blood collection. However, concentration of estrogen varied significantly in both groups. Mean estrogen concentration was found to be significantly higher ($t=3.293$, $p<0.05$) at all stages in Sahiwal cows (Group A) as compared to those of heifers (Group B).

The results revealed that concentrations of progesterone and estrogen (0.42+0.17 ng/ml and 18.50+7.55 pg/ml, respectively) at synchronized estrus (Stage IV) in heifers (Group B) were found to be correlated significantly with the rate of ovulation, i.e., 8.33 ovulations per donor ($R^2=0.902$, $p<0.031$). Similarly, mean concentrations of progesterone and estrogen (1.85+0.76 ng/ml and 12.67+5.17 pg/ml, respectively at the time of $PGF_{2\alpha}$ injection during sov treatment(Stage VI) in heifers were found to be correlated significantly with the rate of ovulation, i.e., 8.33 ovulations/donor ($R^2=0.831$, $p<0.069$). Unlike foregoing, the mean concentrations of progesterone and estrogen at first AI (Stage VII) were not found to be correlated with the rate of ovulation in both the groups.

Findings of the present study indicated that concentrations of estrogen remained highest at sov. estrus in both groups (39.33+16.06 and 18.33+7.48 pg/ml, respectively). Sarvaiya *et al.* (2003) also reported high mean estrogen concentration at sov estrus in pure Jersey cows. Under the present study, mean plasma progesterone concentration declined significantly after PG injection during sov. treatment (Stage VI) and on the day of sov. estrus (Stage VII) in both groups (2.11+0.86 to 0.25+0.10 and 1.85+ 0.76 to 0.73+0.30 ng/ml, respectively). Sugana *et al.* (2001) also reported a drastic decrease in plasma progesterone level following administration of $PGF_{2\alpha}$ in Japanese black cattle superovulated with porcine FSH. Chagas e Silva *et al.* (2002) found significantly higher plasma progesterone concentration on the day of embryo collection (Day 7 of sov. estrus) in dairy heifers than in dairy cows. These findings support the observations made in the present study on progesterone concentrations on the day of embryo collection, which were found to be slightly higher in heifers (3.46+1.41 ng/ml) than in cows (3.28 +1.34 ng/ml).

Mean plasma progesterone levels were highest on the day of embryo collection (3.28+1.34 and 3.46+1.41 ng/ml) and estrogen levels on the day of sov. estrus (39.33+16.06 and 18.33+7.48 pg/ml) in both groups. Ranjana *et al.* (2004) also reported the highest level of progesterone on the day of embryo collection and highest estrogen level on the day of sov. estrus in highly responded (> 6 ovulations) crossbred cows. Their findings support the results found in the present study. However, mean concentrations of progesterone and estrogen in Sahiwal cows (Group A) at the start of sov. treatment (Stage V) and on the day of embryo collection (Stage VIII) were not found to be correlated with the subsequent rate of ovulation. Mutha Rao *et al.* (2005) reported similar findings in purebred Ongole cows.

Further, they have also reported a negative correlation between estrogen concentration at the start of sov treatment and ovulatory response.

Surmising the above observations, it was concluded that better superovulation response and embryo recovery in pure Sahiwal heifers were related with the better profile of circulating ovarian steroids.

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