Final Report

Effects of Equine Chorionic Gonadotropin (eCG) on Ovarian Activity, Fertility And Embryo Loss in Cattle and Sheep

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EXECUTIVE SUMMARY

During the months of May 2010 to December 2012 we conducted a series of experiments to evaluate the effects of administration of eCG (Novormon, Syntex) on ovarian activity and fertility of cows and sheep.
The effect of eCG at ovarian level when used as an ovulation inducer, in addition to its possible effect after ovulation on the maintenance of pregnancy. Different experiments were performed in cows and sheep using a dose of eCG before ovulation and during the first days of gestation.
The results in postpartum anestrous cows showed that administration of 400 IU of eCG at the end of synchronization of ovulation with progesterone and estradiol, causes a greater proportion of cows to ovulate, greater development of the ovulatory follicle, a larger size subsequent corpus luteum, and higher progesterone blood levels during the first weeks of gestation. As a result, the pregnancy rate with this treatment after Fixed Time Artificial Insemination (TAI) increased significantly in postpartum anestrous cows. In addition, we studied the response to a dose of eCG administered after ovulation and before the critical period of gestation (i.e. 14 days after TAI). This treatment increased the activity of the corpus luteum and progesterone concentrations until day 23 of gestation. In a subsequent experiment it was shown that this increase in progesterone concentrations was reflected in increased pregnancy rates in postpartum anestrous cows.
When we gave sheep a dose of 400 IU 10 days after ovulation the size of the corpus luteum increased and progesterone concentrations in blood also increased up to 20 days of gestation. However, treatment with eCG after insemination did not affect pregnancy rates in ewes during the breeding season.

May 10, 2013

This study was carried out in the facilities of IRAUy, Camino Cruz del Sur 2250, Montevideo, Uruguay. Field studies in cows were carried out at the farm San Sebastián, Florida, Uruguay. Field experiments in sheep were carried out in the School of Agronomy, Paysandú, Uruguay, and on the farms San Salvador, Lavalleja, Uruguay, and La Verónica, Salto, Uruguay Hormone determinations were processed at the Nuclear Technique Laboratory of the School of Veterinary Medicine, Universidad de la República, Montevideo, Uruguay. The Directors of the project were: Dr. Alejo Menchaca and Dr. Teresa de Castro, and the Directors of the studies performed in cows were Dr. Richard Núñez and in sheep Dr. Camila García Pintos. Br. Leonardo Fros and Br Enrique Castells participated in the studies performed in sheep. The studies were financed by FUNDACIBA (PEDECIBA Foundation) under the Agreement between IRAUy Foundation and Syntex SA, under the conditions laid down in Law 16,736 of 01/05/96. Section 593.

Alejo Menchaca (PhD) is a researcher at the National Research System (SNI) of the National Agency for Research and Innovation (ANII), is also a researcher of PEDECIBA (Basic SciencesDevelopment Program) and professor of the Graduate Program at the School of Veterinary Medicine, University of the Republic, Uruguay. He is currently Director of the Institute of Animal Reproduction Uruguay. Teresa de Castro (MSc) is a researcher of the SNI of the ANII and President of the IRAUy Foundation. Richard Nunez (DMV) and Camila García Pintos (DMV) are Master students at the National University of Cordoba, Argentina, and the University of the Republic, Uruguay, respectively.

EFFECT OF eCG IN COWS

A series of experiments were carried out to determine the effect of eCG on ovarian activity as inducer of ovulation and as support for the maintenance of pregnancy in anoestrus postpartum cows.

Experiment 1.
eCG as Ovulation Inducer

The objective of this experiment was to evaluate the effect of eCG at the end of a TAI protocol on ovulation and corpus luteum (CL) formation in beef cows in postpartum anoestrus. Hereford multiparous cows were used (n = 46) at 60 to 75 days postpartum with a body condition (BC) of 3.5±0.1 (scale 1-8). All cows were in anestrous with no CL determined by ultrasound during the 10 days prior to start of treatment. Follicular size, weight and BCS were considered for the balanced design of the experimental groups. All females received a TAI protocol using an intravaginal device with 0.5 g of progesterone (0.5 DIB, Syntex, Buenos Aires, Argentina) for 8 days associated with a 2 mg im dose of estradiol benzoate (Estradiol Benzoate, Syntex) at the time of device placement. On device removal 500 micrograms of cloprostenol (Ciclase DL, Syntex) and 0.5 mg of estradiol cypionate (Cipiosyn, Syntex) were administered intramuscularly. At this time an experimental group received an im dose of 400 IU of eCG (n = 23; Novormon, Syntex) while another group received no eCG (n = 23). Insemination was performed between 52 and 56 hours after removal of the device using frozen semen considered fit based on motility and morphology. An anti-suckling nose flap was placed in the calves on the same day the progesterone device was placed in the cows and remained in place until TAI. The dynamics of follicular development and ovulatory CL size were monitored daily by transrectal ultrasonography (Well.D, WED-9618V, Shenzhen, China) from the removal of the device until 14 days after TAI. During the same period, blood samples were taken daily for determination of circulating serum progesterone. The samples were analyzed in duplicate by solid phase radioimmunoassay (Diagnostic Product Co., LA, CA, USA). Ovulation rate (proportion of cows with ovulation/cows synchronized) and the proportion of cows with a CL 14 days after ovulation was compared by chi square, while other continuous variables were compared using Mixed Models (STATA, 2009). The results seen with regards to ovulatory follicle development, ovulation and corpus luteum formation are summarized in Table 1. eCG treatment increased the proportion of cows that ovulated, showed a tendency to increase the diameter of the ovulatory follicle and its daily growth rate, increased the size of the corpus luteum and increased progesterone concentrations during the 14 days after TAI

Table 1. Ovarian response to the administration of eCG (400 UI) after a TAI protocol in anestrous Hereford cows with calf (mean±SD).

	Ovulated cows/synchronized cows	Ovulatory Follicle Diameter (mm)	Rate of Follicular Growth* (mm/day)	CL** area (mm^2)	Progesterone in blood*** (ng/ml)
With eCG	65.2%(15/23)	14.5 ± 0.4	1.4 ± 0.2	344.3 ± 25.1	3.0 ± 0.2
No eCG	30.4%(7/23)	13.1 ± 0.7	0.9 ± 0.2	274.2 ± 23.9	1.8 ± 0.2
P	0.018	0.081	0.077	0.045	0.001

*From dev ice removal to ovulation. **From day 6 to 14 after TAI.
***From day of TAI to 14 days later.

Figure 1 shows the development of the corpus luteum during the first 30 days of gestation in pregnant cows and serum progesterone until day 14 after TAI. eCG treatment on removal of the intravaginal device increased the size of the corpus luteum significantly during the first 23 days after TAI favoring maternal recognition of the embryo and the maintenance of pregnancy. Furthermore, blood progesterone concentrations were higher in cows receiving eCG, both pregnant and not pregnant after TAI.

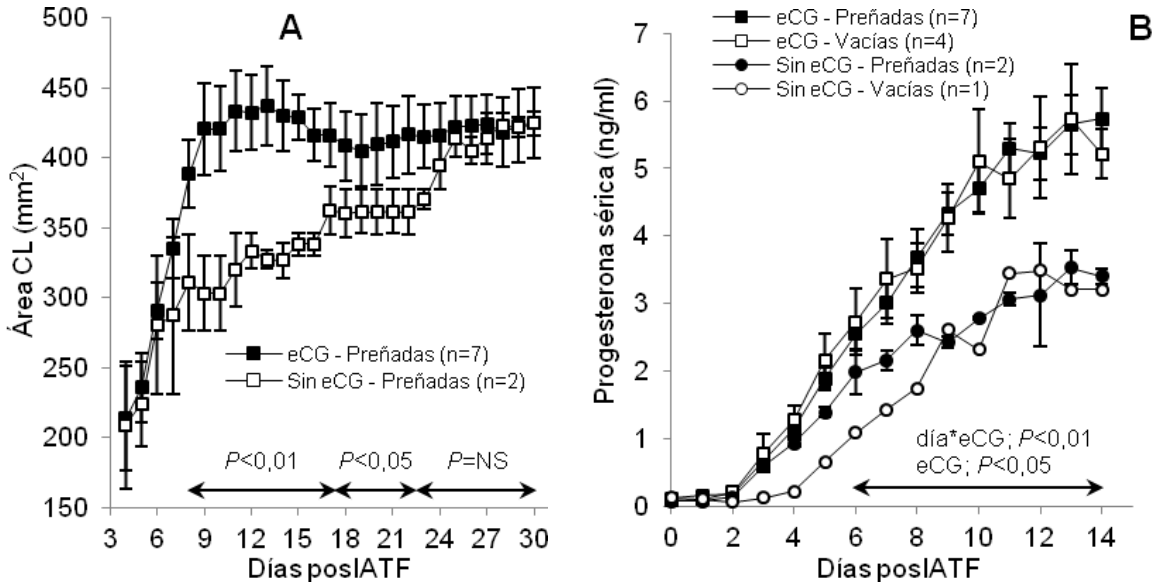

Figure 1. Corpus luteum area in pregnant cows (A) and progesterone serum concentrations in ovulated cows (B) after TAI in response to the administration of 400 IU of eCG at device removal in Hereford anestrous cows with calf.

In cows that ovulated three different patterns were observed with regards to luteal phase after treatment for synchronization of ovulation. One pattern was considered to be the normal one expected after ovulation with concentrations above 1 ng/ml up to 14 days after ovulation, whereas there were two altered response patterns with premature luteal regression, confirmed by progesterone serum concentrations for both experimental groups. In cows that did not receive eCG, this response with subnormal CLs was observed in 57.1% (4/7), whereas in those who received eCG, this response was seen in 26.7% (4/15) cows (P = 0.17). Figure 2 shows the results for this response.

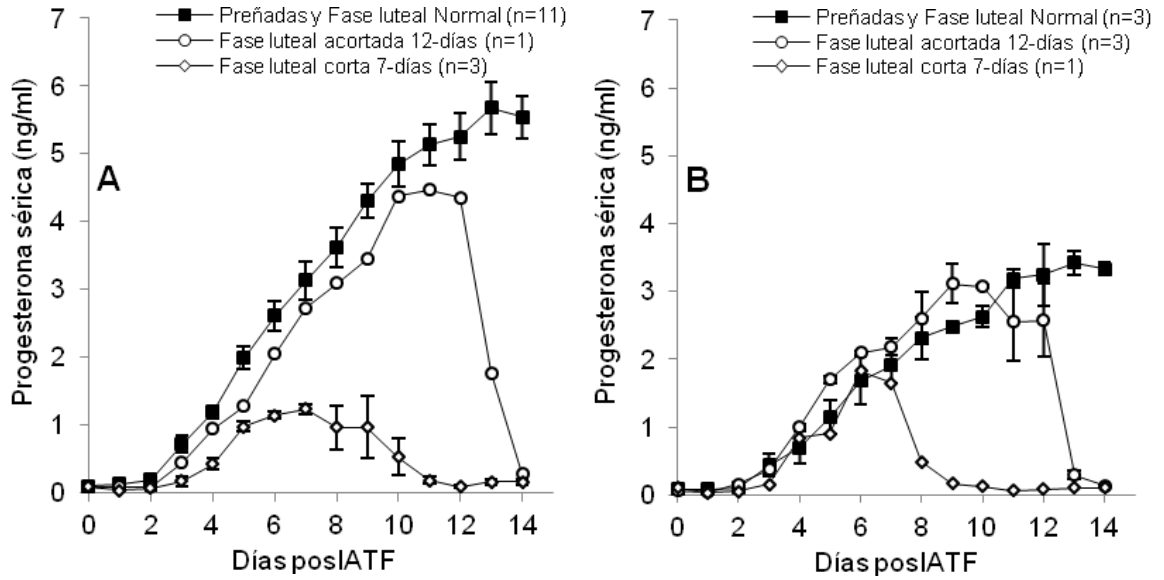

Figure 2. Progesterone serum concentrations in response to administration of 400 IU of eCG (A) or no eCG (B) on removal of progesterone device on Day 14 after TAI in anestrous Hereford cows with calf (mean ±SD).

In conclusion, the administration of 400 IU of eCG on removal of a progesterone device increased the proportion of cows that ovulated and improved subsequent corpus luteum development in anestrous postpartum cows.

Experiment 2.
Effect of eCG 14 days after TAI on Corpus Luteum Activity:

With the aim of providing better luteotrophic support during the critical period of maternal recognition of pregnancy (16-18 days after ovulation) we evaluated the effect of administration of eCG on Day 14 after insemination. We used 60 multiparous Hereford cows that were anestrous determined by ultrasonography, 60 to 75 days postpartum with a body condition of 3.5±0.1 (scale 1-8). All cows entered a TAI protocol with an 0.5 g of progesterone intravaginal device (DIB 0.5, Syntex, Argentina) for 8 days associated with an im dose of 2 mg of estradiol benzoate (Estradiol Benzoate, Syntex) at the time of device placement. On device removal 500 micrograms of cloprostenol (Ciclase DL, Syntex) and 0.5 mg of estradiol cypionate (Cipiosyn, Syntex) were administered intramuscularly At that time the animals were assigned to 4 experimental groups using a 2x2 factorial arrangement to receive or not on im dose of 400 IU of eCG (Novormon, Syntex) at device removal, with or without a dose of 400 IU of eCG at Day 14 after TAI. Insemination was performed between 52 and 56 h after removal of the device. Blood samples were taken daily from day 14 to 22 after insemination to determine concentrations of progesterone and estradiol-17beta. The samples were analyzed in duplicate by solid phase radioimmunoassay (Diagnostic Product Co., LA, CA, USA). Continuous variables were analyzed using Mixed Models (STATA, 2009). Figure 3 shows the development of the corpus luteum and progesterone serum concentrations in pregnant cows from day 14 to 22 after TAI. When the results were analyzed considering the main effect of eCG administration or not at Day 14, there was a significant effect in favor of eCG treatment on progesterone concentrations (P <0.001). This

means that treatment at Day 14 increased progesterone concentrations until day 23 of gestation.

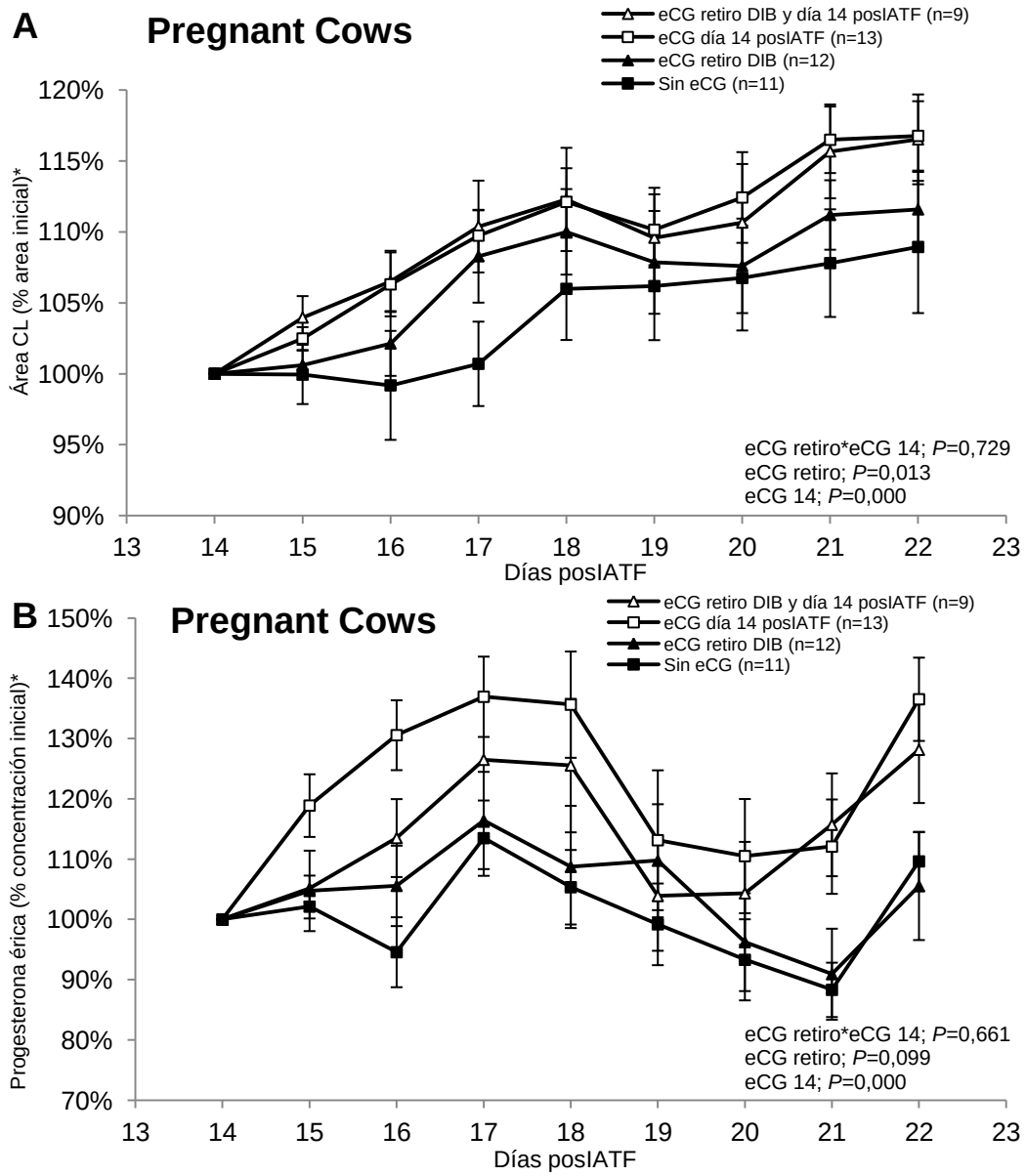

Figure 3. Corpus luteum area (A) and serum progesterone (B) from Day 14 to 22, in response to the administration of 400 IU of eCG on Day 14 after TAI in anestrous Hereford cows with calf.

The development of the largest follicle from day 14 to day 22 in pregnant cows is shown in Figure 4. While greater diameters were achieved after administration of eCG, the difference was not significant. However, serum estradiol concentrations were significantly higher after treatment with eCG (Figure 5). This shows that eCG also has a stimulating effect on follicular activity and estradiol secretion.

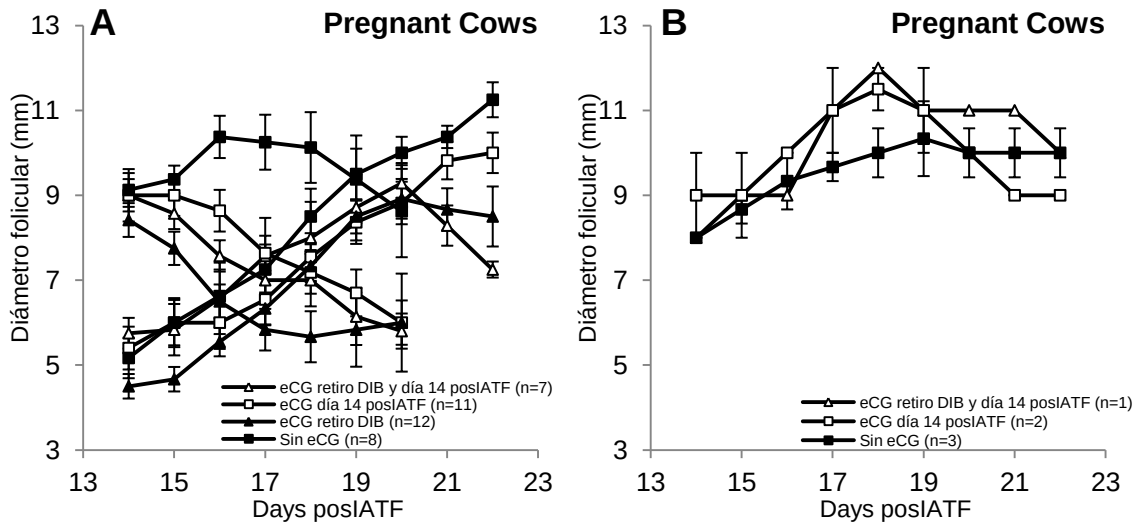

Figure 4. Dominant follicle diameter in cows with 3-wave pattern (A) and cows with 2 waves pattern (B) from Day 14 to 22, in response to the administration of 400 IU of eCG at Day 14 after TAI in anestrous Hereford cows with calf.

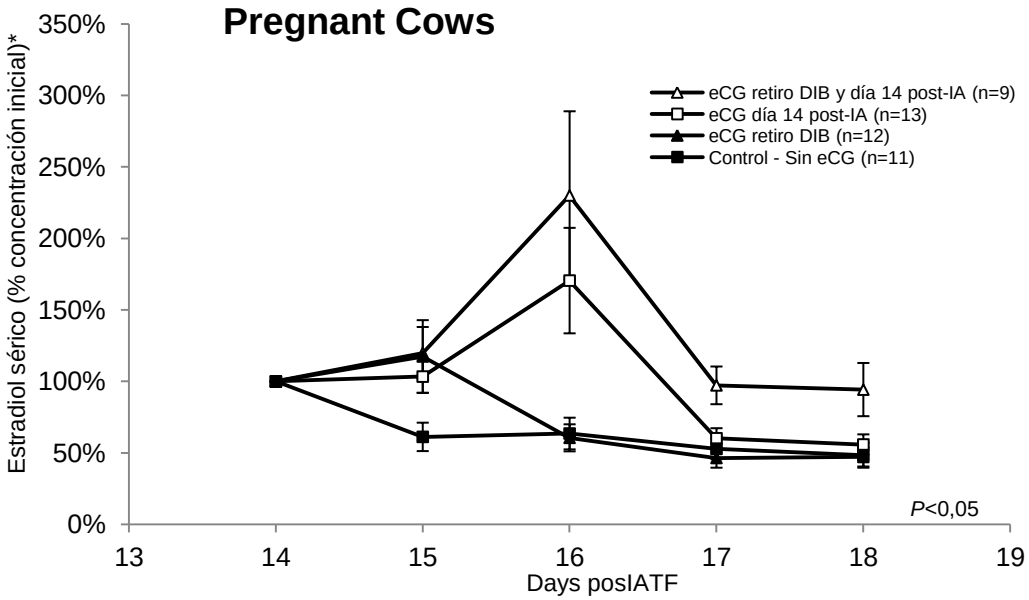

Figure 5. Serum 17β estradiol concentrations from Day 14 to 18, in response to the administration of 400 IU of eCG at Day 14 after TAI in anestrous Hereford cows with calf.

In conclusion, the administration of 400 IU of eCG on Day 14 days after insemination increased progesterone concentrations during the critical period of gestation. This could imply a better luteal support during maternal recognition of pregnancy in beef cows in postpartum anoestrus

Experiment 3.
Pregnancy Rate and Pregnancy Maintenance in Cows with Calf.

The aim was to evaluate the effect of eCG administration prior to ovulation and/or on Day 14 after TAI on pregnancy rate and embryonic loss in cows with calves. Multiparous cows were determined to be in anestrous by ultrasonography (n = 497), with 60 to 90 days postpartum and a body condition (BC) of 3.5±0.1 (scale 1-8) were used. All cows entered a TAI protocol with an 0.5 g of progesterone intravaginal device (DIB 0.5, Syntex, Argentina) for 8 days associated with an im dose of 2 mg of estradiol benzoate (Estradiol Benzoate, Syntex) at the time of device placement. On device removal 500 micrograms of cloprostenol (Ciclase DL, Syntex) and 0.5 mg of estradiol cypionate (Cipiosyn, Syntex) were administered intramuscularly At this time the animals were assigned to 4 experimental groups on a 2x2 factorial arrangement to receive or not an im dose of 400 IU of eCG (Novormon, Syntex) at device removal, with or without a dose of 400 IU of eCG on Day 14 after TAI, balanced by random blocks based on ovarian activity and body condition at baseline. Insemination was performed between 52 and 56 hours after removal of the device. An anti-suckling nose flap was placed in the calves on the same day the progesterone device was placed in the cows and remained in place until one day after TAI. Pregnancy was diagnosed by ultrasonography at 30 and 60 days after TAI. Data was analyzed using logistic regression and the results are summarized in Table 2.
eCG administration on device removal significantly increased pregnancy rates from 48.3% to 62.1%, and a second dose of eCG on Day 14 improved this result up to 78.9% (P <0.05) . There were no differences between experimental groups with regards to embryonic or fetal losses between 30 and 60 days.

Table 2. Pregnancy rate and embryonic/fetal loss after eCG administration on removal of intravaginal progesterone device and/or on Day 14 after insemination in anestrous beef cows.

	Pregnancy Rate at 30 days (*n*=497)	Pregnancy loss between 30 and 60 days (*n*=308)
No eCG (Control)	48.3% (58/120)[a]	0.0% (0/58)[a]
eCG on DIB removal	62.1% (77/124)[b]	3.9% (3/77)[a]
eCG on Day 14 post TAI	57.6% (72/125)[ab]	4.2% (3/72)[a]
eCG on removal and on Day 14	78.9% (101/128)[c]	0.0% (0/101)[a]

For the same column different superscripts indicate $P<0.05$.

In conclusion, the administration of 400 IU of eCG at device removal increased pregnancy rates in anestrous cows with calves. When given a second dose of eCG 14 days after insemination, the result was even better, suggesting a possible beneficial effect on embryo survival and maintenance of pregnancy in cows in anestrous.

Experiment 4.
Pregnancy Rate and Pregnancy Maintenance in Heifers.

The objective was to determine pregnancy rate and embryonic loss after administration of 400 IU of eCG in heifers. We worked with anestrous females in which response to eCG was compared. Initially the physiological status of the ovary (anestrous or cycling) was defined by ultrasound at the time beginning a TAI protocol. 689 nulliparous beef heifers were used with a BCS of 4-5 (scale 1-8). All cows entered a TAI protocol with an 0.5 g of progesterone intravaginal device (DIB 0.5, Syntex, Argentina) for 8 days associated with an im dose of 2 mg of estradiol benzoate (Estradiol Benzoate, Syntex) at the time of device placement. On device removal 500 micrograms of cloprostenol (Ciclase DL, Syntex) were administered and 0.5 mg of estradiol cypionate (Cipiosyn, Syntex) intramuscularly. At this time the animals were assigned to 4 experimental groups on a 2x2 factorial arrangement to receive or not an im dose of 400 IU of eCG (Novormon, Syntex) on device removal, with or without a dose of 400 IU of eCG on Day 14 days after TAI, balanced by random blocks with regards to ovarian activity and body condition at baseline. Insemination was performed between 52 and 56 hours after removal of the device. Pregnancy was diagnosed by ultrasonography at 30 and 60 days after TAI. Data was analyzed using logistic regression and the results are summarized in Table 3.
The pregnancy rate increased with the administration of eCG at device removal, showing no effect of administration at day 14 after TAI. Embryonic or fetal losses between 30 and 60 days was not different between experimental groups.

Table 3. Pregnancy rate and embryonic/fetal loss after eCG administration on removal of intravaginal progesterone device and/or on Day 14 after insemination in anestrous heifers.

	Pregnancy Rate at 30 days (*n*=689)	Pregnancy loss between 30 and 60 days (*n*=292)
No eCG (Control)	36.9% (66/179)[a]	7.6% (5/66)[a]
eCG on DIB removal	46.9% (83/177)[b]	6.0% (5/83)[a]
eCG on Day 14 of TAI	39.0% (64/164)[ab]	3.1% (2/64)[a]
eCG on removal and on Day 14	46.7% (79/169)[b]	2.5% (2/79)[a]

For the same column different superscripts indicate $P<0.05$.

In conclusion, the administration of 400 IU of eCG at the time of device removal increases pregnancy rates in anestrous heifers. Administration of a second dose of 400 IU of eCG on Day 14 days after insemination did not alter the result obtained in heifers.

EFFECT OF eCG IN SHEEP

Two studies were performed to determine the effect of eCG during early pregnancy in sheep.

Experiment 5.
Ovarian Response to eCG on Day 5 or 10 after Insemination.

The aim of this study was to evaluate the ovarian response to eCG administered during early pregnancy in ewes. The experiment was conducted with 30 Corriedale ewes that had an average body condition score of 3.3 ± 0.5 (scale 0-5) during the breeding season (April, 34 ° S, Uruguay). They were treated for 6 days with intravaginal sponges with 60 mg of medroxyprogesterone (Progespon, Syntex) associated with 300 IU of eCG (Novormon, Syntex) and 125 μg of cloprostenol (Ciclase DL, Syntex) administered intramuscularly at the time of sponge removal. TAI was performed between 48 and 56 h after sponge removal (Day 0) by intrauterine insemination with fresh semen diluted in UHT skim milk at a dose of 100 million sperm per ewe. Ewes were randomly assigned to three experimental groups to receive a second dose of 400 IU of eCG at Day 5 (n = 10) or Day 10 (n = 10) and a third group received no eCG after insemination acting as a control group (n = 10). Corpus luteum area and follicular dynamics were determined daily by transrectal ultranosography from the Day of TAI to Day 30 in pregnant ewes and until next ovulation in nonpregnant ewes. During the same period of time blood samples were drawn for determination of serum levels of progesterone. The samples were analyzed in duplicate by solid phase radioimmunoassay (Diagnostic Product Co., LA, CA, USA). Statistical analysis for continuous variables was performed using Mixed Models (STATA, 2009). eCG administration on Day 10 increased corpus luteum area and progesterone concentrations in pregnant ewes ($P < 0.05$) compared to ewes treated on Day 5 and those who did not receive eCG. Treatment on Day 5 showed no significant effect on luteal area and progesterone concentrations. The results are shown in Figure 6.

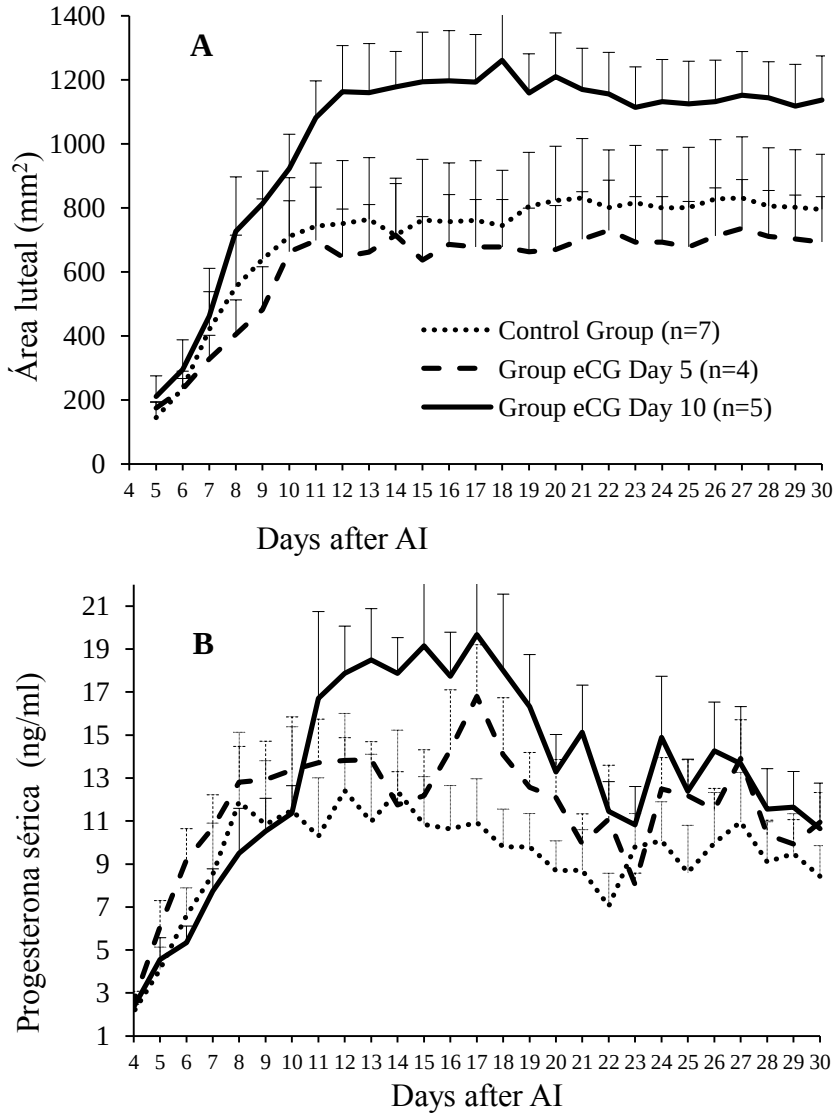

Figure 6. Corpus luteum size (A) and serum progesterone concentrations (B) during early pregnancy in ewes that received a dose of 400 IU of eCG 5 to 10 days after insemination.

eCG treatment had a significant effect on follicular dynamics. When compared to the control group that did not receive eCG after insemination, the administration of eCG at Day 5 or Day 10 prolonged the duration of the follicular wave that was present at the time of treatment ($P < 0.05$). For both groups, there was a tendency to increase the length of the wave that emerged after treatment (Group eCG Day 5, $P = 0.09$, group eCG Day 10, $P = 0.06$). The results are shown in Table 4 and Table 5.

Table 4. Follicular dynamics in ewes treated with 400 IU of eCG 5 days after insemination.

	Duration of growth phase (days)	Maximum follicular diameter (mm)	Day of maximum diameter*	Duration of follicular wave (days)
Wave in regression at the time of eCG administration				
eCG on Day 5 (*n*=9)	3.6 ± 0.4[a]	5.4 ± 0.4[a]	2.9 ± 0.5[a]	6.7 ± 0.7[a]
Control (n=9)	4.3 ± 0.4[a]	5.3 ± 0.2[a]	3.6 ± 0.5[a]	6.9 ± 0.7[a]
Growing wave at the time of eCG administration				
eCG on Day 5 (*n*=9)	5.6 ± 0.6[a]	5.7 ± 0.4[a]	8.1 ± 0.7[a]	8.4 ± 1.1[a]
Control (n=9)	3.6 ± 0.2[a]	5.4 ± 0.2[a]	7.3 ± 0.7[a]	6.0 ± 0.7[b]
Wave that emerged after the administration of eCG.				
eCG on Day 5 (*n*=9)	5.6 ± 1.0[a]	6.3 ± 0.4[a]	10.4 ± 0.5[a]	8.5 ± 0.9[a]
Control (n=9)	4.3 ± 0.6[a]	5.3 ± 0.2[b]	10.3 ± 0.9[a]	6.8 ± 0.4[a]

For the same column different superscripts indicate $P< 0.05$. *Days from insemination.

Table 5. Follicular dynamics in ewes treated with 400 IU of eCG 10 days after insemination.

	Duration of growth phase (days)	Maximum follicular diameter (mm)	Day of maximum diameter*	Duration of follicular wave (days)
Wave in regression at the time of eCG administration				
eCG on Day 10 (*n*=9)	4.7 ± 0.8[a]	5.7 ± 0.3[a]	9.4 ± 0.5[a]	7.8 ± 1.0[a]
Control (n=9)	4.0 ± 0.3[a]	5.4 ± 0.1[a]	8.2 ± 0.6[a]	6.8 ± 0.5[a]
Growing wave at the time of eCG administration				
eCG on Day 10 (*n*=9)	5.6 ± 1.0[a]	5.6 ± 0.3[a]	12.6 ± 0.8[a]	9.3 ± 1.1[a]
Control (n=9)	4.4 ± 0.8[a]	5.1 ± 0.1[a]	10.9 ± 0.5[a]	5.8 ± 0.7[b]
Wave that emerged after the administration of eCG.				
eCG on Day 10 (*n*=9)	5.3 ± 0.4[a]	5.8 ± 0.4[a]	15.7 ± 0.7[a]	9.3 ± 0.6[a]
Control (n=9)	4.5 ± 1.0[a]	5.5 ± 0.2[a]	15.8 ± 0.7[a]	6.6 ± 0.8[a]

For the same column different superscripts indicate $P< 0.05$. *Days from insemination.

In conclusion, the administration of 400 IU of eCG at Day 10 after insemination increases the size and activity of the corpus luteum. This treatment performed at Day 5 did not change the area of the corpus luteum or progesterone serum concentrations. Both treatments at Day 5 or at Day 10 affect follicular dynamics prolonging the duration of the wave that was growing at the time of eCG administration.

Experiment 6.
Pregnancy Rate and Pregnancy Maintenance.

In this experiment, the effect of the administration of eCG on Day 5 or Day 10 on pregnancy rate and embryonic and fetal loss in sheep was determined. We used 408 multiparous meat breed ewes with a BCS 2.8 ± 0.3 (scale 0-5). During the breeding season (April, 33 ° S, Uruguay) the ewes were treated for 6 days with intravaginal sponges containing 60 mg of medroxyprogesterone (Progespon, Syntex) associated with 300 IU of eCG (Novormon, Syntex) and 125 μg of cloprostenol (Ciclase DL , Syntex) administered intramuscularly at the time of sponge removal. TAI was performed (Day 0) between 48 and 56 h after sponge removal with intrauterine insemination with fresh semen diluted in UHT skim milk at a dose of 100 million sperm per ewe. Ewes were randomly assigned to three experimental groups to receive a second dose of 400 IU eCG at Day 5 (n = 137) or Day 10 (n = 138), while a third group received no eCG after insemination acting as the Control Group (n = 133). Between Day 15 and Day 24 day rate of non-return to estrus was determined using vasectomized rams. Pregnancy rate was determined by transrectal ultrasonography (5 MHz, Well-D, China) on Day 30.

Subsequently between Day 140 and Day 155 lambing was checked twice daily. Pregnancy rate was calculated as pregnant ewes on Day 30/inseminated ewes. Lambing rate was calculated as ewes lambing/ewes inseminated; prolificacy rate as fetuses or lambs per pregnant ewes or lambing ewes, respectively. Pregnancy rate and lambing rate were analyzed by logistic regression, while prolificacy rate by Poisson regression. No significant differences in these factors and the results are shown in Table 6.

Table 6. Pregnancy rate and losses during pregnancy in ewes receiving 400 IU of eCG at Day 5 or Day 10 after insemination.

	Non-return to Estrous	Pregnancy Diagnosis		Loss of Pregnancies	
		Pregnancy Rate	Prolificacy Rate	Non-lambing Ewes	Non-born lambs
Control	43.6% (58/133)	36.1% (48/133)	150.0% (72/48)	10.4% (5/48)	9.7% (7/72)
eCG on Day 5	48.2% (66/137)	35.0% (48/137)	131.3% (63/48)	10.4% (5/48)	7.9% (5/63)
eCG on Day 10	42.8% (59/138)	33.3% (46/138)	139.1% (64/46)	10.9% (5/46)	7.8% (5/64)

P=NS.

In conclusion. administration of a second dose of 400 IU of eCG on Day 5 or Day 10 after insemination in cycling ewes does not affect pregnancy rate or embryonic or fetal losses after 30 days of pregnancy.

FINAL COMMENTS

The results allow us to draw the following conclusions.

1) **When eCG is administered at the end of a TAI protocol in cows it:**
   - Increases the proportion of females in anestrous that ovulate, it therefore acts as an ovulation inducer.
   - Promotes the development of the ovulatory follicle.
   - Increases the development of the Corpus Luteum and its subsequent activity.
   - Increases progesterone concentrations during early gestation.
   - Increases pregnancy rates in anestrous females.

2) **2) When eCG is administered on Day 14 after TAI in cows in anestrous it:**
   - Increases the activity of the Corpus Luteum and serum progesterone concentrations.
   - Supports the maintenance of pregnancy during the first 30 days of pregnancy.

3) **When eCG is administered after TAI in sheep it:**
   - Increases the size of the Corpus Luteum and progesterone serum concentrations when administered on Day 10 after insemination.
   - This treatment does not affect pregnancy rates or embryonic/fetal losses after 30 days of gestation.

PUBLICATIONS

The information generated in these experiments resulted in the following published studies.

1) García-Pintos C., dos Santos Neto P.C., Menchaca A. 2011. Pregnancy rate and embryonic/fetal loss after eCG administration given 5 or 10 days after insemination in cycling ewes. Acta Scientiae Veterinariae, 39 (suppl 1), pp. 394.

2) García-Pintos C., Piaggio J., Menchaca A. 2013. Ovarian response to equine chorionic gonadotropin (eCG) treatment after insemination in sheep. *in preparation.*

3) Menchaca A., Núñez R. 2012. Tasa de preñez, retorno al estro y mantenimiento de la gestación en vaquillonas sometidas a diferentes tratamientos con progesterona y eCG durante el período crítico de la gestación. Veterinaria. 48, 178-178.

4) Menchaca A., Núñez R., Wijma R., García Pintos C., Fabini F., de Castro T. 2013. Como mejorar la fertilidad de los tratamientos de IATF en vacas *Bos taurus*. X Simposio Internacional de Reproducción Animal, Córdoba, Argentina, *in press.*

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