

28

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## Diagnosis of Single and Twin Pregnancy, and Early Embryo Mortality Through Progesterone Level Test on Local Does

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### Abstract

The purpose of the study was to determine the levels of progesterone to diagnose single and twin pregnancy, early embryonic mortality and in improving livestock productivity. Twenty does were estrous synchronized using PGF2 $\alpha$  twice and inseminated using fresh semen of etawa buck, and blood sampling for progesterone levels were examined with Radio immunoassay (RIA). The results of the progesterone before pregnancy, at days 14 and 21 after AI in single pregnancy, twins, triplets and quadruplets were 0.84 $\pm$ 1.47 ng/ml up to 1.56 $\pm$ 1.27 ng/ml, 2.5+ 0.16 ng/ml up to 2.91+0.51 ng/ml, 2.64+0.56 ng/ml up to 3.61 + 0.13 ng/ml, 2.89 $\pm$ 0.83 ng/ml up to 4.05 $\pm$ 1.23 ng/ml and 3.08 $\pm$ 0.72 ng/ml up to 4.39 $\pm$ 1.21 ng/ml respectively. Differences in progesterone levels can be used to determine early mortality of embryos and late implantation occurs in single kid and twins <1 ng/ml at 6<sup>th</sup> days.

**Key words** : Goat, Progesterone, Pregnancy, embryo Death

Pregnancy detection in goats is done by conventional method that is based on non return of estrus after mating (Anghel *et al.*, 2011) or abdominal palpation. However this method is less accurate and useful only after 60-70 days (Kharche and Justin, 2015). Estimation of progesterone level on day 14 and 21 post AI was tried to determine the early embryonic mortality or late implantation to determine the pregnancy status.

### Materials and Methods

This research was used in 20 of 2-4 years old indigenous does treated for estrous synchroniza-

tion with PGF2 $\alpha$  4 mg/i.m twice with intervals of 11 days followed by AI twice with an interval of 6 hours using the fresh etawa's goat semen with a concentration of 85x10<sup>9</sup> (Wurlina, 2001). Blood samples (5 ml) were taken from the jugular vein. The initial level of progesterone was taken before doe was injected with PGF2 $\alpha$ . The second and third levels of progesterone were estimated on 14<sup>th</sup> and 21<sup>st</sup> days post AI. The progesterone levels on 60<sup>th</sup>, 90<sup>th</sup>, 120<sup>th</sup>, and 140<sup>th</sup> after AI were also estimated. The measurement of progesterone levels was, performed on goats after 6 hours of kidding. Measurement of serum progesterone levels were done by Radio immunoassay (RIA) technique.

### Results and Discussion

Out of the 20 local does inseminated, 5 does gave birth one lamb each (single); 5 does gave birth twins; 5 does gave birth three lambs (Triplets), 5 does give birth four lambs (Quadruplets). The results of the examination of progesterone levels at various stages before and after pregnancy, and after kidding is presented in Table I.

The progesterone levels before, during pregnancy and after lambing in groups of doe had twins, triplets and quadruplets were always higher than that of the single kid and non significant, before and after lambing. High levels of progesterone began to emerge 60 days after AI and reached a peak in 90 days after AI (significant different  $p < 0.05$  between single kids with twins, triplets, quadruplets) and at 120 days after AI (on single does) followed by a gradual decrease in progesterone levels which is significantly different ( $p < 0.05$ ).

The measurements of average progesterone levels before administration of PGF2 $\alpha$  on

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**Table I.** The progesterone levels before AI, during pregnancy and after kidding with a single, twins, triplets and quadruplets of the experimental does (Mean  $\pm$  SD).

Time of blood samples collected	Progesterone's levels (ng/mL)			
	Singles	Twins	Triplets	Quadruplets
Before AI	0.96 $\pm$ 0.48 <sup>a</sup>	1.56 $\pm$ 1.27 <sup>ab</sup>	0.84 $\pm$ 1.47 <sup>a</sup>	1.44 $\pm$ 1.08 <sup>ab</sup>
After AI 14 days	2.25 $\pm$ 0.16 <sup>b</sup>	2.64 $\pm$ 0.56 <sup>b</sup>	2.89 $\pm$ 0.83 <sup>b</sup>	3.08 $\pm$ 0.72 <sup>b</sup>
After AI 21 days	2.91 $\pm$ 0.51 <sup>b</sup>	3.61 $\pm$ 0.13 <sup>bc</sup>	4.05 $\pm$ 1.23 <sup>bc</sup>	4.39 $\pm$ 1.21 <sup>bc</sup>
After AI 60 days	5.64 $\pm$ 0.58 <sup>c</sup>	9.27 $\pm$ 2.57 <sup>d</sup>	11.03 $\pm$ 2.77 <sup>d</sup>	12.52 $\pm$ 2.85 <sup>d</sup>
After AI 90 days	7.21 $\pm$ 1.28 <sup>cd</sup>	15.76 $\pm$ 0.83 <sup>e</sup>	18.72 $\pm$ 2.51 <sup>e</sup>	19.15 $\pm$ 2.64 <sup>e</sup>
After AI 120 days	10.12 $\pm$ 3.25 <sup>d</sup>	14.16 $\pm$ 3.02 <sup>e</sup>	15.16 $\pm$ 3.42 <sup>e</sup>	16.89 $\pm$ 3.35 <sup>e</sup>
After AI 150 days	8.42 $\pm$ 1.35 <sup>cd</sup>	9.98 $\pm$ 3.16 <sup>d</sup>	12.06 $\pm$ 2.28 <sup>d</sup>	12.12 $\pm$ 2.61 <sup>d</sup>
6 hours after lambing	0.35 $\pm$ 0.05 <sup>a</sup>	0.38 $\pm$ 0.06 <sup>a</sup>	0.43 $\pm$ 0.04 <sup>a</sup>	0.51 $\pm$ 0.06 <sup>a</sup>

Means bearing different superscript in a row differ significantly ( $P < 0.05$ )

does which had single and triplets does before pregnancy were 0.96  $\pm$  0.48 ng / mL and 0.84  $\pm$  1.27 ng / mL suggesting that the ovary is in the follicular phase. While, in does with twins and quadruplet, progesterone levels were 1.56  $\pm$  1.27 ng / mL and 1.44  $\pm$  1.08 ng / mL, suggesting that the ovary was in the luteal phase. This is in line with observation of luteal phase progesterone in sheep and goats 1.5 - 2.5 ng / mL Boscos *et al.* (2003).

The average progesterone levels on 14 days after AI in does with single kid were 2.25  $\pm$  0.16 ng / ml, whereas on doe of twins, triplets and quadruplets were 2.64  $\pm$  0.56 ng / ml, 2, 89  $\pm$  0.83 and 3.08  $\pm$  0.72 ng / ml. According to Boscos *et al.* (*loc cit*) the levels of progesterone as on 21 days pregnancy time on ewe and doe was > 2.5 ng/ml. From the results of the study the levels of progesterone on day 14 after AI can be used to diagnose early pregnancy when the progesterone levels is < 2.25 $\pm$ 0.16 ng/ml and < 2.25  $\pm$ 0.16 < levels as non pregnant.

The progesterone level on day 21 after AI in single kid does was 2.91  $\pm$  0.51 ng / mL, whereas in twins, triplets and quadruplets does were 3.61  $\pm$  0.13 ng / ml, 4.05  $\pm$  1.23 and 4.39  $\pm$  1.21 ng / ml. Does with single kid always had lower levels of progesterone than those with twins. It is assumed that local does with single kid also have a single Corpus luteum (CL). The CL is the primary source of progesterone in goats and the CL is more numerous in local does, resulting in more progesterone (Hafez and Hafez, 2013).

Wurlina (*loc cit*) stated that pregnant goats, the levels of progesterone was between 1 to 5 ng/mL, whereas in non pregnant does, the progesterone levels was <1 ng/ml. From the results based on progesterone levels on days 14 and 21 AI will be useful to diagnose the presence of early embryonic mortality in does with single kids or twins to, triplet and quadruplets. When on the 14th day after AI, if progesterone levels was <2.25 + 0.16 ng / mL, the goat is considered not pregnant, whereas on the 21st day after AI if the progesterone levels is > 2.25 + 0.16 ng / mL, the goat is considered pregnant with delayed implantation. Increased levels of progesterone hormone 14-21 days after mating, caused the beginning of pregnancy of the active CL to produce progesterone and continue to increase during the luteal phase (Alwan *et al.*, 2010), whereas the decline that occurs after the luteal phase is due to the shrinking function of the CL, at 12-14 days.

On the 14th day after AI, does with single pregnancy or twins, triplet and quadruplet. Progesterone levels > 2.25 + 0.16 ng / mL, was considered pregnant, but on the 21st day after AI progesterone levels dropped to <2.25 + 0.16 ng / ml, then the doe is declared non pregnant with early embryonic mortality. Embryonic mortality rate during the period of pregnancy is very high, especially 42 days of pregnancy Hariadi *et al.* (2011). The mortality of early embryos are usually marked with non return of estrus so it can extend the kidding interval leading to

economic losses to the breeders.

Average levels of progesterone in does with single twins, triplets and quadruplets at pregnancy 60 to 120 days after AI between  $5.64 \pm 0.58$  to  $19.15 \pm 2.64$  ng/mL are significantly different ( $p < 0.05$ ) between single with twins, triplets, quadruplets. From the results of this study it can also be seen that progesterone levels continue to increase from 60 days to 140 periods of pregnancy and will decline before birth. This is because before birth occurs, the CL will experience luteolysis due to increased concentration of prostaglandin (PGF<sub>2</sub>α) produced by endometrium. It is also caused by the hypothalamus of fetus to produce ACTH-RH which causes a sudden increase in ACTH levels to increase the secretion of cortisol through the placenta so that PGF<sub>2</sub>α will increase. Increased secretion of PGF<sub>2</sub>α leads to constriction of utero ovarica blood vessels, resulting in reduction of luteum cells and causing luteolysis (Hafez and Hafez, *loc cit*).

#### Summary

The progesterone level on day 14 and 21 after AI can be used to diagnose early pregnancy on the does, with levels  $2.25 \pm 0.16$  ng/mL and  $2.91 \pm 0.51$  ng/mL,  $2.64 \pm 0.51$  ng/mL and  $3.61 \pm 0.13$  ng/mL,  $2.89 \pm 0.83$  ng/mL and  $4.05 \pm 1.23$  ng/mL,  $3.08 \pm 0.72$  ng/mL and

$4.39 \pm 1.21$  ng/mL for single, twins, triplets and quadruplets, respectively. The difference in levels of progesterone on day 14 and 21 after AI can be used to determine of the early embryonic mortality or as late implantation

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