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Changes in Biochemical Profile of Superovulated Sahiwal Cows through Hormonal Manipulation at Mid Luteal Phase of Estrous Cycle

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ABSTRACT

In the present study, insulin and insulin-like growth factor-I (IGF-I) administered during mid-luteal phase of the estrous cycle and effect on biochemical profiles of Sahiwal donor cows was noted. Altogether eighteen cows (n=18) were selected and divided into 3 groups; control (n=6, untreated), T-I (n=6, Insulin-treated) and T-II (n=6, IGF-I treated). Superovulatory treatment was started on day 9th of the estrous cycle. With the 6th dose of FSH, prostaglandin was injected to induce superovulatory estrus. The superovulated cows were bred and superovulatory response of each animal was recorded. The collections of embryos were done non-surgically on the 7th day of superovulatory estrus. About 15 ml blood without anticoagulant was collected on days 5,7,9,11,13,15,17,19 and 21st or day of embryo recovery where day 0 of estrous cycle was taken as the day of estrus. Serum was separated, centrifuged at 3000 rpm for 15 minutes and transferred into sterilized serum vials. All samples were stored at -20 ^{o}C till analysis. Serum glucose, cholesterol, total protein, urea, and creatinine were estimated by Span Diagnostic Kits. The concentration of serum glucose increased significantly in insulin-treated and IGF-1 treated Sahiwal donor cows. It may be concluded that exogenous insulin and IGF-1 administration during mid-luteal phase may be helpful in follicular and embryonic development by increasing the level of serum glucose. The concentration of serum cholesterol, total protein, urea, and creatinine remains unaffected.

Keywords: Biochemical profile, Sahiwal, Superovulation etc.

Embryo transfer technology (ETT) in bovines is being widely used for the improvement of breeding stock around the world. Through this technique, elite bulls are produced, and their semen used for the improvement of livestock. In ETT, the genetic contribution of both the male and female are utilized simultaneously to create progeny, which leads to faster genetic improvement. In India, the demand to multiply the genetic material of indigenous *Bos indicus* females has increased. Sahiwal cow is one of our indigenous cow breed which is more suitable to our climatic conditions. But the poor superovulatory response, inappropriate storage and higher embryo mortality which lead to reduce conception rate in the recipient. These problems limit the field application of ETT in large scale.

Insulin application to improve the reproduction is a recent upgradation. The plasma level of insulin and IGF-I was positively correlated with glucose in postpartum Sanga cows. Higher total cholesterol concentrations in the blood may stimulate ovarian steroids' production, such as estradiol in a follicular phase because cholesterol is a precursor of steroid hormones. An elevated estradiol level may promote the development of medium-sized follicles during superovulatory treatment in cows (Takahashi *et al.,* 2013). The total protein concentration in superovulated cow was significantly higher in a good responder as compared to non-responders (Prasad, 2000; Agrawal and Maurya, 2002). In an *in-vitro* study a less number of blastocyst formed on day 8 when exposed to 21 mg/dl urea in culture media (Ocon and Hansen, 2003). It is also found that a less production of transferable embryos was associated with higher activity of creatinine kinase in dairy cattle (Chorfi *et al.*, 2007). Hence, our present work

has been designed to study the effect of insulin and insulin-like growth factor-I treatment on alteration in biochemical profile in embryo donor Sahiwal cows.

MATERIALS AND METHODS

In this experiment, insulin, and insulin-like growth factor-I (IGF-I) administered during mid-luteal phase of estrous cycle and effect were noted on the biochemical profile of Sahiwal donor cows. An embryo donor, Sahiwal cows (n=18) was selected and divided into 3 groups; control (n=6, untreated), T-I (n=6, Insulin-treated) and T-II (n=6, IGF-I treated). Insulin was given @ 0.25 IU / kg body weight and IGF-I @ 10 µg total dose per day by S/C, on 5, 6, 7, and 8th days of estrous cycle to the treatment group I and treatment group II respectively. FSH injection was started on day 9th after the onset of standing estrus (Folltropin-V, Vetoquinol, Canada, 30 mg twice daily at 12 hours intervals for 4 days, total dose 240 mg in 8 divided doses). With the 6th dose of FSH, prostaglandin was injected to induce superovulatory estrus. The superovulated cows were bred 2 times at 12 hours interval through artificial insemination using good quality frozen semen and superovulatory response of each animal was recorded. The embryos were collected non-surgically on the 7th day of superovulatory estrus. About 15 ml blood without anticoagulant was collected on days 5,7,9,11,13,15,17,19 and 21st or day of embryo recovery where day 0 of estrous cycle was taken as day of estrus. The day 9, 11, 13 and 21 corresponds to the day of initiation of FSH treatment, the day of PGF2α injection, the day of superovulatory estrus and day of embryo recovery. The sterilized test tube kept at room temperature as a slant for 1 hour for separation of blood serum. Serum was separated, centrifuged at 3000 rpm for 15 minutes and transferred into sterilized serum vials. All samples were stored at -20^0 C till analysis. Serum glucose, cholesterol, total protein, urea, and creatinine were estimated using Span Diagnostic Kits. The data were analyzed statistically using Analysis of Variance (ANOVA) (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Glucose

The mean serum glucose concentration at different phases of the cycle in Control, T-I (Insulin-treated) and T-II (IGF-I treated) groups have been presented in Table 1. Its concentration differed significantly (P<0.05) between control and T-II groups on the 5th day but non-significant (P>0.05) between groups on the 7th day and within the groups on both 5th and 7th days. Further, its concentration on 9th day i.e., the day of initiation of FSH treatment increased in all the groups, though the differences were non-significant (P>0.05). Again, the glucose concentration increased on the 11th day in all groups and slightly decreased on the day of superovulatory estrus i.e. 13th day in T-I and T-II groups, but the decrease was non-significant (P>0.05). In the control group decrease in the level of glucose was recorded on 15th day which was non-significant. Its concentration then increased in control from 15th day to 21st day and in T-I and T-II from 13th day to 21st day. There were significant variations found in T-I and T-II from control on 15th and 17th day between groups and non-significant within groups. The value of glucose was recorded higher on the 21st day in each group (P>0.05).

Table 1: Serum Glucose concentration (mg/dl) of different groups on different days of estrous cycle/ superovulatory treatment of Sahiwal donors

Days of estrous cycle	Groups		
	Control (n=6)	T-I (n=6, Insulin (@ 0.25 IU/kg b. wt. S/C)	T-II (n=6, IGF-I(@ 10 µg total dose per day S/C)
Day 5	54.27±5.2^{A}	62.32±2.14^{aBA}	67.81±1.34^{B}
Day 7	56.17±7.78	67.39±2.44^{ba}	71.56±1.11
Day 9	61.73±6.57	70.27±3.04^{ba}	73.84±4.23
Day 11	65.72±2.01	69.3±1.09^{ba}	75.00±4.74
Day 13	60.36±4.05	66.21±2.22^{ba}	73.11±6.27
Day 15	52.30±7.8^{A}	73.5±3.96^{baB}	74.33±3.6^{B}
Day 17	56.48±5.71^{A}	75.51±3.6^{baB}	78.2±3.72^{B}
Day 19	64.20±9.85	77.49±5.37^{b}	79.64±3.8
Day 21 or DER	69.89±4.93	78.1±3.71^{b}	81.51±3.36

Means bearing different superscripts differed significantly (P<0.05) within the groups (a, b, c, d) and between the groups (A, B).

In the present experiment, the levels of serum glucose in Sahiwal cows are similar with the previous findings of Sreedhar *et al.* (2013) in Sahiwal cows and Selvaraju *et al.* (2003) in goats. But, our recorded value was higher than reported by Rasolomboahanginjatovo *et al.* (2013). The

concentration of glucose on day 5 of estrous cycle differed significantly within groups in control and T-II groups and non-significantly on day 5 and 7 between groups. The concentration of glucose increased non-significantly on day 9th and 11th of the estrous cycle in accordance with the findings of Barman *et al.* (2013). The level of glucose can plan changes in the insulin and IGF-1 level in blood (Lucy, 2008). According to Kawashima *et al.* (2012), the change of catabolic state to the anabolic state is a key regulator of the reproduction. Its concentration slightly decreased on day 13th or day of superovulatory estrus in T-I and T-II groups similar to the reports of Selvaraju *et al.* (2003), who reported that plasma glucose concentration at estrus in goats. Our findings are also in agreement with the reports of Damptey *et al.* (2013) i.e. the plasma level of insulin and IGF-I was positively correlated with glucose in post-partum Sanga cows.

Cholesterol

The mean serum cholesterol concentration at different phases of cycle in the Control, T-I (Insulin-treated) and T-II (IGF-I treated) groups have been presented in Table 2. The level of serum cholesterol differed non-significantly (P>0.05) on the 5th and 7th day within and between groups. Its concentration on 9th day i.e. day of initiation of FSH treatment increased in all the groups, though the differences were non-significant (P>0.05). Its concentration further increased on the 11th day in all groups and reached a high level on the day of superovulatory estrus i.e. 13th day non-significantly (P>0.05). Then its concentration decreased in all groups from 13th day to 21st day. The lower values of serum cholesterol were recorded on the 21st day in each group and differences were non-significant (P>0.05) between groups. The lowest value of cholesterol was recorded in T-I group than control and T-II groups.

The levels of cholesterol are in agreement with the previous findings of Prasad (1990). However, our values are higher than those reported by Rasolomboahanginjatovo *et al.* (2013) and lower than that reported by Barman *et al.* (2013). The cholesterol concentration increased non-significantly (P>0.05) on day 9th in treated and control groups are in agreement with Kumar (2002). Its concentration increased on day 11th (the day of PGF2α injection) which was similar to the findings of Kharche *et al.* (1989) and Kumar (2002). Its concentration further increased on day 13th (day of superovulatory estrus) in all the groups similarly reported by Barman *et al.* (2013) in Mithun cows. This might be due to increased follicular activity and heavy demand from the positive synthesis of steroid hormones (Kavani *et al.,* 2005). Increased serum total cholesterol concentrations may stimulate the ovarian production of steroids, such as estradiol during the follicular phase because cholesterol is a precursor of steroid sex hormones. The higher estradiol level may promote the medium-sized follicles development during superovulatory treatment (Takahashi *et al.,* 2013). Following superovulatory estrus, the concentration of cholesterol decreased non-significantly in all groups to the day of embryo recovery similar to Kumar (2002).

Table 2: Serum cholesterol concentration (mg/dl) of different groups on different days of estrous cycle/ superovulatory treatment of Sahiwal donors

Days of estrous cycle	Groups		
	Control (n=6)	T-I (n=6, Insulin (@ 0.25 IU/kg b. wt. S/C)	T-II (n=6, IGF-I (@ 10 µg total dose per day S/C)
Day 5	168.98±13.15	147.5±11.51	144.85±8.02
Day 7	174.77±10.64	152.29±13.95	152.17±10.65
Day 9	180.56±20.51	155.99±12.54	156.21±9.91
Day 11	182.42±11.66	160.74±12.14	158.94±11.52
Day 13	184.16±11.78	162.31±15.62	164.05±8.01
Day 15	175.29±9.10	160.97±11.33	156.21±7.83
Day 17	172.22±15.76	156.86±20.41	156.79±4.37
Day 19	164.36±11.19	142.27±15.62	154.2±6.95
Day 21 or DER	157.87±11.37	140.53±13.24	147.51±11.40

Total protein

The mean serum total protein concentration at different phases of the cycle in Control, T-I (Insulin-treated) and T-II (IGF-I treated) groups have been presented in Table 3. The mean concentration of serum total protein on 5th and 7th days differed non-significantly (P>0.05). The concentration of total protein on 9th day i.e. day of initiation of FSH treatment increased non-significant (P>0.05) in all the groups. Its concentration further increased on the 11th day in all groups and reached a high level on the day of superovulatory estrus i.e. 13th day of estrous cycle non-significantly (P>0.05). The mean total protein concentration then decreased in all groups from

13th day to 21st day. The lower value of total protein was recorded on the 21st day in each group and differences were non-significant (P>0.05) between groups. The lowest value of total protein was recorded in control than T-I and T-II groups.

Table 3: Serum total protein concentration (g/dl) of different groups on different days of estrous cycle/ superovulatory treatment of Sahiwal donors

Days of estrous cycle	Groups		
	Control (n=6)	T-I (n=6, Insulin (@ 0.25 IU/kg b. wt. S/C)	T-II (n=6, IGF-I (@ 10 µg total dose per day S/C)
Day 5	4.1±0.53	4.44±0.27	3.98±0.33
Day 7	4.19±0.46	4.71±0.25	4.11±0.27
Day 9	4.27±0.39	4.79±0.28	4.57±0.27
Day 11	4.47±0.40	5.23±0.37	4.71±0.49
Day 13	4.64±0.33	5.42±0.22	4.88±0.19
Day 15	4.47±0.39	5.4±0.20	4.79±0.21
Day 17	4.16±0.35	5.06±0.31	4.58±0.31
Day 19	4.15±0.25	4.82±0.31	4.43±0.16
Day 21 or DER	4.07±0.29	4.77±0.29	4.35±0.20

Total protein levels recorded in the present study were in agreement with Prasad (2000) and Agrawal and Maurya, (2002). Contrary to these findings, higher values were reported by Takahashi *et al.* (2013) and Barman *et al.* (2013). The values of total protein on day 5 and 7th did not differ significantly within and between groups. These findings are in agreement with the reports of Jo (1981). According to him, there was no difference between stages of the estrous cycle in cows. Total protein levels increased on the day of the initiation of gonadotropin treatment and on the day of PGF2α injection non-significantly in all groups and reached peak value at superovulatory estrus. This was in agreement with the findings of Prasad *et al.* (2000) and Barman *et al.* (2013). The higher values of total protein on the first day of superovulatory treatment were recorded by Takahashi *et al.* (2013) in Japanese Black cows in PUFA supplemented diets. The differences in means in all groups were non-significant. After superovulatory estrus, the level of total protein decreased in all groups and reached pretreatment levels in control as reported by Prasad *et al.* (2000) and slightly higher in T-I and T-II groups. However, a total protein concentration in superovulated cow was significantly higher in good responder as compared to non-responders (Prasad, 2000; Agrawal and Maurya, 2002). The plasma level of insulin was positively correlated with total proteins in post-partum beef cows as reported by Damptey *et al.,* 2013.

Urea

The mean serum urea concentration at different phases of the cycle in Control, T-I (Insulin-treated) and T-II (IGF-I treated) groups has been presented in Table 4. The level of serum urea differed non-significantly (P>0.05) on the 5th and 7th day within and between groups. The concentration of urea on the 9th day (the day of initiation of FSH treatment) increased in all the groups though the differences were non-significant (P>0.05). The mean concentration of urea decreased on the 11th day in control and the T-I groups but increased in the T-II group.

Table 4: Serum urea concentration (mg/dl) of different groups on different days of estrous cycle/ superovulatory treatment of Sahiwal donors

Days of estrous cycle	Groups		
	Control (n=6)	T-I (n=6, Insulin (@ 0.25 IU/kg b. wt. S/C)	T-II (n=6, IGF-I (@ 10 µg total dose per day S/C)
Day 5	31.94±1.42	30.1±0.56^{ba}	30.93±0.86
Day 7	33.46±2.56	32.63±0.65^{ba}	33.32±1.47
Day 9	35.42±5.17	35.77±2.33^{b}	34.26±1.4
Day 11	32.74±5.65	33.91±2.91^{ba}	35.37±1.5
Day 13	25.59±4.15	27.23±1.45^{a}	31.59±1.29
Day 15	26.94±2.8	30.06±1.14^{ba}	34.82±2.91
Day 17	34.38±3.79	36.16±1.19^{b}	37.06±1.67
Day 19	36.61±2.39	33.9±1.8^{ba}	34.53±2.35
Day 21 or DER	30.02±2.95	29.66±1.33^{ba}	30.91±1.03

Means bearing different superscripts differed significantly (P<0.05) within the groups (a, b, c, d) and between the groups (A, B).

However, the values were lowest in all groups on the day of superovulatory estrus (the 13th day) and differences were non-significant (P>0.05) between groups and the significant increase on the 13th day from 9th day in T-I group. The mean urea concentration then increased from 13th day to 17th day in T-I and T-II groups and from 13th day to 19th day in control and then decreased on 21st day

in all groups. The lower value of urea was recorded on the 21st day in each group and differences were non-significant (P>0.05) between groups. The lowest value of urea was recorded in T-I group than control and T-II groups.

In the present study, the levels of serum urea on day 7 are in agreement with the previous findings of Velazquez *et al.* (2005) and Rasolomboahanginjatovo *et al.* (2013). Our finding is also in agreement to the reports of Damptey *et al.* (2013), who found that concentration of insulin increased while that of urea decreased during lactation. The serum urea levels on the day of embryo recovery differed non-significantly (P>0.05) between groups are also in agreement with the findings of Rasolomboahanginjatovo *et al.* (2013). In an *in-vitro* study a less number of blastocyst formed on day 8 when exposed to 21 mg/dl urea in culture media (Ocon and Hansen, 2003). They also found in another study that, the embryonic development affected when urea concentration reached 28 mg/dl *in-vitro*. The diets which produce the high amount of blood urea nitrogen (BUN) is responsible for inadequate uterine environment and oocyte quality (Bisinotto *et al.*, 2012; Souza *et al.,* 2014) which leads to poor conception.

Creatinine

The mean serum creatinine concentration at different phases of the cycle in Control, T-I (Insulin-treated) and T-II (IGF-I treated) groups have been presented in Table 5. The levels of serum creatinine in our study ranged from 1.45±0.08 to 1.98±0.11 mg/dl. There was significant (P>0.05) difference between control from T-I and T-II groups on the 5th day and Control and T-I on the 7th day in mean serum creatinine concentration. There was non-significant (P>0.05) difference within each group on 5th and 7th day. The concentration of creatinine on 9th day i.e. day of initiation of FSH treatment decreased in all the groups and difference was significant (P<0.05) between control and T-I group (Table 5). The mean concentration of creatinine decreased on the 11th day in T-I group but increased in control and T-II group and lowest in all groups on the day of superovulatory estrus i.e. 13th day were non-significant (P>0.05). The non-significant (P>0.05) difference also found on day 15th, 17th, 19th and 21st between control, T-I and T-II groups.

The levels of serum creatinine in our study are in agreement with previous reports of Saraiva *et al.* (2014). The serum creatinine levels (mg/dl) are also in concurrence with reports of Sreedhar *et al.* (2013) in Sahiwal heifers, cows, and Jersey × Sahiwal cows, but their upper range was far higher than our reports (1.25±0.07mg/dl to 9.81±0.13 mg/dl, 1.29±0.09 mg/dl to 9.90±0.17 mg/dl and 1.43±0.10 mg/dl to 16.18±0.15 mg/dl, respectively). However lower values of serum creatinine were reported by Abd Ellah *et al.* (2010) in the serum of buffaloes during different stages of the estrus cycle were comparable with the present study. It is also found that a less production of transferable embryos was associated with higher activity of creatinine kinase in dairy cattle (Chorfi *et al.*, 2007) and serum concentration of creatinine is higher in high milk producer cow as compared to lower one (Nozad *et al.*, 2012).

Table 5: Serum creatinine concentration (mg/dl) of different groups on different days of estrous cycle/ superovulatory treatment of Sahiwal donors

Days of estrous cycle	Groups		
	Control (n=6)	T-I (n=6, Insulin (@ 0.25 IU/kg b. wt. S/C)	T-II (n=6, IGF-I (@ 10 µg total dose per day S/C)
Day 5	1.56±0.07^{A}	1.95±0.11^{B}	1.98±0.11^{B}
Day 7	1.51±0.05^{A}	1.92±0.04^{B}	1.74±0.14^{AB}
Day 9	1.45±0.08^{A}	1.84±0.12^{B}	1.65±0.1^{AB}
Day 11	1.54±0.09	1.81±0.11	1.71±0.15
Day 13	1.65±0.11	1.89±0.13	1.84±0.16
Day 15	1.67±0.18	1.88±0.19	1.79±0.17
Day 17	1.52±0.08	1.85±0.05	1.77±0.19
Day 19	1.52±0.05	1.79±0.13	1.74±0.12
Day 21 or DER	1.63±0.13	1.83±0.08	1.85±0.12

Means bearing different superscripts (A, B) differed significantly (P<0.05) between the groups.

In conclusion, the concentration of serum glucose increased significantly in insulin-treated and IGF-1 treated Sahiwal donor cows which may be helpful in follicular and embryonic development by increasing the level of serum glucose. The concentration of serum cholesterol, total protein, urea, and creatinine remains unaffected.

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