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Plasma progesterone concentration during estrus cycle detected through ELIZA kit method in Kamohri goats

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Abstract: Study was conducted at Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam to detect estrus in time in Kamohri goat. In goats measuring plasma P_4 concentration is most important to monitor luteal functions. In present study the forty adult Kamohri goats were selected and utilized in study. The goats were divided at random in to four equal treatment groups. Goats of group A received progesterone on day 0 and PGF2α along with GnRH both on day 10th, goats in group B were given PGF2α on D-0 and repeat dose of PGF2α along with GnRH on D-10th. While animals of group C were offered 50 gm of dry date fruit with 50 gm of Ajwain both daily for 10 days, whereas goats of group D received no any treatment and served as control. The goat does were treated accordingly and closely observed for estrus. The blood samples were collected from each goat to determine the estrus on the basis of plasma progesterone concentration in Kamohri goat. The serum progesterone concentration was measured by Enzyme Linkage Immuno Sorbent Assay (ELISA) kit. The results revealed that the goats of treatment groups A, B and C induced estrus 100%, irrespective of estrus synchronization treatment. However none of the goat in group D shown estrus during trial time period. The mean progesterone concentration level at day of estrus was recorded as 0.46±0.07, 0.34±0.06 and 0.36±0.05 ng/ml in treatment group A, B and C respectively and 1.38±0.04 ng/ml in control group of animal in Kamohri goat does. There were no any significant difference ($P \geq 0.05$) was found between the treated and control group. It was concluded that the, P4 concentration provides rapid and simple method and it could be applied to monitor estrous cycles and P4 analysis can be applied as a useful tool to help to herds' men and veterinarians in the determination of estrus so that more efficient breeding program could be developed and implemented in the flock at farm level.
Keywords: GnRH, PGF2α, Enzyme Linkage Immuno Sorbent Assay (ELISA)

INTRODUCTION

Pakistan is an agricultural country and livestock is an important sub-sector of Agriculture. It plays a vital role in the rural economy and provides sole source of livelihood for millions of landless and poorer in many countries including Pakistan. Pakistan is bestowed with some of the fine breeds of cattle, buffaloes, sheep and goat. The province of Sindh is very rich in goat wealth and breeds of goats. These goat breeds are classified into dairy and meat and breeds and primarily reared for meat and milk purpose, secondary for hairs and skins (FOASTAT, 2010; Macha and Mbaga in 2009 [1]. Hence considered as dual purpose [2-6]. Goat is integral part of the livestock production in the tropics and subtropics. Goat has ability well adaptation to harsh tropical environmental conditions. They play an important role in the economic activity especially in arid and semi-arid region of various countries including Pakistan [7]. They serve as a sustainable economic source of income in assisting to reducing poverty especially among the poorer families of rural areas [8, 9, 6].

The Kamohri in one of the most popular goat breed due to its high milk yield heavy body weight and its beautiful color, hence goat farmer prefer this breed over the other goat breeds. Kamohri goat is considered as non-seasonal breed. In tropical countries like Pakistan the breeding season is extended round the year and peak breeding season during the months of rainy season (August and September). It is important to know the reproductive status of the goat through the knowledge of reproductive physiology which will help in improvement of reproductive efficiency and management of the flock [10, 11]. Estrus is the behavioral manifestation of sexual receptivity in female; characterized by willingness for opposite sex [12, 13]. The estrus signs are due to the action of estradiol and do not occurs during luteal phase of the cycle [14]. In goats the progesterone (P-4) concentration in peripheral circulation periodically

changes throughout the various stages of estrous cycle. The P_4 concentrations in cyclic goats decline to reach minimum concentrations level during estrus, than after it gradually increase and reach at maximum level in the luteal phase. The C. L. produces the hormone P_4 in female animals that prohibits the fmale to show estrus and maintained the pregnancy in pregnant females [15-18]. In goats measuring plasma P_4 concentration is most important to monitor the luteal functions, because it reflects the development and regression of the corpus luteum to predict the estrus [19, 16-18]. Detection of estrus is important in breeding but is difficult to detect properly with visual observation [20]. Assessment of progesterone level is a management tools to characterize and detect estrus, ovarian cyclicity and pregnancy in goat [16, 20]. The timing of estrus can be estimated with the progesterone concentration in blood or in milk but it tends to be more accurate in blood as compared to other traditional methods of estrus [21, 18]. The concentrations of progesterone (P4) in plasma/milk could be determined with using Enzyme Immuno Assay kits. P4 analysis can be applied as a useful tool to help to herds' men and veterinarians and help in the determination of estrus (heat) detection in goats [22-24].

MATERIAL AND METHODS

Study was conducted at Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, with the objective to determine the timing of estrus on the basis of plasma progesterone concentration level in Kamohri goat. The blood samples were collected from each goat and the serum progesterone concentration level in blood was measured by Enzyme Linkage Immuno Sorbent Assay (ELISA) kit.

Management of the animals:

The goat flock was raised in the sheds, which were scientifically designed to provide an adequate space, ventilation and sanitation. The goat flock was allowed for grazing in day time and stall feeding were practiced on return back of animals to the sheds of the farm. The seasonal green fodders available according to the season were offered to goats. The concentrates ration (barley, cotton seed cake and wheat bran) at the rate of 250 gm were given daily/animal and common salt blocks were placed in mangers for licking. Water was provided ad-libitum in plastic tubs in the shed and from nearby irrigation channel during the grazing period. All goats were identified with ear tags or numbers. The vaccination of goat flock at the farm was performed regularly as per scheduled against various contagious diseases. The deworming was also practiced at regular interval (twice a year) against gastrointestinal parasites.

Collection of blood sample:

The experimental goats were brought at collection point and restrained in standing position with the help of two assistants. The jugular vein was made prominent by applying digital pressure and cleaned with cotton swab dipped in sprit to minimize the contamination. Blood samples of 3 ml were collected from jugular venipuncture in vacuoateized plain glass tubes with disposable syringes under aseptic conditions carefully avoiding hemolysis. The blood samples were collected early in the morning, kept in isothermal container (5°C) and brought to the laboratory. And were analyzed for progesterone concentration to detect estrus. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored in capped plastic tubes (apple drape tubes/ serum cups) at -20°C till progesterone analysis. The plasma progesterone concentration level was analyzed using double-antibody Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) kit 96 wells (Monobind ®, USA) as per instruction of manufactures.

Hormone analysis procedure:

Before proceeding assay, all reagents, serum references and controls were brought at room temperature, (20- 27°C).

- Microplate wells were formatted for serum reference, control and patient specimen to be assayed.
- First of all 0.025 ml (25 µL) of appropriate serum reference, control and specimen pipetted into each assigned well.
- The 0.050 ml (50µl) of Progesterone Enzyme Reagent was added in all wells.
- Microplate was swirled gently for 10-20 seconds to mix the contents.
- Then 0.050 ml (50µl) Progesterone Biotin Reagent was added in to all wells.
- The microplate was swirled gently for 10-20 seconds to mix both diluents.
- Microplate was covered and allowed to incubate for 60 minutes at room temperature.
- Then 350µl of buffer wash was added and decant or aspirate. It was repeat two additional times for a total for three time wash. For washing an automatic plate washer was used to wash the microplates.
- Then 0.100 ml (100µl) of Substrate solution was added in each well. Not shanked the plate after substrate addition.
- The microplate was allowed to incubate at room temperature for twenty minutes.
- During incubation the color was turned into light blue to deeper blue. Then 0.050 ml (50µl) of stop solution was added in to each well and mix gently for 15-20 seconds.
- The color of microplate wells were turned blue to yellow after adding the stop solution.
- Reagents were added in the same order to minimize reaction time differences between the wells.

- The plasma progesterone was measured through ELISA (Buck Man, Cultrus, 430) reader using progesterone kit (Monobind, Accu-bind, USA) at standard of 0.0, 0.3, 2.0, 5.0, 15.0, 30.0, and 60.0 at rate of 450 ng/ml. Result was recorded reading the absorbance in each well at 450nm (using a reference wavelength of 620-630nm). The results were recorded within thirty minutes of adding the stop solution.
- **Breeding of goats:** Estrus was detected on the basis of progesterone hormone concentration level. The goats were also visually monitored for signs of estrus for 30 minutes daily in morning and evening (6.00 a.m. and 6.00 p.m.). Goats observed in estrus were allowed for natural breeding.

RESULTS AND DISCUSSION:

Progesterone concentration on day of estrus:

Estrus is the behavioral manifestation of sexual receptivity in female. An early detection of estrus is the important event in breeding management but it is difficult to patronized and detect at proper time and observe the changes which occurs in estrus[13]. Present study was conducted to evaluate the effect of different estrus synchronization protocols in terms to induce estrus in Kamohri goat breed. The estrus was determined on the basis of plasma progesterone concentration level in estrus synchronized goat. In present study the mean progesterone concentration level at day of estrus as recorded was 0.46±0.07, 0.34±0.06 and 0.36±0.05 ng/ml in treatment group A, B and C respectively and 1.38±0.04 ng/ml in control group of animal in Kamohri goat does (Table-1). There was no any significant difference (P ≥0.05) between the treated and control group. The results of present study are laying in same trend with the results reported by other scientist [25, 17, 11]. They reported that in goat the progesterone (P-4) concentration in peripheral circulation periodically changes throughout the estrous cycle. The measuring of plasma P-4 concentration is most important to monitor the luteal functions [19, 16, 11]. The P4 level were measured with using ELISA method in the present study on the similar procedure of Islam *et al.,* [26] and others[27, 28].

The results observed in current study are in close agreements to the results reported by Tabatabaei *et al.;* in 2014 [19], Błaszczyk *et al.;* in 2004 [18] and Inskeep in 2004 [29]. They reported plasma progesterone concentration was declined from 1.7 to 0.6 ng/ml during days of estrus in synchronized in small ruminant, whereas increasing from 1.4 to 3.0 ng/ml in controlled animals. Furthermore they reported that the progesterone level drops below 1 ng/ml was the indication that the doe in estrus. The findings of present study are also in accordance with the results reported by Gaafar *et al.; in* 2005 [17] and Alwan *et al.;* in 2010 [24]. They reported that plasma progesterone concentration declined up to 0.6 ng/ ml just before and during estrus in does. The mean plasma P4 concentration level during this period was remained low in pro-estrus and estrus phase in goat [17, 18, 12]. These reported results are in consonance with the results of presents study in Kamohri goat. The findings of current study are also in close comparison to the results reported by Kasure *et al.;* in 2008. They reported that the mean plasma P4 concentration level was gradually decreased up to 0.1+0.03 ng/ml in estrus, whereas increased in p-4 concentration level was reported on or after day 6^{th} and continuously increasing trend was recorded and this level reached to an average of 7.7+0.6 ng/ml and remained constant up to the day 15^{th} and then after again declined trend was observed at the end of cycle before the start of next estrus and it again reached to the basal level in goat [17, 18,22].

Table-1: Detection of estrus on the basis of progesterone concentration level in estrus synchronization treated Kamohri goat

Group	Treatment	Number of goat observed	Number of goats induced estrus and served	Progesterone concentration level ng/ml
A	**Progesterone + GnRH+ PGF2@**	10	10	0.46±0.07
B	**PGF2@ +GnRH**	10	10	0.34±0.06
C	**Dry date fruit+ Ajwain**	10	10	0.36±0.05
D	**Normal saline (control)**	10	00	1.38±0.04

In cyclic goats P4 level declined to reach minimum concentrations level during estrus, which gives the path to predict the estrus than after it gradually increase and reach at maximum in the luteal phase [15, 16, 22, 11]. The progesterone level always remains low during estrus and at ovulation. Low progesterone level was reported during proestrus and estrus and it begins to rise slowly after ovulation as the CL develops [16, 29, 8]. In addition it had been reported that the P-4 concertation is related with the onset of growth of CL. Plasma P_{-4} concentration observed in present study was slightly lower than the figures reported by Farshad *et al.; in* 2008 [21]. They reported that the progesterone level was ranged between 2.24-10.95 ng/ml in goats, during mid estrus cycle period. Similar observation was recorded by Bearden *et al.; in* 2004 [12]. in goats. The

measurement of plasma progesterone level is more accurate as compared to the other traditional methods of estrus detection and could be determined with using Enzyme Immuno Assay kits. In addition, P4 concentration provides rapid and simple method and it could be applied as a useful management tool in the determination of estrus, so that more efficient breeding program could be developed and implemented in the flock at farm level.

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