NEW ADVANCES IN HORMONAL PROGRAMS AIMED TO REDUCE EMBRYONIC LOSSES

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Introduction

High embryonic mortality is the main cause of the interval between births in cattle. Most deaths occur during the embryonic period of gestation (<45 d) (Thatcher et al., 1994; Vanroose et al., 2000; Sreenan et al., 2001), and according to Wathes (1992), the majority Of these embryonic losses occur in the first days after fertilization and during implantation of the embryo.
Embryonic mortality refers to the losses that occur during the first 45 days of gestation that coincide with the end of the embryo implantation period (Ayalon, 1978). Embryonic losses at one time can be classified as embryonic mortality, when it occurs within 25 days and late embryonic mortality, between 25 and 45 days (Humbolt, 2002). The term fetal death or abortion refers to losses occurring between 45 and 260 days of gestation (Forar et al., 1996).

In the dairy herds, late embryonic mortality in lactating cows was estimated to be 9.5 - 12.2% (Santos et al., 2004, Stevenson et al., 2003, Vasconcelos et al., 1997 Vasconcelos et al., 2006) In different studies where losses were evaluated between 27 - 31 and days 38 - 50 of gestation. On the other hand, other authors estimated that in the same periods the losses were from 18.6 to 23.4% (Chebel et al., 2003, Fricke et al., 2003, Sartori, 2006). Fetal losses were estimated at 10.7% between 38 and 90 days (López Gatius et al., 2002).

Pharmacological Treatments to Reduce Embryonic Losses

Several therapeutic strategies have been tried in dairy cows in order to induce accessory corpus luteous (CL), increase progesterone levels and reduce follicular growth and estrogen levels during early and late embryonic periods. Induction of ovulation with an implant of a GnRH agonist (Deslorelin, Peptech Animal Health, Australia) could stimulate the formation of a CL with increased production of progesterone. An experiment in Florida used 450 and 750æg deslorelin to induce ovulation in dairy cows and compared it to IATF protocols that used natural GnRH (100æg). Pregnancy rate was assessed at 27 and 41 days post-service and the implant of 750 µg reduced pregnancy rate compared to the 450 µg implant. Pregnancy rates were 40% at 41 days for both control and 450 ug implantation but pregnancy losses between day 27 and 41 tended to be lower for the 450 ug implant than for the control (5 % Vs. 12.7%, Thatcher et al., 2003).

One of the most common treatments in research was the application of GnRH either at 5, 7, 11 or 15 days post-AI or a combination of two of these days. In Florida on high production dairy cows a dose of GnRH (100 ug gonadorelin) was administered on days 5 and 15 post-insemination in a 2x2 design (Bartolome et al., 2005). Approximately 800 animals were used and no beneficial effect was found (4% increase in pregnancy at 55 days for day 15 and 3% at day 5 which was not significant) but administration of both (day 5 And 15) reduced the pregnancy rate to 55 days.
The administration of hCG should have better results due to a more potent LH action with a greater effectiveness in forming accessory luteal bodies and greater stimulation of them. In fact, administration of hCG at day 5 post-service increased the number of luteal bodies and plasma progesterone and conception rates at day 28, 45 and 90 and this increase was even more marked in cows losing body condition (Santos et al. Al., 2001).

Equine Chorionic Gonadotropin as a Strategy to Minimize Embryonic Loss

Equine Chorionic Gonadotrophin (eCG) is a complex glycopore with FSH and LH activity. It has a half-life of approximately 40 hours in the cow and persists for more than 10 days in the bloodstream (Murphy and Martinuk, 1991). The eCG stimulates follicular growth through its action of FSH and LH, increases follicle size, also increases progesterone plasma concentrations, thus improving embryonic development and maintenance of pregnancy (Barucelli et al, 2004). Also, eCG can improve pregnancy rates in foot-raised and poor-body cows (Roche et al., 1992). For its part Kastelic, 1999, says that eCG cannot be effective in cows with good body condition.
The use of 400 IU of eCG at the time of removal of the progesterone delivery device resulted in an increase in plasma progesterone concentration and pregnancy rates in foot-raised cows treated during postpartum anestrus (Baruselli et al. Bó et al., 2007).

An experiment in Brazil revealed that eCG treatments produce higher levels of serum progesterone in the next luteal phase, suggesting that eCG stimulates the development of a more competent corpus luteum (CL). This, in turn, may produce an increase in pregnancy rate (Souza et al., 2006). The eCG, makes an accessory CL (Thatcher, 2002). Circulating progesterone was increased in the luteal phase following treatment with eCG, when injected prior to ovulation (Murphy, 1991).

Consequently, generating alternatives to improve strategies to increase circulating progesterone through eCG injection after ovulation could improve embryo survival in Fixed Time Insemination (IATF) protocols.
A series of studies were recently published (Bartolomé 2009) in which the effect of placement was evaluated at a dose of 400 IU of eCG (Novormón) 22 days after AI in dairy cows. The experiment was performed in 4 replicates in different establishments and the percentage of pregnancy achieved at 29 by means of US was evaluated. In Table 1 it can be seen that a replica x treatment interaction was found. In some establishments the use of eCG after AI significantly improved pregnancy rates.

Table 1. Effect of treatment with 400 IU of eCG 22 post AI on pregnancy rates (PR) and embryonic losses.

Replic x Treatment

		US Day 29	US Day 45
1	Control	26.5 % (17/64)	20.3% (13/64)
	eCG	50% (31/62)	48.4% (30/62)
2	Control	55% (11/20)	45.0% (9/20)
	eCG	50% (9/18)	38.9% (7/18)
3	Control	40,3% (29/72)	27.8% (20/72)
	eCG	39.0% (25/64)	34.4$ (22/64)
4	Control	39.1% (18/46)	34.8% (16/46)
	eCG	47.7% (21/44)	43.2% (19/44)

eCG*Rep (P<0,05)

An effect of the decrease in embryonic mortality after day 29 can also be observed in these results, since in some of the replicates the percentage of pregnancy decreased more in the control group than in the eCG at 45 days.

In another experiment (Vilches, personal communication) we evaluated the use of 400 IU of eCG but in this case applied to the 14 days after IATF. All cows were treated with a standard IATF protocol and were divided to receive or not receive eCG on day 14 pos IATF.

In Table 2 it can be seen that a higher percentage of pregnancy was obtained in the diagnosis of day 33 as well as that the percentages of embryonic death were higher in the cows of the control group than in the eCG group at day 75.

Table 2. PR in dairy cows treated with 400 IU of eCG 14 days post FTAI.

	PR Day 33		PR Day 75		Embryonic Losses (Day 33 to 75)	
	%	n	%	n	$	n
Control	28.7	29 of 101	23.8	24 of 101	17.2	5 of 29
eCG	38.7	36 of 93	37.6	35 of 93	2.7	1 of 36

Use of eCG post FTAI in beef cows.

An experiment was designed with the objective of evaluating the effect of the application of a dose of 400 IU of eCG (Novormon, Syntex SA, Argentina) 14 days after the IATF on the percentages of pregnancy in Cebu x Bonsmara sulked cows, Inseminated at fixed time (IATF). A secondary objective was to evaluate the effect of the use of oestradiol benzoate (OEB) or GnRH as an ovulation inducer. 260 second-serving cows were used, between 60 and 120 days postpartum (DPP) and with a body condition between 2.0 and 2.5. On the day of treatment initiation (Day 0), the cows were palpated and a percentage of cyclicity of 11% was determined.

All animals received an intravaginal device with progesterone (DIB 0.5, 0.5g progesterone, Syntex SA) at Day 0. At that time the cows were divided to receive one of three treatments. Group E2 cows received 2 mg EB and cows from the GnRH and ½ GnRH Groups received a dose of 100 µg Gonadorelin (Gonasyn GDR, Syntex). On Day 7 the DIBs were removed from all cows and a dose of 500 µg cloprostenol (Cyclase DL, Syntex) was applied along with 400 IU of eCG (Novormon, Syntex) and the E2 Group cows received 0.5 mg of oestradiol cypionate (Cipiosyn, Syntex). All cows were IATF using semen from one of two bulls between 52 and 56 h after DIB withdrawal.

In addition, the cows of the GnRH Group received a dose of 100 µg of Gonadorelin and those of the Group ½ GnRH 50 µg of Gonadorelin at the time of the IATF. On Day 14 post IATF the cows of the three groups were subdivided into two (3 x 2 factorial) to receive or not a dose of 400 IU of eCG. The diagnosis of gestation was performed at 30 days post IATF by ultrasonography. The data were analyzed by logistic regression, taking into account the effect of the treatment and semen groups used. There were no differences (P = 0.16) between the pregnancy percentages of Groups E2 (39/87, 43.7%), GnRH (38/92, 41.3%) and ½ GnRH (25/81; 30.9%).

However, a greater percentage (P = 0.02) of pregnancy was obtained in cows treated with eCG on Day 14 post IATF (60/127; 47.2%) versus the Control Group (41/133; 30.8 %). On the other hand, Toro A (77/155; 47.1%) resulted in greater pregnancy (P = 0.009) than Toro B (28/107; 26.2%). No interactions were found between the variables analyzed (P> 0.1).

Table 3. Effect of the kind of ovulation inducer used and the treatment with eCG 14 days post FTAI sulked cows.

	eCG Day 14	**Control**	***Overall***
E_2	21/45 (46,7%)	17/42 (40,5%)	***39/87 (43,7%)***
GnRH	25/45 (55,6%)	13/47 (27,7%)	***38/92 (41,3%)***
1/2 GnRH	14/37 (37,8%)	11/44 (25,0%)	***25/81 (30,9%)***
Overall	***60/127 (47,2%)[a]***	***41/133 (30,8%)[b]***	

P=0,02

Conclusions

The use of eCG post-FTAI in beef and dairy cows may be an interesting alternative to reduce embryo mortality. It remains to be determined whether the positive effect of post-FTAI eCG is due to an increase in P4 levels. A series of experiments designed for this purpose are now underway. Likewise, experiments are carried out to determine the effect of treatment on heifers and embryo recipients.

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