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RESEARCH ARTICLE

Effect of exogenous progesterone and gonadotropin-releasing hormone application on maintenance of pregnancy in early pregnant ewes after prostaglandin F2 alpha injection

Mehmet Köse[1]*, Mehmet Salih Kaya[2], Mehmet Osman Atlı[1]

[1]Department of Obstetrics and Gynaecology, [2]Department of Physiology,
Faculty of Veterinary Medicine, Dicle University, Diyarbakır, Turkey
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*mehmetkose1977@gmail.com

Gebeliğin erken dönemindeki koyunlar-da prostaglandin F2 alfa enjeksiyonu sonrası eksojen progesteron ve gonadotropin salınım uyarıcı hormon uygulamasının gebeliğin devamlılığı üzerine etkisi

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Öz

Amaç: Sunulan bu deneysel çalışmada, koyunlarda gebeliğin erken döneminde prostaglandin F2alfa (PGF2α) enjeksiyonu sonrası eksojen progesteron (P4) ve gonadotropin releasing hormon (GnRH) uygulamalarıyla plazma P4 düzeyinin yüksekliğinin sürdürülerek gebeliğin devamlılığının sağlanabileceği test edildi.

Gereç ve Yöntem: Bu amaçla, 9 gebe koyun 18. günde (çiftleşme günü 0. gün olarak kabul edildi) iki gruba ayrıldı; (1) PGF2α grubu (18. gün 125 mcg d-cloprostenol enjeksiyonu, n=5); (2) PGF2α + P4 + GnRH grup (18. gün 125 mcg d-cloprostenol enjeksiyonu + 20 mg flugestone acetate sponge (P4 sponge), intravaginal 7 gün süreyle, 22. gün 10 mg buserelin acetate (GnRH analoğu) enjeksiyonu, n=4). P4 sponge 25. günde uzaklaştırıldı. Çiftleştirme sonrası 18 ve 19. günlerdeki plazma P4 konsantrasyonu ECLIA ile ölçüldü. Gebelik muayeneleri 18, 22, 25 ve 35. günlerde rektal yolla ultrasonografi ile yapıldı. Gruplar arasındaki istatistiki farklılıklar ki-kare ile analiz edildi.

Bulgular: P4 konsantrasyonu iki grupta da 19. günde 1 ng/mL'nin altına düştü. PGF2α grubundaki koyunların tamamında (5/5) gebelik 25. güne kadar sonlanırken, PGF2α + P4 + GnRH grubundaki koyunlarda (4/4) ise P4 sünger uygulaması gebeliğin sonlanmasını önledi (P<0.02). P4 uygulaması sonlandırıldıktan sonra bu gruptaki koyunların 2'sinde GnRH enjeksiyonuyla gebelik doğuma kadar devam etti.

Öneriler: Sonuçlar eksojen P4 uygulamasının erken gebe koyunlarda korpus luteum (CL) regrese olduğunda da gebeliği devamlılığı için yeterli olduğu gösterdi. Bununla birlikte çiftleştirme sonrası 22. günde GnRH enjeksiyonuyla dominant follikülün luteinize edilebileceği ve bu eksojen P4 uygulaması sonlandırıldığında da gebelik devam etmesi için yeterli olabilir.

Anahtar kelimeler: Koyun, eksojen progesteron, GnRH, gebeliğin devamlılığı

Abstract

Aim: We tested the hypothesis that exogenous progesterone (P4) and gonadotropin-releasing hormone applications (GnRH) would prevent detrimental effect of prostaglandinF2alfa (PGF2α) on early pregnancy in ewes by sustaining high plasma progesterone level.

Materials and Methods: For this purpose, nine pregnant ewes (mating=0) were divided into 2 groups on day 18 as follows: (1) PGF2α group (125 mcg of d-cloprostenol injection on day 18, n=5); (2) PGF2α + P4 + GnRH group (125 mcg of d-cloprostenol injection on day 18 + 20 mg flugestone acetate (P4) for 7 days + 10 mg of buserelin acetate injection, on day 22, n=4). P4 was withdrawn on day 25. P4 concentration was measured on days 18 and 19 by ECLIA. Pregnancies were examined by using transrectal ultrasonography on days 18, 22, 25, and 35. Statistical difference between groups was analysed by chi-squared test.

Results: P4 concentration declined to below 1 ng/mL on day 19 in both groups. While all pregnancies were terminated in PGF2α group by day 25, P4 prevented pregnancy loss in PGF2α + P4 + GnRH group (P<0.02). When P4 was withdrawn on day 25, half of pregnancies continued after GnRH in PGF2α + P4 + GnRH group until birth.

Conclusion: Results suggest that exogenous P4 application is suf-ficient for maintaining early pregnancy in ewes, even if the corpus luteum (CL) is regressed. Furthermore, GnRH application on day 22 might luteinize dominant follicles and could be sufficient to maintain pregnancy after P4 is removed.

Keywords: Ewe, exogenous progesterone, GnRH, maintenance of pregnancy.

Introduction

P4 is an indispensable steroid hormone during pregnancy in mammals due to its effects on uterine functions such as decreasing contraction of myometrium, modulation of immune system and endometrial nutritional secretions for unattached embryo (Hansen 2007, Dorniak and Spencer 2013). Conceptus-maternal interactions for maternal recognition of early pregnancy, regulation of implantation process and formation of placenta for embryonic development and fetal growth until the birth are all regulated by P4 (Spencer et al 2004a, 2004b). During the early pregnancy, CL secretes P4 that is the only source for P4 in many species (Niswender et al 2000, Spencer et al 2004c).

Until the full formation of placenta that occurs between day 50 and 60 of pregnancy in ewes, P4 sourced from CL are responsible for all the functions mentioned above. P4 has very short half-life and any reasons that cause regression of CL before the beginning of placental P4 production rapidly decreases plasma P4 concentrations and result in pregnancy termination (Weems et al 2006, Sammin et al 2009).

Early embryo when not attached to endometrium is nourished with uterine milk (histotroph) that is secreted from endometrial glands in intercaruncular area and transferred into uterine lumen (Spencer et al 2004a, Bazer et al 2014). Growing embryo needs more histotroph. Therefore, endometrial glands must enlarge. Uterus must be exposed to estrogen and then P4 to complete all the endometrial morphogenic changes (Spencer and Bazer 2004). Specifically, P4 induces many gene expressions in endometrium including galectin 15 (LGALS15), insulin-like growth factor binding protein 1 (IGFBP1), cystatin C 3, gastrin releasing polypeptide (GRP), sodium-dependent glucose transporter (SLC5A11) that are related to embryonic growth and implantation (Bazer et al 2010, Bazer et al 2011). Moreover, interferone-tau, placental lactogen and placental growth hormone are all regulated by maintenance of P4 secretion (Spencer et al 2004b).

GnRH and its analogs are widely used to induce ovulation due to its effects on the release of the gonadotropic hormones from the anterior pituitary (Schneider et al 2006, Paksoy and Kalkan 2010). Studies in cows and ewes have shown that luteal structure formed after GnRH injection is capable of producing P4. Therefore, GnRH and its analogues could be used to induce ovulation of dominant follicles in the luteal phase thus may reduce pregnancy losses in farm animals due to P4 deficiency (Peters 2005).

Although an application of P4, GnRH and PGF2α for estrous synchronization are very well documented in ewes, effects of those hormones on early pregnancy need to be elucidated. Therefore, in this experimental study, we tested the hypothesis that exogenous P4 and GnRH applications would prevent the detrimental effect of PGF2α on early pregnancy in ewes by sustaining high plasma P4 level.

Materials and Methods

This study was conducted in Research and Application farm of Faculty of Veterinary Medicine, Dicle University, Turkey and was approved by local animal ethic committee (2011-66). A total of nine pregnant ewes were used as animal material. Briefly, estrus of ewes were synchronized with intravaginal sponges containing 20 mg flugestone acetate (Chronogest CR, Intervet, Turkey) for 9 days, and mating day was recorded as day zero (d 0). Ewes were mated with fertile rams. Pregnancy were detected by transrectal ultrasonography on day of 18 after mating according to observation of an echoic embryo in embryonic vesicle and then pregnant ewes were divided into 2 groups on day 18 as follows: (1) PGF2α group (on day 18, 125 mcg of d-cloprostenol injection (Dalmazin, Vetas, Turkey), n=5); (2) PGF2α + P4 + GnRH group (125 mcg of d-cloprostenol injection on day 18 + 20 mg flugestone acetate via intra vaginal for 7 days, n=4) Moreover, 10 mg of buserelin acetate (a GnRH analog, Receptal, Intervet, Turkey) was injected intramuscularly to PGF2α + P4+ GnRH group ewes on day 22 after mating to induce luteinization in dominant follicles. The P4 sponge was withdrawn on day 25 (n=4). Blood samples were collected from pregnant ewes on days of 18 and 19 in tube containing Na-ETDA and centrifuged at 4000 rpm for 10 min. Plasma samples were stored at -20°C until P4 assay. Plasma P4 concentration was measured using the electro chemiluminescence immunoassay (ECLIA) kit obtained from Roche Diagnostics GmbH, D-68298 Mannheim, Germany. Inter assay and intra-assay coefficients of variation (CV) were 10.3% and 6.4% for P4, respectively. Pregnancies were re-examined by using transrectal ultrasonography (8 MHz probe, PIE Medical Falco; Esaote, Maastricht, The Netherlands) on days 22, 25, and 35 after mating, and the size and wholeness of embryonic vesicle and embryo were evaluated in two group ewes. Statistical difference between groups for pregnancy was analyzed by chi-squared test and $P<0.05$ was considered as significant.

Results

Serum P4 levels were higher in both groups on day 18 whereas after PGF2α injection, P4 concentration declined to below 1 ng/mL on day 19 in both groups. Plasma progesterone concentration decreased from 3.07±0.27 ng/mL on day 18 to 0.52±003 ng/mL on day 19 in PGF2α group. Similarly, plasma progesterone dropped from 3.15±0.53 ng/mL on day 18 to 0.45±0.04 ng/mL on day 19 in PGF2α + P4 + GnRH group. It is clearly indicated that luteolytic dose of PGF2α immediately (within 1 day) decreased plasma P4 levels in pregnant ewes. After PGF2α injection, pregnancy status of ewes on day of 25 was shown on Table 1. While all pregnancies were terminated (5/5) in the PGF2α group by day 25, P4 sponge

Table1. Pregnancy status of ewes during examined days in groups.

Groups	Examined days				
	18	22	25	35	Birth
PGF2α	5/5	0/5[a]	0/5[a]	-	-
PGF2α + P4+ GnRH	4/4	4/4[b]	4/4[b]	2/4	2/4

[a, b]: Means with different superscripts in the same column are significantly different (P < 0.001).

application prevented pregnancy loss (4/4) in the PGF2α + P4 + GnRH group (P<0.02). When the P4 sponge was withdrawn on day 25, half of the pregnancies (2/4) continued in the PGF2α + P4 + GnRH group until birth.

Discussion

In this present study, it is shown that exogenous P4 and GnRH applications could prevent the detrimental effect of PGF2α on early pregnancy in ewes by sustaining high plasma P4 level. Although P4 level declined below 1 ng/ml on day 19 in both groups which clearly indicate this dose of PGF2α injection cause luteolysis, a only source of P4, intra-vaginal P4 sponge (20 mg), was sufficient to maintain pregnancy. Moreover, half of the pregnancies continued after GnRH application in the PGF2α + P4 + GnRH group may indicate that GnRH application on day of 22 could lead the formation of luteal tissue in ovine ovaries which also indicate present dominant follicles on this day. In the present study, dose of PGF2α was administered as same dose for estrus synchronization in ewes. Furthermore, embryo can clearly be observed on day of 18, therefore we chose this day for PGF2α injection.

In non-pregnant ruminants including cows, ewes and goats, pulsative secretion of endometrial PGF2α at the end of the diestrous has been very well documented (Niswender et al 2000). Moreover, when applied exogenously, natural or synthetic derivatives of PGF2α caused functional luteolysis CL much faster than normal luteolysis (Fierro et al 2013). In this study, P4 levels were declined to below 1 ng/mg within 24 hours in both cyclic and pregnant ewes following the injection of PGF2α. In accordance with this study result, plasma P4 levels were declined to below 1 ng/mg in all pregnant ewes within one day in our study.

After early embryonic death, both embryo and surrounding fluids are resorbed by uterus or expelled through cervix uteri and restart of new estrous cycle varies due to timing of embryonic death (Thwaites 1972, Sawyer and Knight 1975, Silvia and Niswender 1989, Fierro et al 2013). In the present study, both embryo and embryonic fluids were resorbed within 7 days in PGF2α group. In contrast, although PGF2α injection causes luteal regression in PGF2α + P4 sponge + GnRH group, embryo resorption was prevented by intra-vaginal application flugestone acetate and GnRH in four ewes. Similarly, Zhang and Miller (1989) showed that cronolone acts as natural P4 and is sufficient to maintain pregnancy in overectomized pregnant ewes. It is well known that secretion of P4 from ovarian CL is an essential factor for maintenance of pregnancy during early pregnancy in ruminants, ewes, cows, goats etc (Spencer et al 2004c).

In the present study, ovaries were not monitored to observe follicular status. However, on day 22, GnRH was injected in the PGF2α + P4 + GnRH group to induce ovulation of any dominant follicle. When follicles in ewe reaches ≥ 4 mm on the ovary gain luteinizing hormone (LH) receptors and ovulation that follicle could be induced by human chorionic gonadotropin (hCG) or GnRH (Driancourt 2001). The follicular growth in sheep is continuous, even two days after luteectomia, endogenous or exogenous gonadotropin administration is capable of inducing formation of new functional CL (Munoz-Gutierrez et al 1998). Leyva et al (1998) reported that P4 level increased rapidly after ovulation, and the functional CL was visible on day 2 after ovulation in ewes. Furthermore, it was also detected presence of follicles which are sensitive to GnRH between 25 and 100 of day during pregnancy. GnRH injection on day 22 could possibly induced formation of new CL which secreted P4 to replace sponge-released P4 after withdrawal (Avdi et al 2001).

Conclusion

As conclusion, the present study clearly indicated that PGF2α injection on day 18 after mating ended pregnancy in ewes irreversibly. The results also suggest that exogenous P4 application is sufficient for maintaining early pregnancy in ewes, even if the CL is regressed. Furthermore, GnRH application on day 22 after mating could luteinize dominant follicles and be sufficient to maintain pregnancy after exogenous P4 is removed.

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