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Application of Different Progesterone Protocols on Some Reproductive Hormones during Pregnancy in Awassi Ewes

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Abstract

The present study was conducted to investigate the role of various treatment protocols of Progesterone application in pregnant Awassi ewes. Thirty non-pregnant mature Awassi ewes were divided in 3 groups (n = 10 each), for estrus synchronization, all ewes treated with intra-vaginal sponge for 12 days. Before day of sponge withdrawal, all ewes injected with 250 IU of PMSG, after appearance of estrus sings all ewes were mating with experienced rams. At 4, 12, and 21 days of pregnancy, ewes injected (i.m) with 5 ml of normal saline (Control group; C). At 4 and 12 days of pregnancy, ewes injected (i.m) with 10mg of Progesterone (Progesterone group 1; Pro1) and at 4, 12 and 21 days of pregnancy, ewes injected(i.m) with 10 mg of Progesterone (Progesterone group 2; Pro2). Blood samples were collected, at 30, 60, 90, 120, 140 days of pregnancy, for assessment of Progesterone and Estradiol-17β concentrations. As well as, numbers of newborns after parturition calculated. The statistical analysis registered significant ($p \leq 0.05$) gradual elevation of serum Progesterone concentration in Pro1 and Pro 2 pregnant ewes when compared with control pregnant ewes (C) during the period of pregnancy (60^{th} day, 90^{th} day and 120^{th} day), the results recorded significant ($p \leq 0.05$) decrease in serum Estradiol concentration in groups Pro 1 and Pro 2 at days 30^{th}, 60^{th}, 90^{th} and 120^{th} in comparing with control group. As well as, the results of total numbers of newborns in the Pro 2 group increased significantly ($p \leq 0.05$) when compared with Pro 1 and control groups. Therefore, it was concluded that the injection of Progesterone at days 4, 12 and 21 of pregnancy was efficient method for improving the luteal functions during early development embryos by augmenting some fertility hormones leading to decrease the embryonic death.

Keywords: Progesterone, pregnancy, embryos, ewes.

INTRODUCTION

Most of pregnancy losses in domesticated livestock take place during early stage of pregnancy (1; 2 and 3 and 4). Pre-implantation losses regarded the main cause of decline the reproductive efficiency in mammalian species (1). In ewes, about 20-40% of ova are not characterized by progeny offspring (5). Causing losses up to 63% after induced synchronization and treated with progestagen-PMSG (6). As a result, failure of fertilization accounts for 5–10% of losses (7).

Many factors participate to embryonic losses including inadequate progesterone (P4) production due to insufficient luteal function, as suboptimal P4 production is revealing of maternal failure to maintain pregnancy (8). Several researcher used varies alternative methods like human chorionic gonadotropin (hCG), sponges impregnated with progesterone derivative, immunization against hormones, progesterone hormone and/or analogues and gonadotropin-releasing hormone (GnRH), for decline the embryonic losses and increase pregnancy and fertility rates (9; 10; 11 and 12). These hormones could applied at different days of cycle after insemination or mating like 4^{th}, 5^{th}, 11^{th} and 12^{th} days, these periods regarded the critical time because corpus luteum begin to regress at 12^{th} day after mating (9;10;13 and 14). Such hormonal applications causes increment in secretion of progesterone on day 12 after mating could inhibit luteolysis of the corpus luteum and prostaglandin F2α (PGF2α) secretions (15).

P4 is essential for maintenance and establishment of pregnancy in all mammals. Development and growth of the conceptus (embryo/fetus and associated extraembryonic membranes) requires Progesterone signaling to control pivotal endometrial functions for implantation and placentation (16 and 17).

The present study was designed to investigate various treatment protocols of progesterone injection intramuscularly at 4, 12 and 21 days of pregnancy on hormonal profiles and embryonic survival.

MATERIALS AND METHODS

Animals

The present study was done in Barakat AL- Redah station in Babylon government, during the period from April, 2017 to September, 2017. Awassi ewes (weighed 45-55 kg and aged 2-3 years) were used in the present experiment. The animals were household in semi-opened shade, feeding with concentrated feed supplemented with vitamins and wheat straw as well as green food, Water and minerals were freely available.

Experimental design

Thirty mature Awassi ewes (non-pregnant) were distributed in 3 groups (n = 10 each), all ewes synchronized by intra-vaginal sponge (Medroxy progesterone acetate) for 12 days. Before day of sponge withdrawal, all ewes injected with 250 IU of PMSG. After appearance of estrus signs in ewes, allow matting with (3) experienced rams (aged 2-3 years and weighed 50-60 kg). Control group (C): ewes injected with normal saline (5ml Intra-muscular-i.m) on 4, 12 and 21 days of pregnancy. Progesterone group 1 (Pro1): ewes injected (i.m) with Progesterone (10 mg) on 4 and 12 days of pregnancy. Progesterone group 2 (Pro2): ewes injected (i.m) with Progesterone (10 mg) on 4, 12 and 21 days of pregnancy.

Samples of blood were collected, at 30, 60, 90, 120 and 140 days of pregnancy, for assessment of Progesterone and Estradiol-17β concentrations. Samples were separated by centrifugation at 5000 rpm for 10 minutes, and blood sera kept at -20 ºC until hormonal assay. Pregnancy diagnosis was detected by using pregnancy detector instrument at day 37^{th} -38^{th} of pregnancy. As well as, Number of delivered lambs determined after parturition.

Procedure of ELISA for hormonal assay in serum

Depending on the manufacturer Instructions (Wuhan Fine Biological Technology).

Statistical Analysis:

Data of the experiment were analyzed by using Complete Random Design (C.R.D). Statistical analysis was carried out by using procedure of (18), differences were considered to be significant at the level of $P<0.05$.

RESULTS AND DISCUSSION

Progesterone

Figure (1, 2, 3, 4 and 5) illustrate serum Progesterone concentration (ng/ml) in pregnant ewes during gestation periods. The statistical analysis showed significant ($p \leq 0.05$) elevation in

serum progesterone concentration at 30 day of gestation in Pro2 pregnant ewes when compared with Pro1 and control pregnant ewes. While the results at 30 day of pregnancy in Pro1 pregnant ewes showed non-significant ($p>0.05$) differences when compared with C group (figure 1).

The results revealed significant ($p\leq0.05$) gradual elevation of serum Progesterone concentration in Pro1and Pro2 pregnant ewes when compared with control ewes (C) throughout the period of pregnancy (60 day, 90 day and 120 day) (figure 2,3 and 4). At day 140 day of pregnancy, the results significantly ($p\leq0.05$) decreased in Pro1 and Pro2 when compared with control group (figure 5).

Estradiol-17B (E2)

The results of serum estradiol-17B concentrations (pg/ml) in pregnant Awassi ewes were elucidated in figure (6,7,8,9 and 10). The statistical analysis showed declined significantly ($p\leq0.05$) in serum Estradiol level in Pro1 and Pro2 pregnant group compared with C at 30th , 60th, 90th and 120th days of pregnancy (figure 6,7,8 and 9). On the other hand, at 140th day of pregnancy the results revealed a slightly significant ($p\leq0.05$) elevation in Pro1 and Pro2 pregnant ewes in comparison with that of control groups (figure 10).

Average number of newborns

Average numbers of newborns/ ewe were clarified in figure (11). Number of newborns in groups Pro2 showed significant elevation ($p\leq0.05$) in comparing to the number of newborns in groups Pro 1 and control.

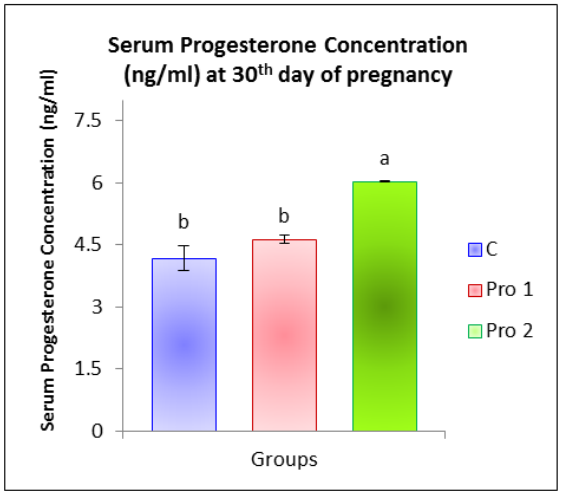

Figure 1: Serum Progesterone concentration (ng/ml) after 30th days of pregnancy in Awassi ewes. C= control; Pro 1= Progesterone group 1 and Pro2= Progesterone group 2. The values represented as M±SE. Different letters denote significant difference ($p<0.05$) between groups.

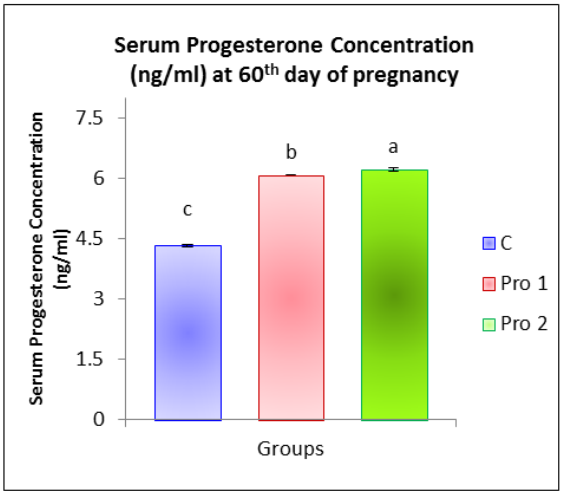

Figure 2: Serum Progesterone concentration (ng/ml) after 60th days of pregnancy in Awassi ewes. C= control; Pro 1= Progesterone group 1 and Pro2= Progesterone group 2.The values represented as M±SE. Different letters denote significant difference ($p<0.05$) between groups.

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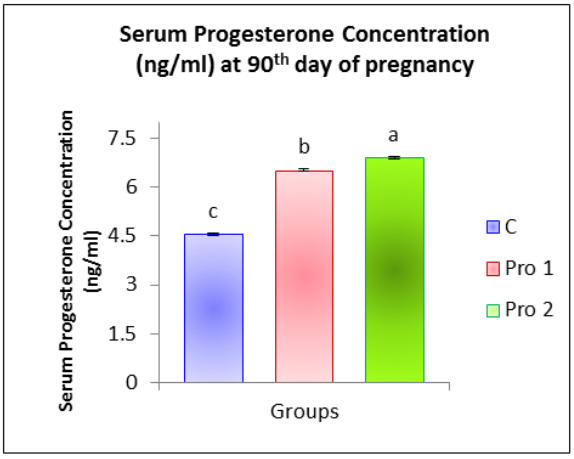

Figure (3): Serum Progesterone concentration (ng/ml) after 90th days of pregnancy in Awassi ewes.
C= control; Pro 1= Progesterone group 1and Pro2= Progesterone group 2. The values represented as M±SE. Different letters denote significant difference ($p<0.05$) between groups.

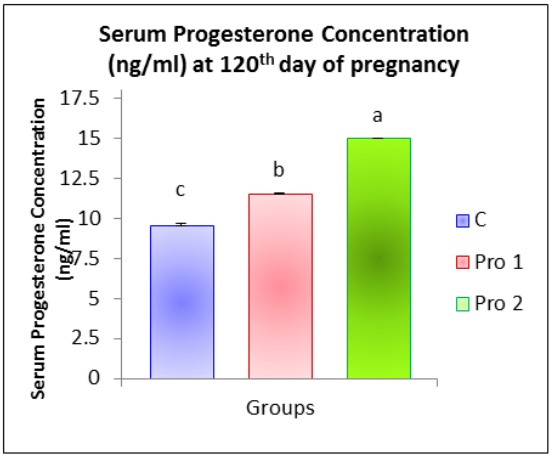

Figure (4): Serum Progesterone concentration (ng/ml) after 120th days of pregnancy in Awassi ewes.
C= control; Pro 1= Progesterone group 1 and Pro2= Progesterone group 2. The values represented as M±SE. Different letters denote significant difference ($p<0.05$) between groups.

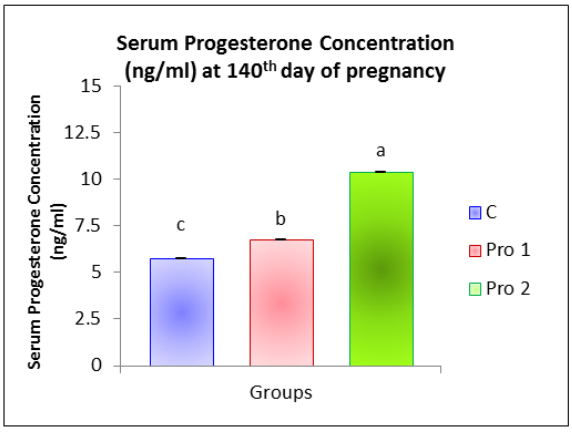

Figure 5: Serum Progesterone concentration (ng/ml) after 140th days of pregnancy in Awassi ewes.
C= control; Pro 1= Progesterone group 1 and Pro2= Progesterone group 2. The values represented as M±SE. Different letters denote significant difference ($p<0.05$) between groups.

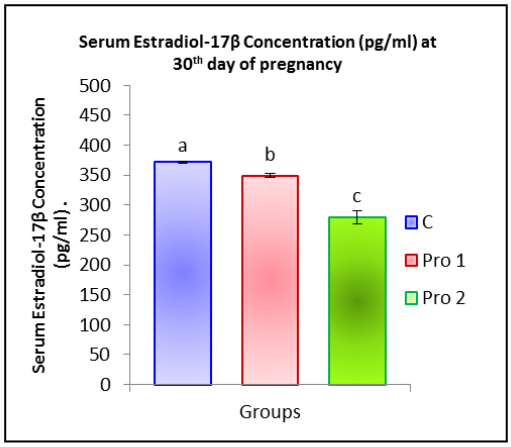

Figure 6: Serum Estradiol-17β concentration (pg/ml) after 30th days of pregnancy in Awassi ewes. C= control; Pro 1= Progesterone group 1 and Pro2= Progesterone group 2. The values represented as M±SE. Different letters denote significant difference ($p<0.05$) between groups.

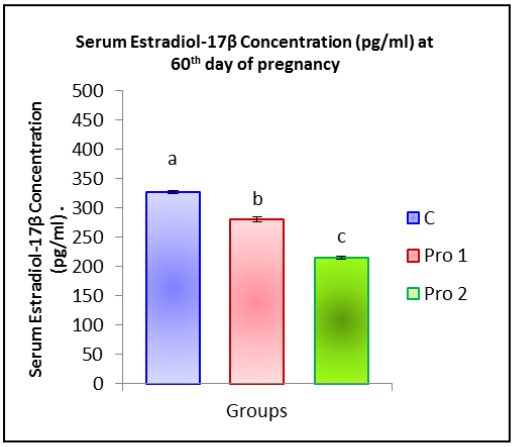

Figure 7: Serum Estradiol-17β concentration (pg/ml) after 60th days of pregnancy in Awassi ewes. C= control; Pro 1= Progesterone group 1 and Pro2= Progesterone group 2. The values represented as M±SE.
Different letters denote significant difference ($p<0.05$) between groups.

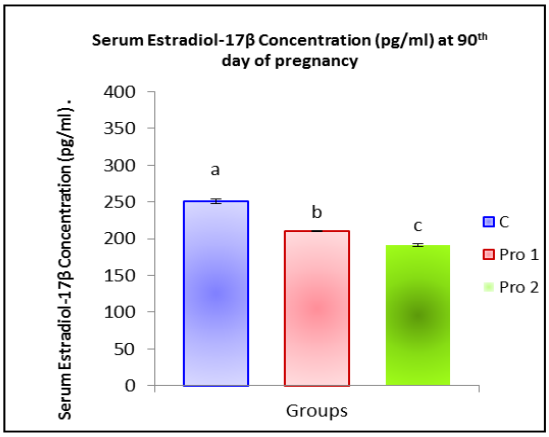

Figure 8: Serum Estradiol-17β concentration (pg/ml) after 90th days of pregnancy in Awassi ewes. C= control; Pro 1= Progesterone group 1 and Pro2= Progesterone group 2.
The values represented as M±SE. Different letters denote significant difference ($p<0.05$) between groups.

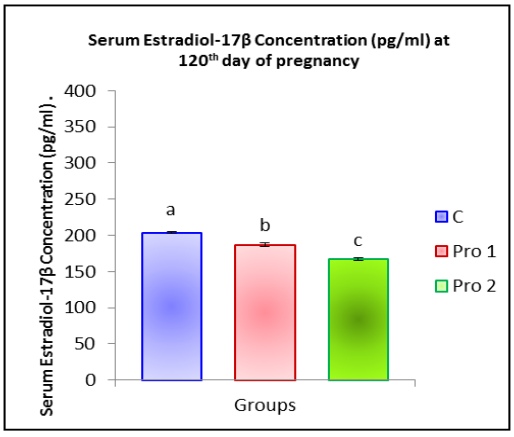

Figure 9: Serum Estradiol-17β concentration (pg/ml) after 120th days of pregnancy in Awassi ewes. C= control; Pro 1= Progesterone group 1 and Pro2= Progesterone group 2. The values represented as M±SE. Different letters denote significant difference ($p<0.05$) between groups.

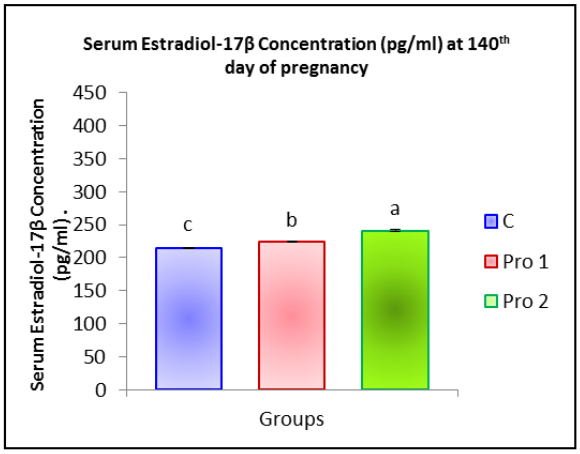

Figure 10: Serum Estradiol-17β concentration (pg/ml) after 140thdays of pregnancy in Awassi ewes. C= control; Pro 1= Progesterone group 1 and Pro2= Progesterone group 2. The values represented as M±SE. Different letters denote significant difference ($p<0.05$) between groups.

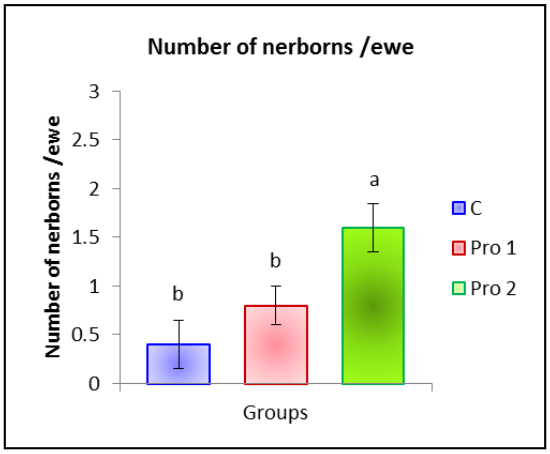

Figure 11: Mean number of newborns/ ewe. C= control; Pro 1= Progesterone group 1 and Pro2= Progesterone group 2. The values represented as M±SE. Different letters denote significant difference ($p<0.05$) between groups

The current study attempted to find effective method for reduction embryonic loss during early embryonic development by using augmented hormone (Progesterone) in Awassi ewes in Iraq.

The present results showed significant gradual elevation of progesterone concentration ($p\leq0.05$) in Pro2 pregnant group during month 1st, 2nd, 3rd and 4th of gestation, when compared with Pro1 and control, this might be attributed to the efficacy of administrated quantities of progesterone during early stage of embryos development in the present study for reducing the embryonic losses rate through improved luteal functions.

Beside, days 4, 12 and 21 after mating considered the critical period of gestation because maternal recognition and embryos implantation, which accompanied with the onset the corpus luteum regression in the estrous cycle (15), progesterone administration on days 4; 12 and 21 could elevate production of interferon-T (INF-T) (19), which in turn inhibits luteolysis by preventing PGF2α secretion (15). Furthermore, Luteolysis could be prevented by Progesterone administration, hence decline follicles ability for production of estradiol hormone. The reduction in estradiol secretion causes inhibiting of oxytocin receptors synthesis on uterine endometrium leading to prevent PGF2α production, this mechanism is necessary for pregnancy maintenance (20). The elevation in blood progesterone concentration could stimulate synthesis and secretion important embryonic proteins on days 4; 12 and 21 of gestation, called Ovine trophoplast protein (OTP-1) then recently named ovine interferone-T (OIF-T) could prevent luteolysis (21). The changing in progesterone concentration after mating was proportion with the extent and life span of luteal cells in turns with the account of embryos and embryonic mortality, so the ewes with lower concentration of progesterone suffering from embryo loss (22) due to decrease secretion of progesterone at periovulatory period causes abnormal actions of uterine and/or poorly development of oocytes and decline synthesis and secretion of steroid due to atresia of follicles (8), the current results agreement with (11).

Wilmut *et al*.,(23) found the conceptus development can be accelerated by exogenous progesterone. According to above informations, this supports the present results about the number of newborn delivered in Pro 2 group higher than Pro 1 and control groups, may attributed to decrease embryonic death during early stage of pregnancy (3 weeks post mating) due to injected of sufficient amount of progesterone at days 4, 12 and 21 of pregnancy leading to improve the luteal functions that enhancement maternal recognition; implantation of embryos and placentation (21), the current results agreement with (11) who concluded the using GnRH and sponges impregnated with fluorogestone acetate on 4 and 12 days after mating were effective for decreasing embryonic death by providing of progesterone during early developing embryos.

The current study revealed gradual decline in estradiol concentration along gestation period (30th, 60th, 90th and 120th days), as a result of gradual increment of progesterone concentration leading to effect on hypothalamus – pituitary glands by negative feedback action causing prevent synthesis and secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) causing prevent growth of ovarian follicles and/or estradiol production (24), the present results agreement with (25).

Conclusion

The results presented in this study recorded that the injection of Progesterone on days 4, 12 and 21 of pregnancy was efficient method for improving embryonic survival by augmenting some fertility hormones specially progesterone hormone leading to decline the embryonic losses .

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