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Estrogen and progesterone concentration during super ovulatory treatment and embryo collection in crossbred cows

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Abstract
Sahiwal x Jersey crossbred cows were super ovulated with 250 (G1) and 200 mg (GII) pFSH from d10 to 13 of PGF2α synchronized estrous cycle in divided doses and luteolysis on 72h after initiation of super ovulatory treatment. The concentrations of progesterone at the time of embryo collection were significantly ($p<0.05$) different between Group I and II. But the difference in the progesterone concentrations at remaining period of study was not significant ($p>0.05$) between the groups. Concentrations of progesterone during synchronization was negatively and significantly ($p<0.05$) correlated with recovered ova, anovulation's, total follicles and total ovulations. Whereas the progesterone concentrations at superovulation PG, super estrus and embryo collection days were positively and significantly ($p<0.05$) correlated with anovulation's. Similarly, there was positive and significant ($p<0.05$) correlation between the total number of follicles and number of ovulations and between total number of anovulation's and number of embryos recovered in group I. While the progesterone concentrations during synchronization was negatively and significantly ($p<0.05$) correlated with transferable embryos, total follicles, anovulation's, recovered ova and embryo collection except between the concentration of progesterone at embryo collection with transferable embryos where the correlation was positive and significant ($p<0.05$). Similarly, there was positive and significant ($p<0.05$) correlation between total number of anovulation's and number of embryos recovered in group II. The concentrations of estrogen at the time of embryo collection were significantly ($p<0.05$) different between Group I and II. But the difference in the estrogen concentrations at remaining period of study were not significant ($p>0.05$) between the groups. In the present study concentrations of estrogen during synchronization was negatively and significantly ($p<0.05$) correlated with total follicles, recovered ova and anovulation's, while the same was positively and significantly ($p<0.05$) correlated with recovered ova. Similarly positive and significant ($p<0.05$) correlation was observed between total number of follicles and ovulations and between total number of anovulation's and embryos recovered in group I. While during synchronization the estrogen concentration was positively and significantly ($p<0.05$) correlated with total ovulations, recovered embryos, total follicles and also at super estrus with recovered embryos and transferable embryos in group II. Similarly, there was positive and significant ($p<0.05$) correlation between total number of anovulation's and number of embryos recovered in group II.

Keywords: progesterone, ovulatory, embryo collection, crossbred

Introduction
India, the home of rich livestock biodiversity, possesses more than 200 million cattle and about 90 million buffaloes. Spread over an area of 329 million hectares there are more than 30 recognized cattle breeds contributing about 55% of total milk production in country besides providing draught power of 40 million Horse power worth Rs 6000 crore per year (Ramaswamy, 2006). In Andhra Pradesh, Chittoor district is known for crossbreeding which made the district as one of the leading cow milk producing districts in the country. However, indiscriminate crossbreeding resulted in more exotic inheritance (>75%) brought many managemental and disease problems resulting in low productivity, conception rates, feeding problems etc. In order to stabilize the exotic blood level, Sahiwal x Jersey crossbred cows have been introduced keeping in view the high milk producing efficiency of indigenous Sahiwal breed of cattle. In order to uphold the population of Sahiwal x Jersey crossbred cows one of the best methods is Multiple Ovulation and Embryo Transfer Technology has been attempted from among biotechnological tools. Superovulation is a key element of this technology which is being used in conjunction with embryo transfer.

Materials and Methods

The present study was conducted in dairy farm maintained by Livestock Production and Management department in the College of Veterinary Science, Tirupati. Twelve lactating / dry, multiparous, clinically normal, cyclic Sahiwal x Jersey crossbred cows aged 6-14 years and weighing between 350 and 450 kg were used as donors. Based on milk yield daily ration of each animal consisted of 3-5 Kg high protein feed containing 20% DCP, 70% TDN and 20-30 kg chopped fodder and paddy straw ad-libitum. Water was available continuously. In general, animals were maintained under hygienic and optimum management conditions in loose housing system with a large, open paddock for free movement. They were sent for grazing daily between 09.00 and 15.00 hrs. To the adjoining pasture lands. Calves were allowed to suckle the dams and hand milking was practiced twice a day. After balancing for age, weight, parity and stage of lactation the donors were randomly divided into 2 groups. Animals in group 1 were given 250 mg of FSH and in group 2 were given 200 mg of FSH.

Protocol of Superovulation treatment

Twelve Sahiwal x Jersey crossbred cows without any reproductive abnormalities were selected as embryo donors and divided in to two groups (six in each group) based on the total dose of FSH. All the selected donor cows were synchronized with double dose of prostaglandins (25mg) 11 days apart. In group-I, superovulation was initiated on the day 10 of estrous cycle (estrous-0) by administering 250 mg of FSH intramuscularly, twice daily doses (50/50, 35/35, 25/25 and 15/15) at 12h interval for 4 consecutive days. In group II, superovulation was initiated on day 10 of estrous cycle (estrous-0) by administering 200 mg of FSH intramuscularly, twice daily doses (40/40, 30/30, 20/20 and 10/10).

Results

Estimation of Hormones

After initiation of super ovulation of Sahiwal x Jersey crossbred cows, blood samples were collected in to Eppendorf tubes with the help of disposable syringe to estimate the progesterone concentrations. Samples were collected before administration of drug into the animal. In this manner samples were collected from the day of initiation of superovulation with FSH administration i.e., day 13 both in the morning and evening. Blood samples were also collected from the donor on the day of super estrous and on the day of embryo collection. The serum separated from blood samples were transferred into sterile Eppendorf tube, labelled and stored in deep- freeze (-20^{0}C) until hormonal assay was carried out.

Progesterone

The progesterone concentrations in the Sahiwal x Jersey crossbred donor cow super ovulated with two different doses (Group I: 250mg and Group II: 200 mg) of pFSH were presented in tables 1 and 2.

In Group I the overall mean progesterone concentrations recorded on different days of synchronization prior to superovulation and during and after superovulation i.e., at 1st $PGF_{2\alpha}$, 2nd $PGF_{2\alpha}$, synchronized estrus, initiation of superovulation, $PGF_{2\alpha}$ administration on 3rd day of superovulation, super estrus and embryo collection were 3.37±0.15, 2.90±0.10, 1.03±0.10, 3.62±0.21, 3.27±0.23, 0.90±0.16 and 3.72±0.26 ng/ml, respectively (Table. 1).

In Group II the overall mean progesterone concentrations recorded on different days of synchronization prior to superovulation and during and after superovulation i.e., at1st $PGF_{2\alpha}$, 2nd $PGF_{2\alpha}$, synchronized estrus, initiation of superovulation, $PGF_{2\alpha}$ administration on 3rd day of superovulation, super estrus and embryo collection were 3.22±0.14, 2.77±0.18, 1.06±0.08, 3.87±0.09, 3.22±0.23, 1.05±0.15 and 4.78±0.21 ng/ml, respectively (Table. 2).

The concentrations of progesterone at the time of embryo collection were significantly (p<0.05) different between Group I and II. But the difference in the progesterone concentrations between the groups at 1ST $PGF_{2\alpha}$, 2nd $PGF_{2\alpha}$ administration and synchronized estrus and after administration of $PGF_{2\alpha}$, at the time initiation of superovulation, $PGF_{2\alpha}$ administration, super estrus and embryo collection were not significant (p>0.05).

Table 1: Progesterone concentration in the serum of Sahiwal x Jersey crossbred cows super ovulated with 250 mg pFSH.

Group I (250 mg of pFSH)							
Cow No	**1st PG**	**2nd PG**	**Estrus PG**	**Initiation of Super-ovulation**	**Super-ovulation PG**	**Super estrus**	**Embryo collection**
1	3.16	2.51	0.74	3.98	4.19	1.63	4.88
2	3.95	2.77	1.38	3.49	3.65	0.9	3.43
3	3.65	2.84	1.21	3.76	2.98	0.48	3.08
4	3.27	3.14	0.72	3.44	3.01	0.96	4.02
5	2.95	3.17	0.96	3.59	3.22	0.67	3.23
6	3.26	2.98	1.14	3.46	2.59	0.76	3.69
Overall Mean	3.37 ±0.15	2.90 ±0.10	1.03 ±0.10	3.62 ±0.21	3.27 ±0.23	0.90 ±0.16	3.72 ±0.26

Table 2: Progesterone concentration in the serum of Sahiwal x Jersey crossbred cows super ovulated with 200 mg pFSH.

Group II (200 mg of pFSH)							
Cow No	**1st PG**	**2nd PG**	**Estrus PG**	**Initiation of Super-ovulation**	**Super-ovulation PG**	**Super estrus**	**Embryo collection**
1	3.18	2.94	0.82	4.16	3.71	1.27	5.71
2	3.27	2.13	1.24	3.64	3.17	1.32	4.26
3	3.41	2.84	1.29	4.12	2.5	0.9	4.36
4	2.82	3.18	0.8	3.7	3.26	1.38	4.85
5	2.89	3.23	1.04	3.85	2.74	1.09	4.52
6	3.74	2.34	1.22	3.72	3.96	0.38	4.99
Overall Mean	3.22 ±0.14	2.77 ±0.18	1.06 ±0.08	3.87 ±0.09	3.22 ±0.23	1.05 ±0.15	4.78 ±0.21

Estrogen

The estrogen concentrations in the Sahiwal x Jersey crossbred donor cow super-ovulated with two different doses (Group I: 250mg and Group II: 200 mg) of pFSH were presented in tables 3 and 4.

In Group I the overall mean estrogen concentrations on the day of synchronization prior to superovulation i.e., 1st $PGF_{2\alpha}$, 2nd $PGF_{2\alpha}$ and synchronized estrus and at initiation of superovulation, at the time of $PGF_{2\alpha}$ administration, at super estrus and at embryo collection were 14.19±1.08, 20.25±1.17, 24.19±1.02, 21.81±0.88, 32.46±2.46, 38.27±1.02 and 23.01±0.10 pg/ml, respectively (Table. 3).

In Group II the overall mean estrogen concentrations on the day of synchronization prior to superovulation i.e., 1st $PGF_{2\alpha}$, 2nd $PGF_{2\alpha}$ and synchronized estrus and at initiation of superovulation, at the time of $PGF_{2\alpha}$ administration, at super estrus and at embryo collection were 14.14±0.91, 20.20±0.99, 24.14±0.86, 21.76±0.74, 32.41±2.08, 38.22±0.86 and 20.05±1.03 pg/ml, respectively (Table. 4).

The concentrations of estrogen at the time of embryo collection were significantly (p <0.05) different between Group I and II. But the difference in the estrogen concentrations between the groups at 1st $PGF_{2\alpha}$, 2nd $PGF_{2\alpha}$ administration and synchronized estrus and after administration of $PGF_{2\alpha}$, at the time initiation of superovulation, $PGF_{2\alpha}$ administration, super estrus and embryo collection were not significant (p>0.05).

Table 3: Estrogen concentration in the serum of Sahiwal x Jersey crossbred cows super-ovulated with pFSH.

Group I (250 mg of pFSH)							
Cow No	**1st PG**	**2nd PG**	**Estrus PG**	**Initiation of Super-ovulation**	**Super-ovulation PG**	**Super estrus**	**Embryo collection**
1	10.58	18.38	24.99	18.37	35.86	38.64	24.98
2	15.30	20.03	26.99	22.37	40.76	34.74	23.48
3	18.39	22.03	26.46	21.87	25.06	36.64	19.58
4	12.68	25.03	24.26	23.87	26.56	39.54	22.68
5	13.51	19.03	21.46	23.97	35.46	42.04	24.88
6	14.70	17.03	20.99	20.37	31.06	38.04	22.98
Overall Mean	14.19 ±1.08	20.25 ±1.17	24.19 ±1.02	21.81 ±0.88	32.46 ±2.46	38.27 ±1.02	23.01 ±0.10

Table 4: Estrogen concentration in the serum of Sahiwal x Jersey crossbred cows super-ovulated with pFSH.

Group II (200 mg of pFSH)							
Cow No	**1st PG**	**2nd PG**	**Estrus PG**	**Initiation of Super-ovulation**	**Super-ovulation PG**	**Super estrus**	**Embryo collection**
1	10.53	18.33	24.94	18.32	35.81	38.59	16.93
2	15.25	19.98	26.94	22.32	40.71	34.69	17.43
3	18.34	21.98	26.41	21.82	25.01	36.59	19.53
4	12.63	24.98	24.21	23.82	26.51	39.49	22.63
5	13.46	18.98	21.41	22.32	40.71	34.69	20.83
6	15.25	19.98	20.94	21.82	25.01	36.59	22.93
Overall Mean	14.14 ±0.91	20.20 ±0.99	24.14 ±0.86	21.76 ±0.74	32.41 ±2.08	38.22. ±0.86	20.05 ±1.03

Discussion

Progesterone

The serum progesterone concentration in group I and II at day 0, day 11 of prostaglandin injection and PG heat were 3.42±0.20, 3.02±0.19 and 1.03±0.11: 3.01±0.14, 2.38±0.19 and 1.11±0.11, respectively and the same during initiation of superovulation, prostaglandin injection, super heat and day of embryo collection were 4.09±0.16, 3.27±0.23, 1.32±0.16 & 4.78±0.22: 3.75±0.12, 3.22±0.23, 0.90±0.16 & 3.72±0.27 in group I and II, respectively. Though there was no significant difference in mean serum progesterone concentration on different days of superovulation except on the day embryo collection an insignificant fall in progesterone levels on the day of heat is similar to the findings of Prakash *et al*. (1995) [7], Dugwekar *et al*. (2003) [3] and Siddiqui *et al*. (2011) [13] who have reported higher progesterone concentrations on the day of $PGF_{2\alpha}$ administration and same was decreased to lower level at the time of heat. At the same time the significant increase in concentrations of progesterone from super heat to embryo collection is also akin to the reports of Siddiqui *et al*. (2011) [13], Rao *et al*. (2005) [8], Rawat *et al*. (2007) [9] where the concentrations of progesterone were decreased from initiation of super-ovulatory treatment to day of collection with a sudden rise in progesterone concentration from super heat to day of embryo collection. These higher plasma P4 concentrations reflect not only differences in ovulation rate as well as the competence of the corpus luteum, which is potentialized by gonadotrophin stimulation Silva *et al*. (2002) [14].

The increase in the progesterone concentration is higher on the day of embryo collection but the values are slightly and significantly lesser in group I than group II though the follicular development is higher in group I and it may be attributed to the presence of more anovulated follicles in group I. These findings have indicated that there is a need to tackle the ovulatory problems to further boost the embryo recovery though the recovery percentage is higher in group I.

In the present study, group I donor cow's concentrations of progesterone at 1st PG with recovered ova, 2nd PG with anovulation and estrus PG with total follicles, total ovulations and recovered ova were negatively and significantly correlated. While in group II the progesterone concentrations at estrus PG with transferable embryos; at superovulation PG with total follicles, anovulation and recovered ova and at embryo collection with recovered ova were negatively and significantly correlated except between the concentration of progesterone at embryo collection with transferable embryos in group I and between total number of anovulation and number of embryos recovered in group II the correlation was positive and significant (Table.5) Saumande and Batra (1985) [11] also found negative and significant correlation between the progesterone concentration and viable embryos recovered, while, Yadav *et al*. (1986) [18] observed positive and significant correlation. Whereas, the progesterone concentrations at superovulation PG, super estrus and embryo collection day were positively and significantly correlated with anovulation.

Similar to the present findings, Wubishet *et al*. (1986) [17] also noticed positive and high correlation of plasma progesterone concentration with the number of corpora lutea palpated and with the number of ova and/or embryos recovered. Silva *et al*. (2002) [14] also observed significant correlation between plasma P4 concentrations at 5 days after the super estrus and at embryo recovery. There was positive and significant correlation between the total number of follicles and number of ovulations and between total number of anovulation and number of embryos recovered in group I. However, Samunde, (1980) [12], Greeve *et al*. (1983) [5] and Bevers and Dieleman, (1987) [1], Tamboura *et al*. (1985) [15] did not demonstrate the relationship of progesterone concentrations at the initiation of superovulation with ovulation. Whereas, Testart *et al*. (1977) [16] and Suguno *et al*. (2001) have related the progesterone concentrations with the numbers of corpora lutea and also to the numbers of anovulated follicles.

Estrogen

The serum estrogen concentration in group I at day 0, day 11 of prostaglandin injection and PG heat were 14.19±1.08, 20.25±1.17 and 24.19±1.02, respectively and in group II were 14.14±0.91, 20.20±0.99 and 24.14±0.86, respectively with no significant difference between the groups but with an increase in estrogen concentration from day 0 to induced heat which is similar to results obtained by Siddiqui *et al*. (2011) [13] who observed that there was an increase in concentration of estrogen from day 0 to day of induced heat (12.67±5.17 to 20.17±8.23).

Serum estrogen concentrations in group I during initiation of superovulation, prostaglandin injection, super heat and day of embryo collection were 21.81±0.88, 32.46±2.46, 38.27±1.02 and 22.10±1.22 and in group II were 21.76±0.88, 32.41±2.08,

38.22±0.86 and 22.05±1.03, respectively with a significant difference between two groups in the estrogen concentration during super heat and day of collection but there was no significant difference between two groups during initiation of superovulation and prostaglandin injection. Similar to this study, Siddiqui *et al*. (2011) [13] observed more or less equal mean estrogen concentrations at 1st PGF, 2nd PGF, estrous, 1st day of FSH treatment, 3rd day of superovulation, super heat and day of collection 12.67±5.17, 19.00±7.76, 20.17±8.23, 22.67±9.25, 30.17±12.32, 39.33±16.06 and 28.50±11.64 pg/ml, respectively.

On contrary to the present findings Saumande and Batra, (1985) [11] and Rao *et al*. (2005) [8] recorded higher values (29.70±8.80 and 41.90±30.10 pg/ml on 1st day of FSH treatment and day of PGF2α injection administration in Friesian cows, respectively and 37.88±4.08, 88.28±3.71, 149.48±35.40 and 35.98±9.74 pg/ml on day of initiation of FSH treatment, 3rd day of treatment, super estrus and embryo collection, respectively in Ongole cyclic cows). While, at before FSH treatment and at super heat Chauhan *et al*. (1994) [2] and Gradela *et al*. (1996) [4] recorded lower values of estrogen (5.57±2.23 and 30.47±8.28 pg/ml with 44 mg FSH, respectively and 6.93±3.02 and 70.65±42.03 pg/ml 35 mg FSH, respectively in Jersey x Kankrej crossbred cows and 11.34±1.22 and 37.45±3.50 pg/ml in Nellore cows, respectively). Sarvaiya *et al*. (2003) [10] also reported lower mean estrogen concentration at 1st day of FSH treatment and on the day of embryo collection (7.72±2.50, and 8.19±2.48 pg/ml, respectively) but with higher values at super heat (85.03±36.94) in Jersey cows and reports of Maurya and Mathur (2010) [6] also are higher (148.0±54.44 pg/ml) in Frieswal cows.

The significant increase in the concentrations of estrogen from day of initiation of superovulation to day of super heat in this study might be due to folliculogenesis and decrease in the concentration noticed on day of collection may be attributed to ovulations and formation of corpora lutea (Sarvaiya *et al*., 2003 [10], Rao *et al*., 2005 [8] and Siddiqui *et al*., 2011) [13] who have also observed similar increase from initiation of super-ovulatory treatment to day of super heat with a sudden fall from super heat to day of embryo collection.

In the present study, concentrations of estrogen at 1st PG with total follicles and recovered ova, estrus PG with recovered ova and at initiation of superovulation with anovulation were negatively and significantly correlated, while the concentration of progesterone at 2nd PG with recovered embryos and at super estrus with recovered ova were positively and significantly correlated. Similarly, there was positive and significant correlation between the total number of follicles and number of ovulations and between total number of anovulation and number of embryos recovered in group I.

While the estrogen concentrations at 2nd PG with total ovulations and recovered embryos; at estrus PG with total follicles and at super estrus with recovered embryos and transferable embryos were positively and significantly correlated except between the concentration of estrogen at super estrus with recovered ova in group II. Similarly, there was positive and significant correlation between total number of anovulation and number of embryos recovered in group II (Table.5)

Table 5: Mean concentrations of Progesterone and Estrogen in the serum of Sahiwal x Jersey crossbred cows super-ovulated with 250 and 200 mg pFSH.

Time of serum collection	Progesterone		Estrogen	
	250 mg FSH	200 mg FSH	250 mg FSH	200 mg FSH
1st PG	3.37±0.15 [a]	3.22±0.14 [a]	14.19±1.08 [a]	14.14±0.91 [a]
2nd PG	2.90±0.10 [a]	2.77±0.18 [a]	20.25±1.17 [a]	20.20±0.99 [a]
Estrus PG	1.03±0.10 [a]	1.06±0.08 [a]	24.19±1.02 [a]	24.14±0.86 [a]
Initiation of Superovulation	3.62±0.21 [a]	3.87±0.09 [a]	21.81±0.88 [a]	21.76±0.74 [a]
Superovulation PG	3.27±0.23 [a]	3.22±0.23 [a]	32.46±2.46 [a]	32.41±2.08 [a]
Super estrus	0.90±0.16 [a]	1.05±0.15 [a]	38.27±1.02 [a]	38.22±0.86 [a]
Embryo collection	3.72±0.26[a]	4.78±0.21[b]	23.01±0.10 [a]	20.05±1.03[b]

Means bearing different superscripts within a row differ significantly ($p<0.05$)

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