

Effect of two prostaglandin F2 α injections administered 24 hours apart on the pregnancy rate of Simmental cows subjected to the Ovsynch or Ovsynch + Controlled internal drug release (CIDR) protocols

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Summary

The aim of the present study was to determine the effects of one or two doses of prostaglandin F2 α (PGF) on the pregnancy rate (PR) in Simmental cows subjected to the Ovsynch or Ovsynch + Controlled internal drug release (CIDR) protocols. On d 0, 100 μ g gonadorelin diacetate tetrahydrate (GnRH) was injected into cows in group 1 (n = 50) and group 2 (n = 40) and CIDR was intravaginally inserted. On d 7, 25 mg dinoprost tromethamine (PGF2 α , PGF) was administered, and GnRH was injected 56 h later. Fixed-time artificial insemination (TAI) was performed 16 h after GnRH injection. In group 3 (n = 62) and group 4 (n = 63), PGF was administered 7 d following GnRH treatment, and GnRH was injected 56 h later. TAI was performed 16 h after GnRH injection. In groups 2 and 4, a second dose of PGF was injected 24 h after the first dose. Pregnancy was determined by transrectal ultrasonography on d 30 and 60 post-TAI. The pregnancy rate (PR) on d 30 post-TAI was significantly different among the groups: 46%, 55%, 29%, and 36.5%, in groups 1, 2, 3, and 4, respectively (P = 0.048). In addition, the PR was 36.3% and 43.7% in the single PGF and double PGF injection groups, respectively. There was no statistical difference in the PR on d 60 post-TAI (P > 0.05). Pregnancy loss was between 4.34% and 11.11% (P > 0.05). The highest PR in the primiparous cows was observed in group 3 (57.7%). The PR was not significantly different among multiparous cows (P > 0.05). In conclusion, two injections of PGF administered 24 h apart in Simmental cows may increase the PR in the Ovsynch or Ovsynch + CIDR protocols.

Keywords: CIDR, ovsynch, pregnancy, second dose PGF2 α , Simmental cow

Many estrus or ovulation synchronization protocols have been developed to increase reproductive efficiency in cows and heifers (2, 20, 32, 36). Reproductive performance decreases due to factors such as high milk yield and insufficient care-feeding in cows. The weakness of estrus symptoms or poor estrus detection in dairy cows reduces the fertility or pregnancy rate (PR). Therefore, the PR is increased using fixed-time artificial insemination (TAI) protocols. Although TAI protocols such as Ovsynch in cows and heifers have been developed, the PR may not still reach the desired level (4, 32, 36).

The use of a progesterone device improves synchronization and may also enhance oocyte competence in

cattle (22, 30). In the Ovsynch protocol, progesterone treatment can be performed between d 0 and 7 to increase the pregnancy rate (PR) (1, 2, 12, 18, 25). Hence, premature estrus does not occur and full synchronization is ensured (8, 17).

The newly formed corpus luteum (CL) may increase circulating progesterone (P4) concentration during the preovulatory follicle growth phase, increasing the PR and decreasing the double ovulation rate. The increased ovulation rate in response to the first hormonal administration in the Ovsynch protocol has a significant positive effect on the PR in cyclic and acyclic cows (1, 3, 38). Interestingly, human chorionic gonadotropin (hCG) does not increase the PR when

used in the Ovsynch protocol as an alternative to the first administration of gonadotropin releasing hormone (GnRH) (16). However, hCG may be an alternative to the second GnRH administration in the ovulation synchronization protocols performed in cyclic heifers (20).

The new CL induced by the first GnRH injection in the ovulation synchronization protocols can be resistant to a single prostaglandin F₂ α (PGF) injection. During the ovulation synchronization protocols, the percentage of cows and heifers that do not have complete regression following the PGF injection has been reported to range from 1.69% to 30%, which causes a high concentration of circulating progesterone during artificial insemination, decreasing the PR (5, 20, 24, 36, 38).

Single or double PGF treatments are performed in heifers and cows in the 5-d Cosynch or Ovsynch protocol, during which it is important to use a second dose of PGF due to the resistance of the developing CL (19, 27, 29, 31-33, 36). Even in the classical 7-d Ovsynch protocol, CL regression may be impaired, negatively affecting fertility (5, 24, 38). PGF injections may be ineffective in cows with a new CL compared to cows that ovulate after the first GnRH injection (11). In the middle of the luteal phase (7-12 d of the estrous cycle), PGF treatment reduces the plasma P4 concentration and CL volume; however, luteolysis does not occur in the early luteal phase (until d 5 of the estrous cycle) (21). Luteolysis is an important event in ovulation synchronization programs such as Ovsynch in dairy cows. In addition, delayed or incomplete luteolysis has been observed following PGF injection in 10-25% of cows treated with Ovsynch (5, 11, 31, 37). In general, two strategies are employed to increase luteolysis: the first is to increase the dose of PGF and the second is to inject two doses of PGF 24 h apart (5, 10, 29, 38). In the 5-d Cosynch protocol in dairy cows, two injections of PGF 24 h apart result in a higher rate of luteolysis (29).

The aim of the present study was to determine the effects of one PGF injection or two PGF injections administered 24 h apart on the PR in Simmental cows subjected to the Ovsynch or Ovsynch + Controlled internal drug release (CIDR) protocols.

Material and methods

The present study was conducted after obtaining the approval of the Ethics Committee of Animal Experiments at Kafkas University, Kars, Turkey (KAÜ-HADYEK – 2018/022).

Group 1 (Ovsynch + CIDR with single PGF, n=50)



Group 2 (Ovsynch + CIDR with 24-h interval two PGF injection, n=40)



Group 3 (Ovsynch with single PGF, n=62)



Group 4 (Ovsynch with 24-h interval two PGF injection, n=63)



Fig. 1. Schematic of the synchronization protocols

Explanations: PGF – prostaglandin F₂ α , CIDR – controlled internal drug release, GnRH – gonadotropin-releasing hormone, TAI – timed artificial insemination, US – transrectal ultrasonography, d – day, h – hour.

Animal material. The study was conducted with 215 clinically healthy, lactating Simmental cows that were at least 60 d postpartum (postpartum between 60-120 d) and displayed body condition scores (BCS) between 2.5 and 3.5 (9). Milk yields of Simmental cows were between 15-20 L. The ovarian cyclic activity of the cows was not determined prior to starting the study. The animals used in the study were fed with corn silage, dry meadow grass, wheat straw, and concentrate feed (barley, corn, bran, sunflower seed meal, calcium carbonate, salt, vitamin-mineral premix) with 18 CP and 2700 kcal/kg ME twice a day and water was provided *ad libitum*.

Synchronization protocols. Group 1 (Ovsynch + CIDR with single PGF injection, n = 50): On d 0, gonadorelin diacetate tetrahydrate (100 mg, 2 mL, i.m., GnRH, Ovarelin[®], Ceva, Turkey) was injected and the controlled internal drug release (CIDR 1380[®], Zoetis, Turkey) was intravaginally inserted. The CIDR was removed on d 7, and dinoprost tromethamine (25 mg, 5 mL, PGF, i.m., Dinolytic[®], Zoetis, Turkey) was injected. The second GnRH dose was administered 56 h later. TAI was performed 16 h after the second GnRH injection (Fig. 1).

Group 2 (Ovsynch + CIDR with two PGF injections administered 24 h apart, n = 40): On d 0, GnRH was injected and CIDR was intravaginally inserted. CIDR was removed on d 7. PGF was injected on d 7 and 8. GnRH was injected 32 h after the second PGF injection, and TAI was performed 16 h after the second GnRH injection (Fig. 1).

Group 3 (Ovsynch with single PGF injection, n = 62): GnRH was injected on d 0 and PGF was injected on d 7. The second GnRH injection was administered 56 h after PGF injection. TAI was performed 16 h after the second GnRH injection (Fig. 1).

Group 4 (Ovsynch with two PGF injections administered 24 h apart, n = 63): GnRH was injected on d 0 and PGF was injected on d 7 and 8. The second GnRH injection was administered 32 h after the last PGF injection, and TAI was performed 16 h after the second GnRH injection (Fig. 1).

Pregnancy diagnosis. Pregnancy was diagnosed by transrectal ultrasonography 30 \pm 2 and 60 \pm 2 d following TAI (15). The pregnancy loss rates (embryonic and fetal deaths) during this period were compared among the groups (20).

Statistical analysis. The SPSS 20 (SPSS®, Chicago, IL, USA) software package was used for statistical analysis. The PR among the groups were compared using the chi-square test. $P < 0.05$ is considered statistically significant.

Results and discussion

There was no statistically significant difference in postpartum day, milk yield, days to first service, calving to conception interval between groups ($P > 0.05$).

The PR diagnosed by transrectal ultrasonography 30 d following TAI was significantly different among the groups: 46%, 55%, 29%, and 36.5%, in groups 1, 2, 3, and 4, respectively ($P = 0.048$). In particular, the PR between groups 2 and 3 was significantly differ-

ent ($P = 0.009$). No statistically significant difference was found between groups 1 and 3 ($P = 0.064$) and groups 2 and 4 in terms of PR ($P = 0.065$). The highest PR was found in group 2 (Ovsynch + CIDR with two PGF injections administered 24 h apart group; Fig. 2A).

The PR, as diagnosed by transrectal ultrasonography 30 d following TAI, was 36.3% and 43.7% in the groups with the single PGF injection and two PGF injections administered 24 h apart, respectively. The second PGF injection increased the PR in both the Ovsynch + CIDR and the Ovsynch protocols, but the difference was statistically insignificant ($P > 0.05$, Fig. 2B).

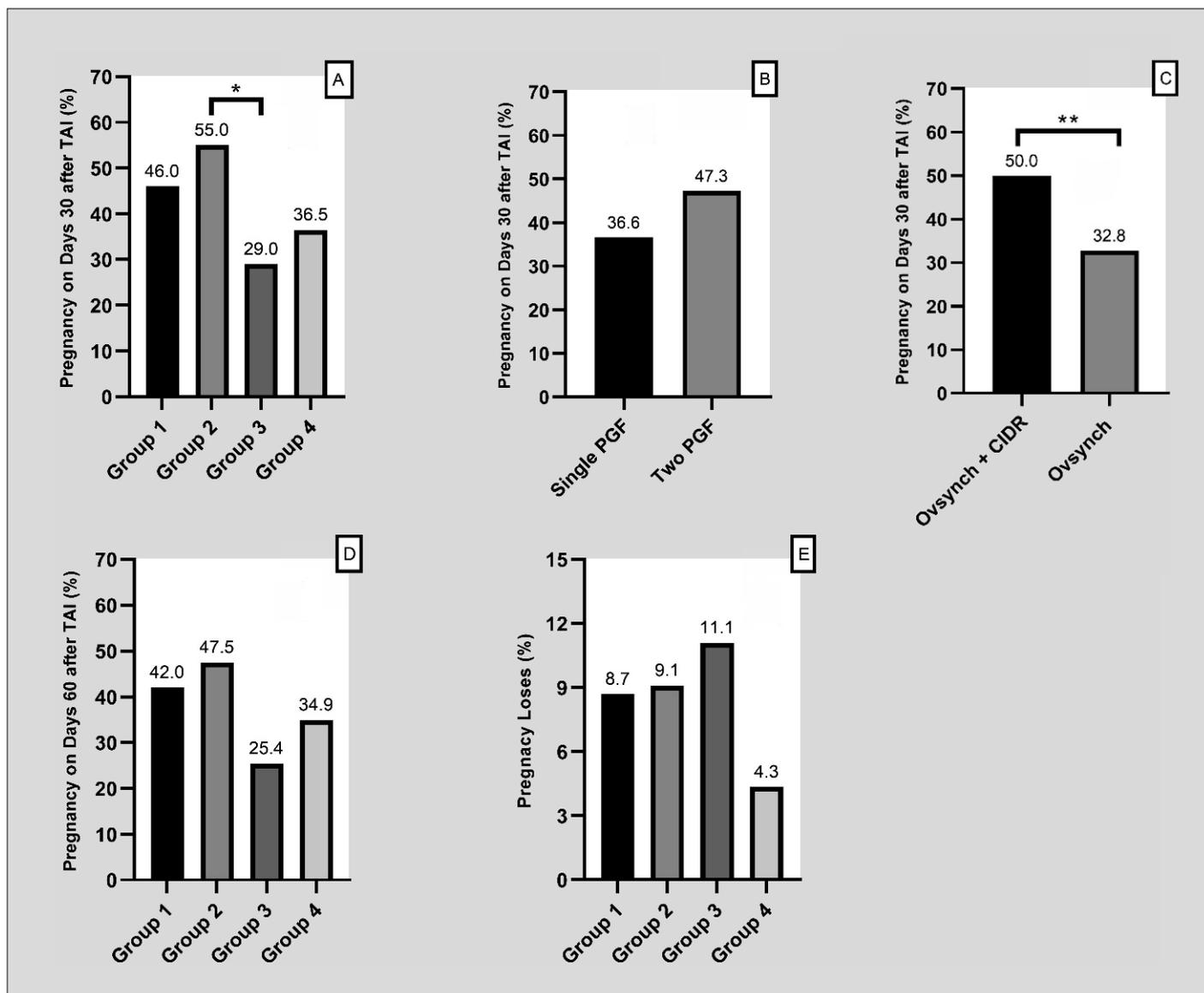


Fig. 2. Pregnancy rates and pregnancy loss in groups

Explanations: A: pregnancy rates on d 30 following timed artificial insemination. The highest pregnancy rate was determined in group 2. * – there was a significant difference between groups 2 and 3 ($P = 0.009$). B: pregnancy rates determined by transrectal ultrasonography on d 30 following fixed-time artificial insemination in single or double PGF injection groups ($P > 0.05$). C: pregnancy rates determined by transrectal ultrasonography on d 30 following fixed-time artificial insemination in Ovsynch + CIDR (single or double PGF) and Ovsynch (single or double PGF) groups. ** – significant differences between groups ($P = 0.011$). D: pregnancy rates determined by transrectal ultrasonography on d 60 following fixed-time artificial insemination ($P > 0.05$). E: pregnancy loss in groups ($P > 0.05$). Explanations: group 1: Ovsynch + CIDR with a single PGF dose; group 2: Ovsynch + CIDR with two doses of PGF administered 24 h apart; group 3: Ovsynch with a single PGF dose; group 4: Ovsynch with two doses of PGF administered 24 h apart.

Comparison of the groups with (groups 1 and 2) and without (groups 2 and 3) CIDR in Simmental cows showed that progesterone treatment significantly increased the PR ($P = 0.011$). The PR was 50.0% and 32.8% on d 30 following TAI in the groups with (groups 1 and 2) and without (groups 2 and 3) CIDR, respectively (Fig. 2C).

The PR diagnosed by transrectal ultrasonography 60 d following TAI was similar to that on d 30. The highest PR was determined in group 2 and the lowest in group 3; however, the difference among the groups was not statistically significant ($P > 0.05$, Fig. 2D). The pregnancy loss ranged from 4.34% to 11.11%, but there was no significant difference among the groups ($P > 0.05$). The highest pregnancy loss was determined in the Ovsynch group (group 3), while the lowest was determined in group 4, in which the cows were administered a second PGF injection (Fig. 2E).

The most successful protocol for primiparous cows was Ovsynch + CIDR with two PGF injections administered 24 h apart (PR: 57.7%) (group 2), and the lowest PR (25.6%) was found in the Ovsynch group (group 3). The PR between these two groups (groups 2 and 3) was significantly different ($P = 0.009$). Moreover, the PR in primiparous cows was significantly different among groups ($P = 0.026$). The numerical difference found in the PR in multiparous cows among the groups was insignificant ($P > 0.05$, Tab. 1). There was a higher PR in the groups with the two PGF injections administered 24 h apart than in the single PGF injection groups.

The comparison based on the BCS (Tab. 2) showed that the PR was not significantly different among the groups ($P > 0.05$). In BCS = 3, the highest PR was obtained in progesterone-supported protocols, yielding a statistical significance ($P = 0.068$).

Many ovulation synchronization protocols have been developed to increase reproductive performance in cows; and certain additional hormonal treatments have been used in these synchronization protocols to increase the PR (2, 4, 7, 13, 18, 32, 36-38). To prevent the decrease in PR caused by delayed or incomplete luteolysis, two PGF injections have been performed 24 h apart in ovulation synchronization protocols (5, 6, 38). In the present study, the 7-d Ovsynch + CIDR protocol was evaluated following the incorporation of a second PGF injection after a 24-h interval.

In the Ovsynch protocol, two PGF injections administered 24 h apart in dairy cows have been shown to increase the PR. In a previous study, the PR following a single PGF dose or two doses of PGF administered 24 h apart in the Ovsynch protocol was 33.3% and 36.1%, respectively. Two doses of PGF administered

Tab. 1. Pregnancy rates on d 30 following fixed-time artificial insemination

Parity	Group 1	Group 2	Group 3	Group 4	P value
Primiparous, % (n/total)	51.9 (14/27) ^{ac}	57.7 (15/26) ^a	25.6 (10/39) ^b	33.3 (16/48) ^{bc}	0.026
Multiparous, % (n/total)	39.1 (9/14)	50.0 (7/14)	34.8 (8/23)	46.7 (7/15)	NS
P value	NS	NS	NS	NS	-

Explanations: group 1 – Ovsynch + CIDR with a single PGF dose; group 2 – Ovsynch + CIDR with two doses of PGF administered 24 h apart; group 3 – Ovsynch with a single PGF dose; group 4 – Ovsynch with two doses of PGF administered 24 h apart, NS – not significant, ^{abc} – statistical differences in the same row ($P = 0.026$)

Tab. 2. Pregnancy rates according to the body condition score (BCS)

BCS	Pregnancy rate, % (n/total)				P value
	Group 1	Group 2	Group 3	Group 4	
< 3	20.0 (2/10)	30.0 (3/10)	14.3 (2/14)	17.6 (3/17)	NS
3	53.3 (16/30)	66.7 (14/21)	32.4 (12/37)	42.9 (15/35)	NS
> 3	50.0 (5/10)	55.6 (5/9)	36.4 (4/11)	45.5 (5/11)	NS
P value	NS	NS	NS	NS	-

Explanation: as in Tab. 1

24 h apart in the double-Ovsynch protocol has been shown to have no effect on the PR (36). Similarly, another study using the Ovsynch protocol also found that a second PGF injection did not significantly increase the PR (double PGF 44.7%, single PGF 41.5%) in dairy cows (5). Moreover, the PR in Cosynch-72 and 5-d Cosynch protocols with a second PGF injection after 24 h was 30.9% and 37.9%, respectively, in pre-synchronized (11-d interval) dairy cows, which shows that a second PGF injection significantly increased the PR (31). In the present study, the PR was 29% and 36.5% in the Ovsynch protocol with a single PGF dose (group 3) and two doses of PGF administered 24 h apart (group 4), respectively; however, there was no significant difference between the groups ($P > 0.05$). In both the present and previously reported studies (31, 36), the effect of two doses of PGF in the Ovsynch protocol on the PR was limited. Two doses of PGF, especially administered 24 h apart, increase luteolysis and may be more effective on immature CL. In addition, administration of two doses of PGF increases the PR by reducing the rate of delay or incomplete luteolysis. Therefore, a second PGF injection can be used in the Ovsynch or Ovsynch + progesterone protocol.

The use of progesterone-releasing devices in the Ovsynch protocol has been shown to increase the PR by approximately 5% to 9%. Particularly at the beginning of the Ovsynch protocol, progesterone treatment is effective in cows without CL (23, 26, 37); therefore, the use of controlled progesterone-releasing devices may be useful in increasing the PR in cows (32, 34). In the 5-d Cosynch + CIDR protocol in dairy cows, the PR has been shown to be 30.2% and 34.3% following a single dose of PGF or two doses of PGF administered 24 h apart, respectively; thus, a second PGF injection did not increase the PR (29). In the G6G protocol with single and double PGF injections, the PR has

been shown to be 28.7% and 45.4%, respectively. An interaction has been found between synchronization and luteolytic treatment with respect to the PR on d 35 following TAI. Although the PGF injection (single or double doses) in CIDR-inserted cows has been shown to have no effect on the PR, two PGF injections in the G6G protocol did improve the PR (29). In another study, the PR was determined following two doses of PGF administered 6 h apart in TAI protocols (double PGF, PR 58.1%; single PGF, PR 55.1%) (35). In the present study, the PR on d 30 following TAI was higher in the CIDR groups (groups 1 and 2) than in the groups without CIDR (groups 3 and 4). However, statistically significant differences were observed between the Ovsynch + CIDR and the Ovsynch protocols following the two doses of PGF (55% and 29%, respectively) ($P = 0.009$). In the present study, two doses of PGF administered 24 h apart increased the PR (47.3% and 36.6%) as compared with a single dose. The use of CIDR in the Ovsynch protocol increased the PR (50% and 32.8%, respectively) ($P = 0.011$). In particular, the second PGF injection, which increases the rate of complete luteolysis, decreases progesterone synthesis and increases fertility; therefore, two doses of PGF administered 24 h apart in the Ovsynch protocols, with or without CIDR, can help to increase the PR.

In the present study, two doses of PGF administered 24 h apart (groups 2 and 4) had a positive effect on the PR 60 d following TAI. Particularly in the Ovsynch protocols (groups 3 and 4), two doses of PGF had a positive effect on the PR (25.4% and 34.9%, respectively). A previous study showed that the second PGF injection increased the PR on d 64 following TAI in the G6G (single PGF: 27.7%, double PGF: 40%) and 5-d Cosynch (single PGF: 26.1%, double PGF: 29.2%) protocols performed in dairy cows (29). Another study also showed that in ovulation synchronization protocols (Cosynch-72 and 5-d Cosynch), two doses of PGF treatment increased the PR 66 d following TAI in dairy cattle ($P = 0.05$) (31). In the present study, we determined the positive effect of two doses of PGF administered 24 h apart on the PR. Two PGF injections can be administered for higher luteolysis in TAI protocols performed in dairy cattle with undetermined ovarian cyclic activity.

Pregnancy loss has been shown to range from 3.6% to 19.5% in single or double PGF injection TAI protocols (10, 20, 29). Single and double PGF injections have been shown to have no effect on pregnancy loss in dairy cows (4.2% and 6.3%, respectively) (31). In the modified Ovsynch protocol with single or double PGF injections, the pregnancy loss has been determined to range between 6% and 16% (6). In addition, pregnancy loss was found to be similar in the 5-d Cosynch protocols with single or double PGF injections in heifers (14). In the present study, the pregnancy loss was between 4.34% and 11.1% and two doses of PGF did not affect these rates.

In primiparous and multiparous cows, the PR was similar in the Ovsynch protocols with a single PGF dose or two doses of PGF administered 24 h apart. The PR following single or double PGF injections in the Ovsynch protocol performed in primiparous or multiparous cows was found to be 37.1% and 38.2% and 32% and 35.4%, respectively (36). In the present study, there was a statistically significant difference between single and double PGF injections (Ovsynch or Ovsynch + CIDR) ($P < 0.05$); however, the PR in multiparous cows was not significantly different. Two doses of PGF administered 24 h apart in primiparous and multiparous cows had a positive effect on the PR in both the Ovsynch and Ovsynch + CIDR protocols.

In conclusion, two doses of PGF administered 24 h apart in the Ovsynch or Ovsynch + CIDR protocols performed in Simmental cows may be useful for increasing PR. In addition, fertility can be increased by the administration of a second PGF injection to provide a complete luteolysis in TAI protocols.

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