# Effect of Recombinant Luteinizing Hormon (rLH) on Some Fertility Parameters in Awassi Ewes Synchronized with $PGF_2\alpha$ in the Breeding Season

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**Abstract:** The aim of this study is to investigate the effects of three different doses of recombinant Luteinizing hormone (rLH), applied 48 hours after the second prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) injection apart 9 days in oestrus synchronization on reproductive performance of Awassi ewes. Eighty purebred Awassi ewes given birth to at least one lamb were used. All ewes were treated with $PGF_{2\alpha}$ consisting of two IM doses of a $PGF_{2\alpha}$ analogue (1.25 ml, 0.093 mg–D-Cloprostenol of per dose, Dalmazin®, Vetas,Turkey) administered 9 days apart. Animals were the randomly allocated to four equal groups and 48 h later a single intramuscular (IM) dose of rLH (Group1 0.5 IU; Group 2, 7.5 IU; Group 3, 10 IU) control group was injected with 1 ml normal saline solution. The oestrus was controlled at 48 hours later. Artificial insemination was performed with fresh diluted semen collected from fertile Awassi rams after observing oestrus behaviors. The pregnancies were determined with transrectal ultrasonography at 40$^{th}$ day after artificial insemination. There were no statistically significant differences (P>0.05) between the treatment groups and the control group for estrus response (Group 1, 90.0 %; Group 2, 85.0 %; Group 3, 80.0 %; Control group, 90.0 %). Pregnancy rates were 50.0 %, 47.06%, 50.0 % and 50.0 % in groups 1, 2, 3 and the control group, respectively. Lambing rates in treatment groups (89, 100-%) were similar to control group (100 %). Lambing rates in Groups 1, 2, 3 and control group were 89.90 %, 100 %, 100 %, and 100 % respectively. Single birth rates and twinning rates in three different rLH groups were similar to control group. There were no statistically significant differences on the lambing rates, single and twinning rate among the treatment groups and control group. These results indicated that the three different doses of rLH after oestrus synchronization with PGF2α did not affect the fertility parameters in Awassi ewes.

***Key words:*** *Awassi Ewes, Luteinizing hormone, $PGF_2\alpha$*

## Aşım Sezonunda $PGF_2\alpha$ ile Senkronize edilen İvesi Koyunlarında Bazı Fertilite Parametreleri Üzerine Rekombinant Luteinize Hormonun Etkisi

**Özet:** Bu çalışmanın amacı, İvesi koyunlarda, aşım sezonunda, östrus senkronizasyonu amacıyla 9 gün arayla çift doz prostaglandin ($PGF_{2\alpha}$) uygulamasını takiben 48 saat sonra üç farklı dozda rekombinant luteinizing hormonunun (rLH) üreme performansı üzerine etkilerini araştırmaktır. Çalışmada; en az bir doğum yapmış toplam 80 baş İvesi koyun kullanıldı. Koyunlara 9 gün arayla i.m. yolla çift doz $PGF_{2\alpha}$ (1.25 ml, 0.093 mg–D-Cloprostenol of per dose, Dalmazin®, Vetas,Turkey) enjeksiyonlarını takiben, rastgele 20 şerli gruplar halinde biri kontrol grubu olmak üzere dört eşit gruba ayrıldı. Son PGF2α uygulamalarından 48 saat sonra farklı dozlarda sırasıyla, Grup 1; 0,5., Grup 2; 7,5 ve Grup 3; 10 IU rLH İ.M enjekte edildi. Kontrol grubuna ise 1 ml serum fizyolojik uygulandı. Östruslar 48. saatten sonra araştırıldı. Östrus gösteren koyunlar; fertil İvesi koçlarından toplanan taze spermayla suni tohumlandı. Gebelikler, tohumlamayı takiben 40. günde transrektal ultrasonografi ile belirlendi. Koyunlarda östrus görülme oranları grup 1, 2, 3 ve kontrolde sırasıyla % 90.085.0; 80.0; 90.0. olarak tespit edildi. Gruplar arasında istatistiki açıdan bir fark bulunmadı. Gebelik oranları sırasıyla grup 1, 2, 3 ve kontrolde % 50.0;47.0; 50.0;50.0. olarak tespit edildi. Kuzulama oranları rLH uygulama gruplarında (% 89- 100), kontrol grubunda ise % 100 olarak belirlendi. Tekil ve ikiz doğum oranları ise farklı rLH uygulama gruplarında ve kontrol grubunda benzer bulundu. Gruplar arasında gebelik, tekillik ve ikizlik oranları istatistiksel açıdan önemsiz bulundu. Sonuç olarak, aşım sezonu içerisinde bulunan İvesi koyunlara çift doz $PGF_{2\alpha}$ uygulamaları takiben 48. saatte yapılan farklı dozlardaki rLH enjeksiyonları İvesi koyunlarında fertilite parametrelerini arttırmadığı kanaatine varıldı.

***Anahtar kelimeler****: İvesi koyun, Luteinize hormon, $PGF_2\alpha$*

## Introduction

Oestrus synchronization can be an effective means of increasing the proportion of ewes that become pregnant over a short time, resulting in more lamb crops. Precise control of estrus cycles requires the manipulation of both follicular growth and luteal life span (Husein and Kridli, 2003). Over the years protocols have been developed for oestrus synchronization in ewes and the most successful of these have been based on the temporary suppression of oestrus with the aid of

progestin (Maxwell and Barnes, 1986). The most frequently used oestrus synchronization protocols are based on the use of equine chorionic gonadotropin after a pretreatment with progesterone or progestagen intravaginal devices (Evans and Maxwell, 1987, Menchaca and Rubianes, 2004). Alternative applications have been developed in order to manipulate follicular development and provide oestrus synchronization. Oestrus synchronization methods in ewes include fluorogestone acetate (FGA) or medroxyprogesterone acetate (MAP) (Aköz et al., 2006; Husein and Kridli, 2003) GnRH-PG, or PG-Recombinant Follikül stimulating Hormone (Zonturlu et al., 2009) and GnRH-PMSG injections (Luther et al., 2007). Progesterone and/or its analogue treatment combined with the injection of PGF2a are also used (Dixon et al., 2006).

Sponges impregnated with progesterone provide ES by extending the luteal phase during the treatment period in ewes (Whitley and Jackson, 2004; Wildeus, 1999). PGF2a is the luteolytic factor for sheep, and the use of PGF2a and/or one of its analogues causes faster regression of CL than normal luteolysis in sheep having a functional CL at the time of treatment (Gordon, 1999). The use of PGF2a and/or its analogue alone, a double injection system 9 and/or 11 days apart, is the most widely used approach in sheep (Wildeus, 1999).

Prostaglandin $F_{2\alpha}$ a great many of strategies have been developed in order to control ovarium function in sheep. Oestrus synchronization can be controlled by manipulating either follicular development or Corpus Luteum (Husein and Kridli, 2003). $PGF_{2\alpha}$ can be applied to the ewe to provide oestrus synchronization. Oestrus and ovulation formed following $PGF_{2\alpha}$ application can change depending on the existence of follicle developed in ovarian during the application of exogenous hormone (Husein and Kridli, 2003).

Ovulation rates in ewes are increased by injection of gonadotropins such as equine chorionic gonadotropin (eCG), follicle stimulating hormone (FSH), hCG (Didarkhah and Danesh Mesgaran, 2013; Simonetti et al., 2008; Yavuzer et al., 2010). Hormonal control of the reproductive function is secreted by the anterior pituitary gland: FSH and LH, consisting of an alpha and beta subunit. The alpha subunit is common to LH-HCG (Goochee et al., 1991). Recently, recombinant LH (r-LH) by recombinant DNA technology became available and now is a new option among the protocols for ovarian stimulation (The European Recombinant LH Study Group, 1998).

Using the IVT technique allows us to obtain a large number of mammalian embryos for research, genetic improvement or commercial purposes. The successful culture and embryonic development of in vitro-matured oocytes has been reported in cattle, sheep and other species (Eppig et al., 1996; Ptak et al., 1999). The administration of low doses of r-LH observed higher pregnancy and implantation rates positively influences ovarian response and oocyte quality (Humaidan et al., 2002).

The Gonadotrophin hormone regulates the secretion of Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) which plays the main role during the periovulatory period of the oestrus cycle and oocyte maturation (Moor and Trouson, 1997). They have been shown to enhance the in vitro fertilization and embryonic development of immature oocytes of sheep, cattle and other mammals (Accardo et al., 2004). LH pulse frequency and mean LH concentrations increased significantly on every occasion on which animals were treated with steroids. In females, an acute rise of LH called the LH surge triggers ovulation. After the progesterone decline and regression of corpus luteum from the previous oestrus cycle, oestradiol secretion from the maturing Graafian follicle increases and induces the preovulatory LH surge (Evans et al., 1994). LH alone decreased numbers of small follicles and stimulated growth of only a few large follicles in prepubertal gilts (Guthrie at al, 1990). Another study confirmed that when using exogenous hormones, pigs require significant LH support in order to induce large follicle development, oestrus and ovulation (Breen and Knox, 2012). Human recombinant gonadotrophins (r-FSH and r-LH) have been demonstrated to stimulate maturation and ovulation in rats (Törnell et al., 1995). The LH surge is well known is a very important regulator of the timing of ovulation and that the variability oestrus synchronization after PGF2α injections includes.

The aim of present research was to study the effect on reproductive performance of different doses of rLH after oestrus synchronization with PGF2α 9 d protocol consisting of two IM doses of a PGF2α analogue in Awassi ewes in breeding season.

## Material and Methods

### Animals and Location

The present study was carried out between June and August which is the period accepted as breeding season for ewes in Sanliurfa province of Turkey (located at latitude of 37°10'N, at longitude of 39°03'E and at altitude of 518 m above sea level). The Ewes were located at the Animal

Research Station, Harran University, Sanliurfa. A total of 80 cyclic Awassi ewes, 2-5 years of age and weighing 45-55 kg body weight, were used in this study. All animals fed consisted of a concentrate composed of barley straw (1000 g per animal daily) and barley grain (800 g per animal daily). Water and mineral licks were available ad libitum.

**Study Design**

All ewes were treated with $PGF_{2\alpha}$ 9 d protocol consisting of two IM doses of a $PGF_{2\alpha}$ analogue (1.25 ml, 0.093 mg–D-Cloprostenol of per dose, Dalmazin®, Vetas, Turkey) administered 9 days apart. Animals were the randomly allocated to four equal groups.

In group 1 (n=20), group 2 (n=20) and group 3 (n=20) 5 IU, 7.5 IU and 10 IU of recombinant LH (Luveris, 75 IU, IM, Sereno®) were administered 48 hours after the second $PGF_{2\alpha}$ injection, respectively, In the control group (n=20), ewes were injected 1.0 ml 0.9 % saline 48 hours after the second.

**Artificial insemination**

Artificial insemination was performed with fresh diluted semen collected from a single ejaculate of the fertile Awassi rams after observing oestrus behaviors.

Ejaculates of four fertile Awassi rams were pooled and diluted 1:2 at room temperature with skimmed milk extender. Cow skim-milk (SM) based solution was prepared from 10 g non-fatty milk powder dissolved in 100 ml bi-distilled water. Thereafter, 500 IU of penicillin and 500 mcg of streptomycin sulphate ⁄ ml were added to The SM extender. All milk based extenders' pH was adjusted to 6.8 with 1 NaOH. Ewes exhibiting estrus were inseminated with 0.2 ml of fresh diluted semen with skimmed milk extender.

**Pregnancy examination**

Pregnancy was determined 40 d after the mating by transrectal ultrasonography using a Scanner LC 100 (Pie Medical scanner LC 100 Vet, Netherlands) provided with a 6 MHz linear probe.

**The following parameters were recorded:**

**Estrus respons**: Number of ewes showing estrus/Total treated ewes in each group x100.
**Pregnancy Rate**: Number of pregnant ewes/Number of mated ewes in each group x100.
**Lambing Rate**: Number of ewes lambing/Number of pregnant ewes in each group x100.
**Single rate**: (Number of single lambing/total number of lambing) x100.
**Twinning rate**: (Number of twin lambing/total number of lambing) x100.

**Statistical analyses**

Statistical analyses were run with Minitab Statistical Software Programme (MINITAB, Version 11.2; Minitab Inc.). The results were analyzed with *Chi-Square* test to compare oestrus response, pregnancy and lambing, and single, twinning rates among the groups.

## Results

The data on reproductive performance of the estrus induced and cervical inseminated ewes in the rLH treated and control groups are set out in Table 1. Oestrus responses were similar in all groups (group 1, 90.0 %; group 2, 85.0 %; group 3, 80.0 %; control group, 90.0 %). There were no statistically significant differences ($P>0.05$) between the treatment groups and the control group for oestrus response. Pregnancy rates were 50.0 %, 47.06 %, 50.0 % and 50.0 % in groups 1, 2, 3 and the control group, respectively. Lambing rates in Groups 1, 2, 3 and control group were 89.00 %, 100 %, 100 %, and 100 % respectively. Single birth rates and twinning rates in three different rLH groups were similar to control group. There were no statistically significant differences on the lambing rates, single and twinning rate among the treatment groups and control group, as demonstrated in Table 1.

## Discussion

Oestrus synchronization in livestock focuses on the manipulation of either the luteal or the follicular phase of the oestrus cycle. In ewes and goat, the opportunity for control is greater during the luteal phase, which is of longer duration and more responsive to manipulation. Strategies can be employed to shorten this phase by prematurely regressing the existing CL (Whitley and Jackson, 2004; Wildeus, 1999). The use of PGF2a and/or its analogue alone, a double injection system 9 or 11 days apart, is the most widely used approach sheep in the breeding season (Wildeus, 1999).

The administration of $PGF_{2\alpha}$ in treatments 9 days apart and injection of three different doses of recombinant LH 48 hours after the second $PGF_{2\alpha}$ injection did not change the oestrus response. Oestrus responses between control and treatment groups were found to be similar.

**Table 1.** Effect of Recombinant Luteinizing hormone (rLH) on some fertility parameters in Awassi ewes.

| Parameters | Group 1; 0.5 IU | Group 2; 7.5 IU | Group 3; 10 IU | Control group; 1 ml saline |
|---|---|---|---|---|
| Estrus response (%) | 90.00 (18/20) | 85.00 (17/20) | 80.00 (16/20) | 90.00 (18/20) |
| Pregnancy rate (%) | 50.00 (9/18) | 47.06 (8/17) | 50.00 (8/16) | 50.00 (9/18) |
| Lambing rate (%) | 89.00 (8/9) | 100 (8/8) | 100 (8/8) | 100.0 (9/9) |
| Single rate (%) | 87.50 (7/8) | 100 (8/8) | 100 (8/8) | 100.0( 9/9) |
| Twining rate (%) | 12.50 (1/8) | 00 (0/8) | 00 (0/8) | 00 (0/9) |

NS: Non-significant (P>0.05).

The results of our investigation did not show any significant differences between the groups. Ultrasonography studies have demonstrated that the variable response to PGF2α injection in ewes was related with the specific ovarian status of each animal at the time when PGF2α was given (Vinoles and Rubianes, 1998). Rubianes et al. (2003), showed that ovulation occurred at a consistent interval (60 h) when a luteolytic dose of PGF2a was applied early in the luteal phase (i.e. 3 days post-ovulation). When a dose of PGF2 was administered together with a dose of GnRH around day 3 after ovulation luteolysis occurred in 85 % of the ewes, and this was followed by ovulation without oestrus (Rubianes et al., 1997).

In the present study, Oestrus induction and synchronization was successful after the PG-9 d protocol Awassi ewes during the breeding season. In addition, oestrus response occurred in a narrow ranges; 80-90 % of total ewes showed oestrus the second dose of PGF2α injection or 48 h after rLH. In the study, no differences in oestrus response beetwen rLH-treated and control group ewes were found. The results were similar to those (80.00 - 93.30 %) obtained by Hashemi et al. (2006), Kridli et al. (2003), Zarkawi et al. (1999), Zonturlu et al. (2011), and lover than 100 % got by Horoz et al. (1999) and Romano, (2004). This difference may be explained by the influence of breed differences on response to the treatment protocol.

Pregnancy rates (47.06 - 50 %) in rLH treatment groups compared with the control group were not observed a significant difference. in 1998, the European Recombinant LH Study Group, published the first randomized study with r-LH and concluded that the dose of 75 I.U. of r-LH may be effective to optimize the procedures of ovarian stimulation. Perhaps this situation may be explained with low dosage of LH at the time of insemination. One reasonable explanation for the overall low pregnancy rate observed in this study might be the generally low body condition score of ewes. (Fukui et al., 1991) reported no influence of GnRH administration on pregnancy rates in sheep. Ataman and Aköz (2006), have suggested that no significant difference was found between Akkaraman cross-bred sheep synchronized with GnRH – PGF2α or two PGF2α injections in the pregnancy rate and the litter size in the breeding season. Pregnancy rates in Awassi ewes treated by equine chorionic gonadotropin (eCG) in outside the normal breeding season and transition period were higher than this research (Zarkawi et al., 1999; Zonturlu et al., 2011). Pregnancy rates in the lowest doses of rLH were similar to its highest doses and control group. Injection of multiple doses of hCG (100 IU) administered on days three-five after artificial insemination did not increase pregnancy rates in artificially inseminated ewes (Fukui et al., 2001). These results are in agrement with studies in sheep (Zamiri and Hosseini, 1998) in which treatment of hCG on the day of artificial insemination or mating was not effective in improving fertility. These results are in contrast with reports by (Khan et al, 2003; Lopez-Gatius, 2000), in which hCG given on day of mating increased the pregnancy and effectively improved the fertility of ewes. Menchaca et al. (2004), reported that the best pregnancy rate was obtained in ewes inseminated at 42 h after a 7-day interval PGF2α protocal. These differences could possibly be due to the different protocols used. But it is also probable that other factors such as nutritional, physiological status, the doses of rLH and breed could also have affected the results. Different doses of rLH in the present experiment (Table 1) had no significant effect on lambing rates. Lambing rates in treatment and control group were 89.00-100 % and 100 % respectively. These were lower than that of Awassi and Polwarth ewes treated with MAP followed by treatment with eCG (Vinoles et al., 2001; Zarkawi et al., 1999), and higher than 70.0%, 63.0% reported by Das et al., 2000 and Knights et al., 2001, which in ewes treated with double PGF2α, intravaginal sponges followed by PMSG and CIDR followed by FSH injection, respectively. LH/hCG induces to an increase in the granulose cells in cows having the late follicular phase (Mihm et al., 2006). However, exogenous LH due to the disappearance of low LH and high receptor affinity lead to stimulation and

high and long-lived of the corpus luteum (Krause and Ohlinger, 2006). Single rates and twinning rates in three different rLH groups were similar to control group. Single rates and twinning rates in rLh groups and control group were similar to a different research in Awassi ewes (Krause et al., 2006). The effect of the LH in follicular development can be only provided by a reduction in the concentration of serum FSH (Balascha and Fábreguesa, 2003). Recombinant LH is a useful tool supporting the final stage of follicular maturation (Balascha and Fábreguesa, 2003). In prepubertal gilts, LH alone decreased numbers of small follicles and stimulated growth of only a few large follicles (Guthrie et al, 1990).

Furthermore, Anderiesz et al. (2000), demonstrated that the join supplementation of rFSH and a high concentration of rLH to in-vitro maturation medium improves the embryonic development of bovine oocytes. Relevant to this, Çoyan et al. (2003), reported that hCG was a good exogenous of LH to accelerate the ovulation.

Results of this experiment showed that estrus response rates, pregnancy and lambing rates in three different rLH groups were similar to controls. These data indicate that the three different doses of rLH after oestrus synchronization with treated $PGF_{2\alpha}$ in Awassi ewes do not effect on the fertility parameters. Nevertheless, further investigation is needed comparing different protocols in order to obtain a higher pregnancy rate, aiming at the optimization of ovarian stimulation or best pregnancy protocols for oestrus synchronization and exogenous rLH support.

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