



STUDIES ON THE ALTERATIONS OF HORMONAL AND BIOCHEMICAL PARAMETERS AND CONCEPTION RATE IN CYCLIC BLACK BENGAL GOATS (*CAPRA HIRCUS*) BY USING DIFFERENT HORMONAL PROTOCOLS

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ABSTRACT

The present research work was conducted on the sixty cyclic goats (*Capra hircus*) divided into four groups viz. group I (control group), group II (received only intravaginal sponge impregnated with 30 mg of Flurgestone acetate for a period of 14 days), group III and group IV (received additional 125 µg of PGF₂ and 0.3 ml of estradiolvalerate respectively on the day of sponge removal), 15 animals in each group to study the alterations of hormonal and biochemical parameters and conception rate. The serum glucose concentration (mg/dl) did not vary significantly between day 0 and day 14 in all groups but its value decreases significantly during the estrus period in all groups. The serum total protein concentration (g/dl) did not show any marked variation in all groups of animals w.r.t the day of sampling. The serum cholesterol level (mg/dl) varied significantly in all experimental groups w.r.t the days of samplings except the group I. It has been marked that there was an elevation in cholesterol concentration during the estrus periods in all trial groups which was more pronounced in group IV. The progesterone concentration (ng/ml) varied significantly in all experimental groups w.r.t the samplings days except the control groups where variation was only observed in day of estrus and 23rd day of post mating and rest other days. In 23rd day of post mating the progesterone concentration was found to be highest in group III animals (6.70 ± 0.15). The estrogen level (pg/ml) was highest during estrus period which varied significantly from rest other days in all groups. In group IV animals the estrogen level was found to be highest (30.16 ± 0.96 pg/ml) during estrus. The efficacy of pregnancy diagnosis was found to be 87.5 percent for serum progesterone estimation on day 23 after mating and 72.9 per cent for abdominal palpation method. The conception rates for group I, II, III and IV were recorded to be 66, 75, 85 and 86 percent respectively. The group IV animals showed a highest number of kidding percentages followed by group II and group III and least in control animals. From the above experiment it was concluded that the treatment protocol involving intravaginal sponges and estradiolvalerate was more efficient in augmenting the conception rate in does. Therefore it can be used in field condition for uplifting the economic status of marginal poor farmers.

KEY WORDS: Hormonal, biochemical, conception rate, goat.

INTRODUCTION

The goats as a source of livelihood security, poverty alleviation and employment generation for the small and marginal land holders that is why this species is mostly called as “poor man’s cow”. The Department of Animal Husbandry and Veterinary Services of the country has undertaken various flagship programmes to uplift the reproductive potential through the adoption of assisted reproductive technologies thereby indirectly helping millions of poor to earn their daily livelihood. Seasonality in reproduction, limits conception and reproductive performances of goats to three parturitions in two years. Manipulation of reproduction by genetics, physiologic and environmental methods could increase the frequency of breeding per year and the litter size in these species. Artificial control of oestrus synchronisation is valuable techniques that have been successfully employed in a view to improve the reproductive efficiency and kidding intervals.

Different hormonal protocols are used in addition to provision of nutrition, trace minerals, antioxidants, melatonin implants and male effects (Fonseca *et al.*, 2008).

So as to achieve a better and prominent estrus synchronisation, progesterone (or its analogues) is usually used along fixed time insemination either by naturally or artificially with a very good conception rate. Progesterone and its analogue exerts their inhibitory actions on releasing of luteinizing hormone (LH) from anterior pituitary by affecting adversely on preovulatory follicle and their ovulation rate. When this hormone is suddenly withdrawn, there is manifestation of prominent oestrus cycle at a definite period of time (Dogan *et al.*, 2004). Administration of exogenous Prostaglandins or its analogues hastens luteal regression and evokes ovulatory heat. In the present study synthetic progesterone has been used intravaginally alone or in combination with estradiol or PGF₂ on or after the removal of progesterone impregnated intravaginal sponge. After coming to the oestrus in a stipulated period of time, the oestrus behaviours, of goats are observed meticulously and allowing them for mating. Taking into consideration all these, the current research work was designed to study the study serum progesterone and estrogen profile during trial, to estimate the glucose, total proteins and cholesterol

in serum, to assess the efficacy of different methods of pregnancy diagnosis and to study the comparative efficiency of different drug trial in response to conception rate and kidding percentage in does.

MATERIALS AND METHOD

The present research work was conducted on the sixty cyclic goats (*Capra hircus*) divided into four groups viz. group I (control group), group II (received only intravaginal sponge impregnated with 30mg of Flurgestone acetate for a period of 14 days), group III and group IV (received additional 125 µg of PGF₂ and 0.3 ml of estradiolvalerate respectively on the day of sponge removal), 15 animals in each group to study the alterations of hormonal (by ELISA kit method) and biochemical parameters and conception rate. Data generated during the present investigation were subjected to statistical analysis as per standard method described by Snedecor and Cochran (1994).

RESULT

The biochemical value for total serum glucose (mg/dl) of Black bangle doe has been shown in Table 1. There is no significant difference between day 0 and day 14 in all

groups of animals except group II. In group II the total glucose concentration was 53.88± 1.03, 55.74± 1.25, 50.68± 0.86, and 49.74± 0.79 for day 0, day 14, day of estrus and 23 day after mating respectively. In a similar fashion the value for group III was 53.13± 0.19, 56.02± 1.56, 50.6± 0.80 and 48.85± 0.82 respectively. In group IV it was 52.86 ± 0.90, 55.94± 1.02, 48.69± 0.77 and 47± 0.77 where as in control group it was found to be 53.44± 0.77, 53.46± 0.90, 50.74± 0.99, 49.52± 0.55 respectively for day 0, 14, day of estrus and 23rd days of post mating. The value of total serum protein (g/dl) has been depicted in the Table 2. In all the groups, the level of serum total protein doesn't vary significantly both among the group and days of treatments. The mean value of serum total protein were 7.25± 0.13, 7.07± 0.17, 7.14± 0.08 and 7.24± 0.11 for group I in day 0, day 14, day of estrus and 23 day of mating respectively. The corresponding value for group II was 7.1± 0.12, 7.00± 0.11, 7.03± 0.10 and 7.18± 0.10 respectively. The overall mean value was 7.20± 0.13, 7.12± 0.19, 6.94± 0.12 and 7.08± 0.12 for group III on the above said days respectively. In a similar fashion for group IV it was 7.23± 0.16, 7.30± 0.16, 7.06± 0.09 and 6.99± 0.09.

TABLE 1: Serum glucose concentration (mg/dl) in different days of sampling in goats

Treatment groups	Days of sampling				“p” value
	Day “0”	Day “14”	Day of estrus	23 days after mating	
I	53.44 ^a ± 0.77	53.46 ^a ± 0.90	50.74 ^b ± 0.99	49.52 ^b ± 0.55	0.05
II	53.88 ^a ± 1.03	55.74 ^b ± 1.25	50.68 ^c ± 0.86	49.74 ^c ± 0.79	0.05
III	53.13 ^a ± 0.19	56.02 ^a ± 1.56	50.6 ^b ± 0.80	48.85 ^c ± 0.82	0.05
IV	52.86 ^a ± 0.90	55.94 ^a ± 1.02	48.69 ^b ± 0.77	47 ^b ± 0.77	0.05

Values are expressed as Mean± S.E.

Means bearing same superscript in a row don't differ significantly

TABLE 2: Serum total protein concentration (g/dl) in different days of sampling in goats

Treatment groups	Days of sampling				“p” value
	Day “0”	Day “14”	Day of estrus	23 days after mating	
I	7.25 ^a ± 0.13	7.07 ^a ± 0.17	7.14 ^a ± 0.08	7.24 ^a ± 0.11	0.05
II	7.1 ^a ± 0.12	7.00 ^a ± 0.11	7.03 ^a ± 0.10	7.18 ^a ± 0.10	0.05
III	7.20 ^a ± 0.13	7.12 ^a ± 0.19	6.94 ^a ± 0.12	7.08 ^a ± 0.12	0.05
IV	7.23 ^a ± 0.16	7.30 ^a ± 0.16	7.06 ^a ± 0.09	6.99 ^a ± 0.09	0.05

Values are expressed as Mean± S.E.

Means bearing same superscript in a row don't differ significantly.

The value for total serum cholesterol (mg/dl) was analysed at different time of interval starting from day 0 to 23 days after mating. There is no significant difference among the groups during pre-treatment periods. In the group I, the value was 87.46 ± 2.10, 83.69± 2.73, 102.28 ± 1.43 and 91.85 ± 1.55 during day 0, day 14, on the day of estrus and 23 days after mating. In group II there was a marked variation in the serum total cholesterol level among the groups whose values were 87.98 ± 2.02, 77.18 ± 2.45, 104.33± 1.19 and 93.89 ± 1.09 whereas for group III the values are 88.34 ± 2.13, 77.07 ± 2.30, 102.18 ± 3.58 and 96.27± 1.5 and for group IV its value were 87.55 ± 3.79, 77.40± 2.32, 106.95 ± 3.02 and 94.14± 1.65 on day 0, day 14, on the day of estrus and 23 days after the mating respectively.

At day 0, *i.e.* on the day of initiation of treatment, the progesterone concentration were estimated to be 1.30 ± 0.08, 1.22± 0.10, 1.35± 0.09 and 1.52± 0.10 in group I, group II, group III and group IV animals respectively. On day 14 *i.e.* on day of withdrawal of sponge, the corresponding value of group II, group III and group IV were observed to be 4.23± 0.12, 4.34± 0.13 and 4.61± 0.08 respectively. This value was 1.29± 0.07 ng/ml in control group.

Sampling of blood was undertaken on day of estrus in group I, group II, group III and group IV animals revealed a concentration of 0.39± 0.04, 0.53± 0.05, 0.54± 0.04 and 0.49± 0.04 respectively. The corresponding value obtained for all four groups on 23 days post mating resulted in a concentration of 5.51 ± 0.24, 6.41± 0.17, 6.70± 0.15 and 6.36± 0.18 respectively. Test for significant on day 0 and

day 14 of the experiment revealed a significant difference between all the sponge impregnated groups except group I. In all groups animals the significant difference was observed with respect to progesterone concentration between day of estrus and 23 days of post mating. Analysis of variance done between different days of sampling *i.e.* day 0, day 14, day of estrus and 23 days of post mating did not revealed a significant difference ($p < 0.05$) between the sponge impregnated groups except the day of estrus for group IV on the day of estrus.

On day 0, *i.e.* on day of initiation of progesterone therapy, the estrogen concentration were estimated to be 13.23 ± 0.66 , 13.93 ± 0.67 , 14.36 ± 0.76 and 14.01 ± 0.62 in group I, II, III and group IV animals respectively. The corresponding value on day 14 after progesterone administration in corresponding group were 12.95 ± 0.66 , 13.30 ± 0.80 , 13.85 ± 0.62 and 13.88 ± 0.80 respectively. The level of estrogen concentration on day of estrus exhibition in control and experimental group were estimated to be 27.13 ± 0.92 , 27.87 ± 0.83 , 28.17 ± 0.79 and 30.16 ± 0.96 in group I, group II, group III, group IV respectively. On 23rd day of post mating the values were 12.10 ± 0.81 , 13.60 ± 0.55 , 14.43 ± 0.73 and 13.61 ± 0.69 groups I, II, III and group IV respectively. Test of significance between group I, group II, group III and group IV revealed a non-significance difference with respect to day 0 and day 14 value of the hormone. Similar non-significant differences were also marked between all the four groups on day of estrus. However, a significant difference ($p < 0.05$) in all groups were marked when concentration of estrogen was compared between day of estrus and rest other days during the experiments.

In abdominal palpation done in 50 goats showed a positive result of 48 numbers which resulted in a kidding 35 number with an overall per cent of 72.9. The estimation of serum progesterone concentration done on 23 days after service in 50 number of does showed a positive result of 40 goats with 35 number of does parturated and efficiency per cent were calculated to be 87.50. Thus the present experiment indicated that estimation of serum progesterone at 21 to 24 days of post mating/service could give a fair indication of conception followed by the normal method practised in field condition *i.e.* through abdominal palpation where 48 does are diagnosed to be positive.

In group II animals 80 per cent of animals responded to treatment with an overall conception rate of 75 % and the resultant kidding percent 66.00. The corresponding results obtained for group III animals were 93.33, 85.00 and 64.50 respectively. In a similar fashion the values for group IV were 100, 86 and 73 respectively. In group I animals it was estimated that out of 15 animals 9 animals (60.00 %) responded and bred which resulted in 6 (66.60 %) animals conceived and the resulted kidding percent were 55.5.

DISCUSSION

Several factors like season, nutrition, estrus detection, genital infections, breed differences and managerial conditions found to exert their respective hindrance effect on the maximum exploitation of the fertility and fecundity in small ruminants. The development of reproductive technologies such as estrus synchronisation are directed to

solve or minimize the effects of these limiting factors as well as to make possible application of more intensive systems of the production and to facilitate the genetic improvement of the reproductive characteristics of the flock. The application of synchronisation techniques not only enhance the reproductive performances of the does but indirectly it also helps in supply of good quantity of meat and milk for human consumption, encouraging the state's economy. So these techniques accelerate the genetic gains of small ruminants by increase of offspring of selected males and females and the reduction of the generation intervals. Leboeuf (1998) reported both the progesterone and its analogues have an inhibitory effect on the maturation of preovulatory follicles and their ovulations are suppressed.

Glucose is an important factor which modulates reproduction and a lower level can be ascribed for decrease in fertility as well as non cyclicity and reduced fertility. The glucose concentration in control and experimental animals is presented in table no-4.

On day "0", the basal value of serum glucose was around 53 mg /dl which is in close agreement with Mohan *et al.* (2013). However this value is lower than the value suggested by Wazri *et al.* (2010). However, a lower basal value for glucose in Black Bengal has been suggested by Tudu *et al.* (2015). There are no significant differences among the groups irrespective of protocols applied. On the day of sponge removal, there is a high surge of progesterone in the circulation which causes a non-significant increase in the glucose concentration in all the groups except group II which differs significantly. In this study, there is a significant decline in the glucose value during the estrus period which is to around 50 mg per dl in first three groups. But in group IV the value is lowest (48.69 ± 0.77) when compared with rest other groups. The reduction in the glucose value during estrus is in close association with the finding of Purohitand Kohli (1977) who reported that the estrogen hormone had an effect on the carbohydrate metabolism in turn cause increase in production of cholesterol in endocrine gland from acetate. Further, Burr and Sharp (1974) elaborated reduction of glucose in estrus due to the effect of prostaglandins, which stimulates the release of insulin from islets of Langerhans by binding with the plasma membrane receptors of pancreases.

During the early month of pregnancy, there is extension of luteal phase of estrus cycle which is under the influences of high progesterone concentration. In this period there is a slight decline in the glucose concentration which is found to be non-significant between day of estrus and 23rd day of post mating in all the groups except group III. The main reason behind this was due to the fetus that has been found to receive a continuous supply of glucose from the mother. Glucose is the major energy substrate for the developing fetus and was also utilized by the utero-placenta. Similar results also found by Sandabeet *al.* (2004) in Sahel goats.

The serum total protein (g/dl) range from 6.94 ± 0.12 (day of estrus in group III) to 7.25 ± 0.13 (day 0 in group I) and they are statistically non different within the pregnant and non-pregnant group. However numerically there is a decrease trend as the pregnancy proceeds which might be

due preparation of reproductive system during pregnancy. This is in agreement with value of Juma *et al.* (2009) in pregnant and non-pregnant animals. Purohit *et al.* (1999) found a decreasing trend in total serum total protein in non-pregnant (6.58 ± 0.46) as compared to pregnant does (6.05 ± 0.16).

Cholesterol as the precursor of all the hormones has been well established and changes during different phases of reproductive cycle had also been well documented. The biosynthesis of steroid hormone from cholesterol is a highly complex and organised process involving multiple enzyme system. In the present investigation the serum cholesterol levels in different phases of experiments are presented in table no 6.

From the analysis of variance, it was clearly seen that there was a significant difference between all the four phases in the experimental groups where as in control only significance was found only between the day of estrus and the rest other days. As the sponge retained *in situ* for a period of 14 days, there was surge of high progesterone concentration. So, excess progesterone may hinder the further synthesis of lipid in body.

As pregnancy advances, the animals are on high progesterone concentration i.e. extension of luteal phases causing decline in the cholesterol level. In this present study the cholesterol level reduced significantly from the value during estrus, but overall higher than the non-pregnant one. This result was in accordance with the findings of Bharali *et al.* (2003), Sandabe *et al.* (2004) in does. The serum progesterone concentration the day "0" did not vary significantly in all four groups of animals. The present finding is similar to those reported by Kusina *et al.* (2000) and lower than those reported by Sharma *et al.* (2015). After initiation of experimental protocol there is a significant increase in Progesterone in all the experimental groups. In cattle it has been described widely that each wave has identified phases of emergency recruitment selection and dominance. The present significant ($p < 0.05$) increase might be due to insertion of sponge which are resulted in sustained and slow release of Progesterone during the period of its retention in vagina while the intra muscular injection might have resulted in the elimination of the metabolite over the period of time. Higher progesterone value on day sponge removal has also been reported by Panicker *et al.* (2015). In does treated with intravaginal sponges, plasma progesterone concentration increases sharply following insertion of sponge to 6-7 ng/ml within 3-5 days after sponge insertion and these level are maintained thereafter until removal of sponge which was observed by Kusina *et al.* (2000). They opined that the sharp increase in Progesterone concentration which was observed at sponge insertion fall sharply to basal level on removal of sponges which was less than 2 ng/ml within 24hrs. The elevation of Progesterone concentration after insertion of vaginal implant suggest that endogenous Progesterone level were augmented indicating intravaginal delivery of Progesterone via sponges cause effective and maintained elevated Progesterone concentration during the duration of the experiment which is in accordance with the observation made by Kusina *et al.* (2000). On the day of

removal of long term progesterone treatments by Silva *et al.* (2011) found a higher concentration of P_4 (7.74 ± 1.10). On the day of estrus, there was a significant ($p < 0.05$) decrease in level of Progesterone in both the experimental and control group when compared with the initial concentration before initiation of any treatment (Naderipour *et al.*, 2012). The present observation is lower than those reported by Salve *et al.* (2016). There was a significant difference of progesterone value between group IV and rest other experimental groups on day of estrus. This may be due to administration of PRID resulted in significantly higher progesterone concentration compared to PRID without estradiolvalerate. This is probably due to modulating effect of estradiol on the absorption of progesterone from vagina in the PRID group and the difference in physicochemical properties between the natural progesterone contained in PRID.

Leyva-ocarizet *et al.* (1995) studied the estrogen concentration on various days of estrous cycle in goats. They referred a range of 12.5 to 15 pg/ml of estrogen level in the luteal phase of estrous cycle in goats which rose to 22.3 ± 4.2 pg/ml on day 19 and it rises to the peak (28 ± 6 pg/ml) on the day of estrus in cyclic goats. During day 18 of luteal phase there is a preovulatory gonadal surge with decline in progesterone concentration and rise of estradiol due to presence of matured follicles. Abrupt rise of estradiol on day 19 might be due to increase in pituitary drive brought by luteolytic effect of PGF_2 . Goats have similar sex hormone and endocrine function to that of bovine species. Increase in estrogen concentration at time of estrus favours positive feedback mechanism of LH release resulting in ovulation. The significant increase in estrogen level on the day of estrus in all the groups might be due to effect of negative feedback of progesterone due to more efficient and control release of progesterone from vaginal impregnated device. But in group IV an overt signs of estrus had been found due to estradiol treatments, as there is a significant rise in estrogen concentration. As pregnancy advances the estrogen concentration declined to its normal basal value and ranges from 12.10 ± 0.81 to 14.43 ± 0.73 pg/ml. This might be due to the negative feedback mechanism of high circulating progesterone.

The easiest and cheapest method applied in field condition is generally non-returning to estrus. But to assess the exact Pregnancy diagnosis, the serum progesterone profile, and abdominal palpation were conducted on does following estrous synchronization by various hormonal protocols. During pregnancy, progesterone is secreted only from corpus luteum throughout the gestation period for maintenance and continuation of pregnancy in goats and level of progesterone in blood is higher than luteal phase and its concentration could be a diagnostic tool for assessment of pregnancy. In the present findings serum progesterone was most efficient method of pregnancy diagnosis recording 87.5 per cent efficacy which is in agreement with Medan *et al.* (2002), who found an efficacy of 80 per cent in goats. Blood plasma concentrations more than 1 ng/ml between days 18–22 in pluriparous goats indicate pregnancy with an accuracy of the assay is 75–86% in pregnant goats (Gaafar *et al.*, 2005).

The conception rate in goats following the application of hormonal protocols revealed a very good result. As the experiment was conducted during breeding seasons, from control group 66.66 percent does got conceived which was lower than Hassanein in *et al.* (1999) who found a 75% conception. The lower conception rate may be due to any unavoidable genital infections. But the use of progesterone impregnated sponge alone and in combination with PGF₂ and estradiolvalerate yields a better picture of conception rate than control group. It is an established fact that lowers progesterone concentration (less than 1ng/ml) and rise in estrogen during estrus is favourable for higher conception rate as observed in sponge treated groups which might be due to the cumulative effect of the drug used. The conception rate for group II, III and IV were found to be 75 per cent, 85 per cent and 86 percent respectively which is in agreement with Hassanein *et al.* (1999) who reported 87.5 per cent in sponge treated groups. The estradiol treated group shows better conception rate (86%) from rest other groups. This might be due to the excessive folliculogenesis by exogenous estrogens which in turn raises the serum progesterone concentration.

Perusal of conception and kidding per cent implied that kidding per cent was slightly lower compared to actual conception rate established by different methods. However there is a discrepancy between conception rate and actual kidding. This might be due to abortion or erroneous detection of pregnancy. The kidding percentage was highest in group IV followed by group II which is followed by group III with an overall percentage of 68 %. The present kidding per cent in goats is comparable to the reports of Bitaraf *et al.* (2007) 60 percent.

CONCLUSION

It can be suggested that sustain progesterone releasing devices like intravaginal sponges have superior therapeutic efficacy in inducing estrus, conception rate and kidding percentages. So, it can be concluded that sustain progesterone releasing devices like intravaginal sponges along with estradiol treatment was superior therapeutic than the other hormonal protocols for establishment of pregnancy and kidding in small ruminants like goats.

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