Estrus syncrhonization in ewes with prostaglandins at different days post CIDR insertion

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ABSTRACT

Objective: To evaluate the effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) application on days 0, 3 and 6 of the synchronized luteal phases on reproductive perfomance and its effect on progesterone concentrations of multiparous ewes.

Design/methodology/approach: The experimental design was a completely randomized. Seventy-five ewes synchronized with control internal drug release devices (CIDR) for six days and 250 UI of equine chorionic gonadotropin (eCG) were used at device removal. The ewes were randomly distributed in three treatments in relation to the days of application of $PGF_{2\alpha}$ (125 μg sodium cloprostenol): in the first group of ewes, $PGF_{2\alpha}$ was applied at the time of insertion of the CIDR (D0, n=25); in the second group $PGF_{2\alpha}$ was applied on day three of insertion of the CIDR (D3, n=25) and in the third group it was applied on day six, at the time of the withdrawal of the CIDR (D3, n=25). The presentation of estrus and the gestation rate were analyzed through the χ^2 test. The onset of estrus and prolificacy by a Tukey analysis of variance and comparison of means. The concentration of P_4 was carried out using the mixed design procedure, which included fixed effects of treatment and day, and interaction of both.

Results: Presentation and onset of estrus were different ($P<0.05$) between treatments due to the effect of the interval in the days of $PGF_{2\alpha}$ application. The progesterone (P_4) concentrations in blood serum showed differences during the synchronized luteal phase (D0: 4.8 ng mL^{-1}; D3: 6.0 ng mL^{-1}, D6: 8.8 ng mL^{-1}). However, no differences were found in gestation rate and prolificacy due to the main effects.

Limitations on study/implications: The application of 125 μg of $PGF_{2\alpha}$ on different days of the synchronized luteal phase does not affect gestation rate and prolificacy. Nevertheless, presentation and onset of estrus were different, so it must be considered in laparoscopic artificial insemination programs.

Findings/conclusions: The application of $PGF_{2\alpha}$ during the synchronized luteal phase at short intervals showed better results at the end of treatment. The corpus luteum (CL) and CIDR increase P_4 concentrations; which modifies the estrus response, and it has a positive effect in reproductive performance.

Keywords: Estrus, synchronization, CIDR, Prostaglandin $F_{2\alpha}$.

INTRODUCTION

Hormonal protocols manipulate physiological moments of the estrous cycle and allow for the presence of estrus in a large number of ewes within a short and defined period, thereby increasing flock fertility (Arya *et al.*, 2023); however, factors such as photoperiod, nutrition, breed, facilities, and other elements affecting animal welfare must be considered (Simões *et al.*, 2021).

In ewes, estrus synchronization protocols primarily involve the use of controlled internal drug release devices (CIDR) for 12 to 14 days to simulate the luteal phase (Hameed *et al.*, 2021). However, when combined with gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), equine chorionic gonadotropin (eCG), and prostaglandins ($PGF_{2\alpha}$), the estrus response is enhanced (González *et al.*, 2012). In this context, some researchers have reduced the exposure time to progestogens, proposing short-term synchronization protocols (5 to 7 days) aimed at synchronizing both estrus and ovulation, as well as the follicular wave (González-Bulnes *et al.*, 2020), which allows for higher fertility compared to conventional protocols during the breeding season (Takci and Kivrak, 2022).

Furthermore, the use of progestogens in synchronization protocols combined with $PGF_{2\alpha}$ for a short period is an economical and flexible alternative for field conditions, yielding promising fertility results (Sinimbu *et al.*, 2022). The use of $PGF_{2\alpha}$ is effective for inducing luteal regression in most ewes, with variability observed in the occurrence of estrus and ovulation (Tsai and Wiltbank, 1997). This variability in response is attributed to the timing of $PGF_{2\alpha}$ administration, as the corpus luteum only responds between days 3 and 14 of the estrous cycle (Rubianes *et al.*, 2003). Conversely, it has been reported that the use of $PGF_{2\alpha}$ at short intervals may be an appropriate alternative for synchronizing estrus and performing artificial insemination (AI) in ewes (Contreras-Solís *et al.*, 2009). The present study was conducted with the assumption that the application of $PGF_{2\alpha}$ on different days during a short synchronization protocol (6 days) in pre-synchronized ewes would allow for the identification of changes in reproductive behavior by lysing the corpus luteum (endogenous source of P_4) at the time of CIDR insertion (CL at 3-4 days), three days after CIDR insertion (CL at 6-7 days), or at the time of CIDR withdrawal (CL at 9-10 days). This would explain whether the exogenous source (CIDR) alone is capable of maintaining a sufficient level of P_4 to improve reproductive variables and sustain gestation.

MATERIALS AND METHODS

This study was conducted in October-November 2022 at the Sheep Experimental Unit of the Colegio de Postgraduados, Montecillo Campus, State of Mexico (19° 27' 18" N and 98° 54' 26" W), at an altitude of 2,220 meters above sea level; the climate is temperate subhumid with rainfall in the summer.

Animal management was conducted in accordance with the ethical and biosecurity standards of the Council for International Organizations of Medical Sciences (CIOMS, 1986), in compliance with Mexican law (NOM-062-ZOO-1999) for the use of animals in experimentation (DOF, 2001), and with regulations for the use and care of research animals, approved by the Animal Welfare Committee of the Colegio de Posgrados, Mexico (COBIAN/007/23).

Animals and Treatments

During the breeding season, 75 multiparous ewes of the Dorset × Katahdin cross were used, with an average weight of 50±2.2 kg and an average body condition score of 3 on a scale of 1 to 5. The ewes were previously dewormed and vitaminized, and an ultrasound was performed to confirm that they were not pregnant (CHISON, Eco 6). The ewes were fed *ad libitum* on oat hay (*Avena sativa*) and given 300 g of commercial feed containing 14% crude protein (CP) and 2.4 Mcal kg^{-1} of metabolizable energy (ME), according to the nutritional requirements for sheep (NRC, 2007), in addition to free access to water. All ewes were pre-synchronized with two doses of prostaglandin $F_{2\alpha}$ (125 μg sodium cloprostenol, Celosil®) at an 8-day interval. Six days after the second dose of this hormone, CIDR devices were inserted to control the lifespan of the CL.

For estrus synchronization, the CIDR device (Zoetis®) was used for 6 days, along with the application of 250 IU of eCG (Gonactive®, Virbac) at the time of its removal. Subsequently, the ewes were randomly distributed into three treatments based on the days of $PGF_{2\alpha}$ (125 μg sodium cloprostenol) application: the first group of ewes received it at the time of CIDR insertion (D0, n=25), the second group received it on day three after CIDR insertion (D3, n=25), and the third group received it on day six, at the time of CIDR removal (D6, n=25).

Estrous presence was determined 24 hours after CIDR removal. The rams were randomly assigned by treatment, and controlled mating was carried out, with each ram mating each ewe two times. The first mating occurred upon detection of estrus, followed by one additional mating 12-hour later. Return to estrus was detected between 14 to 17 days after mating, twice a day (morning and afternoon). Pregnancy was confirmed 30 days post-mating via transrectal ultrasonography.

Sample Collection and Laboratory Analysis

Blood samples (5 mL) were collected via jugular vein puncture at 8:00 a.m. To determine serum P_4 concentration, samples were collected one day before CIDR insertion and subsequently every 48 hours for 17 days. All samples were centrifuged at 1500 g at 5 °C for 20 minutes (International Equipment Company, USA); the blood serum was separated and stored in 1.5 mL microtubes (Axigen™) for preservation at −20 °C in a freezer until hormonal analysis was performed. The P_4 analyses were performed using ELISA with the kit (DRG® Progesterone ELISA). The analytical sensitivity was 0.045 ng/mL, and the intra- and inter-assay coefficients of variation were 7% and 9%, respectively.

Statistical Analysis

The experimental design was completely randomized, where each ewe represented an experimental unit. The percentage of estrus occurrence and pregnancy rate were analyzed using the χ^2 test through PROC NPARIWAY. For the onset of estrus and prolificacy index, an analysis of variance was conducted using PROC GLM and a Tukey's mean comparison test ($P<0.05$). For the concentration of P_4, a repeated measures analysis of variance over time was performed using PROC MIXED, which included fixed effects of treatment

and day, as well as their interaction. All procedures were performed using the Statistical Analysis System (SAS, 2009).

RESULTS AND DISCUSSION

Estrus Presentation and Onset

In the present study, the presentation of estrus differed among treatments ($P<0.05$) due to the timing of $PGF_{2\alpha}$ administration, with 100% observed in the D3 and D6 groups, compared to 60% in the D0 treatment. The onset of estrus also differed among treatments ($P<0.05$) based on the day of $PGF_{2\alpha}$ application, with early estrus observed in the D0 and D3 groups compared to the D6 group (Table 1).

The D0 group exhibited a lower percentage of estrus (60%, $p<0.05$) compared to the D3 and D6 treatments, which can be attributed to the presence of an immature CL in the females that did not show estrus. In this regard, Wiltbank *et al.* (1995) have mentioned that $PGF_{2\alpha}$ does not affect the development and lifespan of the CL when administered before day 5 post-estrus, attributing this to the absence or low presence of receptors in luteal cells; this is like the D0 treatment and is corroborated by the P_4 concentrations following $PGF_{2\alpha}$ administration in this group of ewes (Figure 1).

The percentages of estrus in the D3 and D6 treatments are similar to those obtained by Contreras-Solís *et al.* (2009) and Balan-May *et al.* (2021), who mention that administering $PGF_{2\alpha}$ to ewes on days 3, 5, and 7 of the estrous cycle can achieve up to 100% estrus. Regarding the onset of estrus, various studies reported that approximately 90% occurs within a 26 to 72 h interval after the removal of the CIDR (Rubianes *et al.*, 2003; Contreras-Solís *et al.*, 2009). However, in the present study, the onset of estrus was influenced by the timing of $PGF_{2\alpha}$ administration during the luteal phase synchronized with the CIDR, as $PGF_{2\alpha}$ causes early regression of the CL and consequently decreases the concentration of P_4 in serum.

Contreras-Solís *et al.* (2009) and Balan-May *et al.* (2021), when synchronizing with $PGF_{2\alpha}$ on days 3, 5, and 7, observed an onset of estrus ranging from 28.0±3.1 h to 38.6±3.3 h, with the earliest onset occurring on day 3, which is similar to the findings of this study. In contrast, Urviola *et al.* (2005) reported an onset of estrus of 34.28±4.26 h and 47.4±7.6 h when administering $PGF_{2\alpha}$ on days 4 and 10 of the estrous cycle. This

Table 1. Response of reproductive variables in ewes synchronized with CIDR for a short period (6 days) with the application of $PGF_{2\alpha}$ on days 0, 3, and 6 during the luteal phase.

Reproductive variable	Treatments		
	D0 (n=25)	**D3 (n=25)**	**D6 (n=25)**
Estrus presentation (%)	60 (15/25)^{b}	100 (25/25)^{a}	100 (25/25)^{a}
Estrus onset (h) $^{\dagger 1}$	26.40±1.38^{b}	27.60±1.07^{b}	39.84±1.07^{a}
Gestation (%)^{2}	48 (12/25)	72 (18/25)	60 (15/25)
Prolificacy index$^{\dagger 3}$	1.7±0.1	1.6±0.1	1.6±0.1

[1] Time referred to the withdrawal of the device. [2] Based on serum P_4 profiles and ultrasound on day 30. [3] Number of lambs born per ewe that lambed. [a, b] Values with different letters between columns are different ($P<0.05$). [†] Means ± standard error.

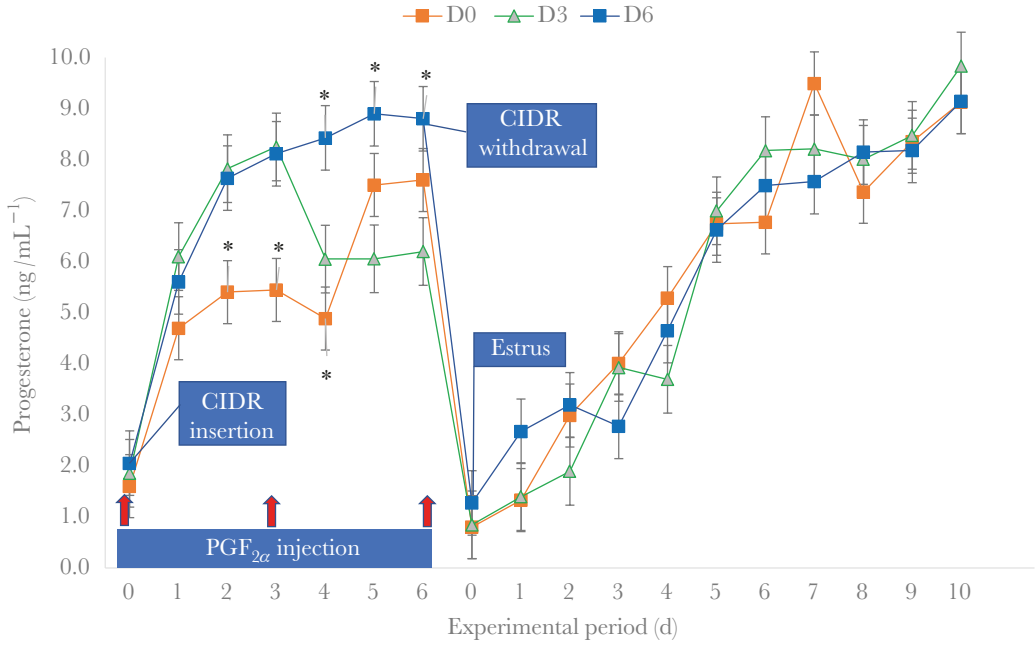

Figure 1. Plasma progesterone concentration (mean ± standard error) during the experimental period in multiparous ewes. * Indicates statistical difference between experimental groups.

variability is attributed to the state of the dominant follicle of the first follicular wave and the developing CL in relation to the timing of synchronization. Champa (2000) reported that estrus and ovulation are less variable during the early luteal phase than during the late luteal phase; that is, when $PGF_{2\alpha}$ induces regression of the CL at earlier stages of its development (Urviola *et al.*, 2005).

Hormonal Profile of Progesterone (P_4)

The concentrations of P_4 in serum were different ($P<0.05$) during the synchronized luteal phase (Figure 1). The results obtained in this study show a higher concentration in the D6 group (8.8 ng mL^{-1}) compared to D3 (6.0 ng mL^{-1}) and D0 (7.5 ng^{-1}). Wheaton *et al.* (1993) reported that serum P_4 concentration rapidly increases after CIDR insertion and decreases after its removal. In the present investigation, P_4 concentrations were above 1 ng/mL prior to device insertion due to prior synchronization and the development of different stages of the CL; the variations in the P_4 curve during the synchronized luteal phase in the D0 and D3 treatments indicated CL lysis following the administration of $PGF_{2\alpha}$ (Figure 1). However, in all three groups, levels fell below 1 ng mL^{-1} 24 h after the CIDR was removed.

Various authors mentioned that the presence of CL and synchronization with CIDR in sheep increases serum P_4 concentrations; Molina-Mendoza *et al.* (2005) reported 8.6 ng mL^{-1} in a group of sheep synchronized for 12 days with the presence of CL; Campero *et al.* (2023) found concentrations of 8.2 ng mL^{-1} in sheep synchronized for 6 days and 10 ng mL^{-1} in sheep synchronized for 12 days, both treatments with the presence of CL. On the other hand, Cordero *et al.* (2023) observed that primiparous ewes have higher concentrations than multiparous ewes.

The increase in P_4 concentrations due to the presence of CL in synchronization protocols must be considered as it modifies the onset of estrus; therefore, $PGF_{2\alpha}$ should preferably be administered before the removal of the CIDR.

Gestation Rate and Prolificacy

The gestation rate showed no differences ($P>0.05$) in the present study; it was 48% for the D0 treatment, while for D3 it was 72%, and for D6 it was 60%. These rates are lower than those reported by Balan-May *et al.* (2021), with 70% and 90% for synchronization protocols in short periods, but similar to those reported by Urviola *et al.* (2005), with gestation rates of 63.6% and 65.0% in ewes synchronized with $PGF_{2\alpha}$ on days 4 and 10 of the estrous cycle; in both studies, the ewes received direct mounting with proven fertile rams. For their part, Contreras-Solís *et al.* (2009) reported rates of 62.5%, 44.0%, and 47.4% in ewes inseminated and synchronized with $PGF_{2\alpha}$ on days 3, 5, and 7, respectively.

Cordero *et al.* (2023) reported a 100% gestation rate in primiparous and multiparous ewes with short synchronization protocols using CIDR (6 days); meanwhile, Ávila *et al.* (2019) mentioned that treatments with $PGF_{2\alpha}$ are effective during the reproductive season, whether in double applications with intervals of 8 days, combined with progestogens, or with GnRH for 6 days. Campero *et al.* (2023) established that regardless of whether a functional CL is present or not, when initiating estrous synchronization in short periods, it is necessary to administer prostaglandin $F_{2\alpha}$ at the end of the treatment to avoid the negative effect of the CL on the estrous response and to achieve steroid feedback.

In the present study, the prolificacy index did not show differences between treatments ($P>0.05$), although it is similar to that reported by Balan-May *et al.* (2021), with prolificacy rates of 1.42, 1.44, and 1.55 for hormonal treatments with $PGF_{2\alpha}$ applied on days 3, 5, and 7. Similarly, Almadaly *et al.* (2023) mentioned that the application of $PGF_{2\alpha}$ on days 7 and 14 results in prolificacy indices of 1.3 and 1.6, respectively.

CONCLUSIONS

The luteolysis triggered by the application of $PGF_{2\alpha}$ at the time of CIDR insertion limits the increase of P_4 during the first 4 days in short synchronization protocols (6 days) in ewes. Meanwhile, the application of $PGF_{2\alpha}$ at 3 days post-CIDR insertion reduces and limits the peak of P_4 concentration, which implies that the presence of the CL exerts a positive effect in synchronization protocols, as lower concentrations of P_4 have been associated with a negative effect on reproductive variables.

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REFERENCES

Almadaly, E. A., Sahwan, F., Wael B, E. D., M. Fawzy, A., Shukry, M., & Farrag, F. (2023). Comparison of estrus response and subsequent fertility following estrus synchronization with six protocols in Ossimi

ewes during the early summer season: Ossimi ewe fertility during the early summer season. *Veterinaria*. México. doi:10.22201/fmvz.24486760e.2023.1058.

Arya, D Goswami, R., Sharma, M. (2023). Estrous synchronization in cattle, sheep and goat. *Multidisciplinary Reviews. 6*(1): 2023001-2023001. doi:10.31893/multirev.2023001.

Ávila-Castillo, BR., García-Flores, E.O., Molina-Mendoza, P., German Peralta-Ortiz, J.G., & Sánchez-Torres-Esqueda, M.T. (2019). Sincronización del estro en ovejas de pelo mediante protocolo basado en prostaglandinas + GnRH. *Biotecnología y Ciencias Agropecuarias. 13*(2): 141-151. doi: 10.29059/cienciauat.v13i2.1012.

Balan-May, Daniel, Chiquini-Medina, Ricardo, Flota-Bañuelos, Carolina, Hernández-Marín, Antonio, Rosales-Martínez, Verónica, & Fraire-Cordero, Silvia. (2021). Short-term protocols for the estrus synchronization in hair sheep in Campeche, México. *Abanico veterinario. 11*(123). doi:10.21929/abavet2021.34

Campero-Cruz, A., Sánchez-Torres, M.T., Figueroa-Velasco, J.L., Martínez-Aispuro, J.A., Nieto-Aquino, R., Cordero-Mora, J.L., López-García, S., Martínez-Cruz, I., & Cárdenas-León, M. (2023) Importancia del cuerpo lúteo al iniciar protocolos para sincronizar estro en ovejas. *Ecosistemas y Recursos Agropecuarios 10*(3): e3312. doi: 10.19136/era.a10n3.3312.

Champa, L. (2000). Efecto de la ovulación del folículo dominante del día 7 y 13 del ciclo estral con PGF2α sobre las tasas reproductivas en vacas. Tesis de Médico Veterinario. Facultad de Medicina Veterinaria, Univ. Nacional Mayor de San Marcos. Lima. 56 p.

CIOMS (Council for international Organizations of Medical Sciences). (1986). "International Guiding Principles for Biomedical Research Involving Animals". CIOMS, Geneva, Switzerland.

Contreras-Solis, I., Vasquez, B., Diaz, T., Letelier, C., Lopez-Sebastian, A., & Gonzalez-Bulnes, A. (2009). Efficiency of estrous synchronization in tropical sheep by combining short-interval cloprostenol-based protocols and "male effect". *Theriogenology. 71*(6): 1018-1025. doi: 10.1016/j.theriogenology.2008.11.004.

Cordero-Mora, J.L., Sánchez-Torres, T., Nieto-Aquino, R., Salinas-Ríos, T., Hernández-Bautista, J., Figueroa-Velasco, J.L., & Martínez-Aispuro, J.A. (2023). Synchronization of the estrous during a short period, using a low dose of equine chorionic gonadotropin (eCG) in primiparous and multiparous ewes. *Agro Productividad*. doi: 10.32854/agrop. v16i2.2263.

DOF (Diario Oficial de la Federación). (2001). "Norma Oficial Mexicana NOM-062-ZOO-1999: Especificaciones técnicas para la producción, cuidado y uso de animales de laboratorio". México, D.F.

González, J. F. L., Velásquez, L. F. U., & Osorio, J. H. (2012). Control hormonal de la reproducción en hembras ovinas (Ovisaries). *Revista Veterinaria y Zootecnia. 6*(2): 134-147.

González-Bulnes, A., Menchaca, A., Martin, G. B., & Martinez-Ros, P. (2020). Seventy years of progestagen treatments for management of the sheep oestrous cycle: Where we are and where we should go. *Reproduction. Fertility and Development. 32*(5): 441-452. doi:10.1071/RD18477.

Hameed, N., Khan, M. Lu, R., & Zubair, M. (2021). Approaches of estrous synchronization in sheep: developments during the last two decades: a review. *Trop Anim Health Prod. 53*(485) doi: 10.1007/s11250-021-02932-8.

Molina-Mendoza, P., Sánchez-Torres-Esqueda T., García-Flores, E.O., Martínez-García, A., Cárdenas-León, M., Peralta-Ortiz, J., Cordero-Mora, J.L., Hizarza-Espinoza, A., & Ortega-Cerrilla, M.E. (2005) Manipulación de la presencia del cuerpo lúteo en la sincronización de estro en ovejas Dorset. *Agrociencia* 39: 11-18.

National Research Council (NRC). (2007). Nutrient requirements of small ruminants. Sheep, goats, cervids and new world camelids. National Academy Press, Washington D.C.

Rubianes, E., Menchaca, A., & Carbajal, B. (2003). Response of the 1–5 day-aged ovine corpus luteum to prostaglandin $F_{2\alpha}$. *Animal Reproduction Science. 78*(1-2): 47-55. doi: 10.1016/S0378-4320(03)00046-0.

Simões, J., Abecia, J. A., Cannas, A., Delgadillo, J. A., Lacasta, D., Voigt, K., & Chemineau, P. (2021). Managing sheep and goats for sustainable high yield production. *Animal*. 15, 100293. doi:10.1016/j.animal.2021.100293.

Sinimbu, A.P., Ferreira, E.M., Denadai, R. (2022). The effect of progesterone length in timed AI in ewes. *Trop Anim Health Prod*. 54, 258. doi: 10.1007/s11250-022-03265-w.

Statistical Analysis System Institute (SAS) (2009). SAS User´s Guide: Statiscs (Version 5). Cary, N.C. U.S.A. Ins.Inc.pp.584.

Takci A, & Kivrak M.B. (2022). The effect on reproductive performance of different synchronization methods applied in early postpartum process that kangal sheep lambing in the breeding season. *Turkish Veterinary Journal. 4*(2): 46-50. doi: 10.51755/turkvetj.1224767.

Tsai, S. J., & Wiltbank, M. C. (1997). Prostaglandin $F_{2\alpha}$ induces expression of prostaglandin G/H synthase-2 in the ovine corpus luteum: a potential positive feedback loop during luteolysis. *Biology of reproduction. 57*(5), 1016-1022.

Urviola, M., Leyva, V., Huamán, H., & García, W. (2005). Manipulación de la ovulación del folículo dominante con prostaglandina en diferentes estadios del ciclo estrual sobre las tasas reproductivas en ovinos Corriedale. *Revista de Investigaciones Veterinarias del Perú. 16*(2), 103-113.

Wheaton, J. E., Carlson, K. M., Windels, H.F., & Johnston, L.J. (1993). CIDR: A new progesterone-releasing intravaginal device for induction of estrus and cycle control in sheep and goats. *Animal Reproduction Science. 33*(1-4), 127-141.

Wiltbank, M.C., T.F. Shiao; D.R., Bergfelt; O.J., & Ginther. (1995). Prostaglandin F2 alpha receptors in the early bovine corpus luteum. *Biology Reproduction.* 52: 74-78.