Conceptus Survival In Cattle

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It has been estimated that rates of fertilization failure were approximately 13%, whereas rates of embryonic mortality, as a percentage of cattle inseminated, averaged about 15%. These are the two major causes of reproductive failure in dairy cattle that contribute to an overall failure rate of approximately 40%. During the summer periods in Florida, these losses are much greater due to heat stress effects on the maternal unit.

Maintenance of the corpus luteum (CL) in cattle is required if pregnancy is to persist. Uterine production of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) causes CL regression in cyclic cattle. Maintenance of the CL and hence survival of the conceptus or embryo depends upon the ability of conceptus produced products to "signal" the uterine endometrium and/or ovary to maintain the CL. The signal is initiated or produced by the conceptus between 15 to 17 days after estrus. A protein produced by the conceptus is involved because we have demonstrated that conceptus secretory proteins (produced by the conceptus during a 24 hour incubation in a culture dish at 37C) will extend life span of the CL when injected twice daily into the uterine lumen from day 15.5 to day 21. Cows given the conceptus secretory proteins returned to estrus at a mean of 33.4 days (n=3 cows) versus 23.5 days (n=3 cows) for the control group. These results combined with plasma progesterone analyses indicate that the conceptus is able to prevent regression of the CL which is essential for maintenance of pregnancy. Research is being continued to characterize and purify the specific protein so that we may be able to improve conceptus survival in the future. At the present time, we know that the conceptus secretory proteins reduce the ability of the uterus to secrete $PGF_{2\alpha}$ when estradiol is injected at day 18 postestrus.

Research in our and other laboratories indicates that injection of Human Chorionic Gonadotrophin (HCG) will extend estrous cycles in cattle. We have conducted a series of experiments to examine if HCG delays CL regression, if it does how does it act, and if injection of HCG will improve pregnancy rates.

In all of our experiments, HCG (3300 IU) was injected intravenously at day 15 of the estrous cycle; day 15 just precedes the critical time when the conceptus "signals" or initiates the pregnancy recognition process or extends life span of the CL. In experiment I, injection of HCG caused animals (n=15) to have an estrous cycle length of 24.0 days versus 20.0 days for control animals (n=15). Analyses of plasma progesterone indicated that CL regression was delayed by approximately 3

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days for animals injected with HCG. HCG may delay CL regression via several possible mechanisms such as: blocking the luteolytic action of $PGF_{2\alpha}$, reducing uterine secretion of $PGF_{2\alpha}$, altering ovarian activity that delays ovarian induced secretion of uterine $PGF_{2\alpha}$, or induction of a new ovulation and CL formation.

Experiment II indicated that injection of HCG on day 15 did not block the luteolytic effect of a $PGF_{2\alpha}$ injection (25 mg) given 24 hours later on day 16. Both groups of cows (HCG or Saline treatments) had an abrupt decrease in plasma progesterone associated with CL regression that occurred on day 17 and 18 following the injection of $PGF_{2\alpha}$. All control cows (n=5) had an induced estrus on day 18.5. In contrast none of the HCG treated cows had a detected estrus by day 25. Although HCG treated cows had an abrupt decrease in plasma progesterone concentrations, values did not go below 1 ng/ml, and these values were sustained until at least day 28. When additional cows (n=3) were treated (HCG on day 15 plus $PGF_{2\alpha}$ on day 16) and ovariectomized on day 18, the ovarian responses were clear. The original CL was regressed, several follicles were luteinized and a new CL (≅2.5 days old) was present. Consequently, HCG did not block the luteolytic effect of exogenous $PGF_{2\alpha}$ and ovulated a follicle that was present on day 15. Formation of a new CL and luteinization of follicles were probably responsible for the sustained basal levels of P_4 in the range of 1 to 2.5 ng/ml. The important point of the experiment was that HCG did not block the luteolytic effect of $PGF_{2\alpha}$ (25 mg) that was injected.

Experiment III was conducted to determine if HCG reduced the uterine secretion of $PGF_{2\alpha}$ when ovariectomized cows were injected intravenously with estradiol (3 mg). The experimental model was that ten cows were ovariectomized on day 14 of the estrous cycle, injected with either HCG (n=5 cows) or saline (n=5 cows) on day 15, and all were injected with estradiol on day 16. Progesterone concentrations of 2 ng/ml of plasma were maintained by installation of a "Progesterone Release Intravaginal Device" (PRID) on day 13 or 24 hours prior to ovariectomy (day 14). The uterine secretion of $PGF_{2\alpha}$ induced by estradiol injected on day 16 was monitored over a 10 hour period (30 min sample) in which plasma concentrations of 15 keto-13,14 dihydro-$PGF_{2\alpha}$ were measured. Results indicated that estradiol induced $PGF_{2\alpha}$ secretion, but the response was the same for cows treated with either HCG or saline. Implications are that HCG does not directly inhibit uterine secretion of $PGF_{2\alpha}$ in ovariectomized cows, and that a delay of CL regression in intact cows (Experiment I) is likely due to some ovarian effect (e.g. follicle luteinization) which delays the normal ovarian induced secretion of $PGF_{2\alpha}$ from the uterus that would cause CL regression. It is important to recognize that mechanisms of luteal maintenance brought about by HCG and conceptus secretory proteins are probably different. The reason for this assumption is that we know uterine $PGF_{2\alpha}$ secretion in response to an injection of estradiol is reduced in cows treated with conceptus secretory proteins but not when treated with HCG.

At this point, a series of field experiments (IV and V) was conducted at Larson's Dairy, Inc., Okeechobee, FL to determine if injection of HCG on day 15 postinsemination affected pregnancy rates.

Our rationale for treatment was that an induced delay in CL regression may permit developing conceptuses an additional 24 to 48 hours to expand so they would have a greater probability of blocking the normal cyclic release of $PGF_{2\alpha}$ that causes CL regression. Two hundred and four heifers were injected randomly with either HCG or saline on day 15 postinsemination. Injections were completed over a 7 day period (October 9 to 16, 1983), and all heifers palpated for pregnancy on 12-14-83 between 74 to 81 days of pregnancy. Pregnancy rates for Experiment IV are presented in Table I.

TABLE 1. PREGNANCY PERCENT IN 204 HEIFERS TREATED WITH HCG[a] OR SALINE AT DAY 15 POSTINSEMINATION: TRIAL I.

GROUP	ALL SERVICES	SERVICE	
		1	2-5 (POOLED)
OVERALL MEAN	46.6%	53.2%	31.7%
HCG	51.9% (54/104)	59.7% (43/72)	34.4% (11/32)
SALINE	41.0% (41/100)	46.4% (32/69)	29.8% (9/31)
DIFFERENCE	+10.9%	+13.3%	+5.4%

[a] 3,300 International Units (IU) given intravenously.

Overall pregnancy rate was 46.6%, and a clear difference was detected between heifers bred to their first service versus services 2 to 5 (53.2% > 31.7%). Pregnancy rates were lower than expected and may be due to a continued heat stress effect during early fall in South Florida. HCG caused a significant ($P<.10$) overall increase in pregnancy rate of 10.9%, and the benefit was greater in first service heifers (13.3%) than those presented for second to fifth services (5.4%). In heifers that were not pregnant, interestrous intervals were 29.0 and 24.0 days for HCG (n=36) and saline (n=49) treated heifers, respectively. If interestrous intervals are restricted to cycles less than 38 days (to avoid the bias of possible missed heats), then interestrous intervals for HCG (n=27) and saline (n=42) groups were 24.3 and 21.3 days, respectively. This 3 day delay agrees closely with the earlier results in Experiment I.

An exact replicate of Experiment IV was repeated during a 20 day period of February 18 to March 9, 1984 (Experiment V). Reasons for replicating the experiment were two fold: to repeat or further document the beneficial effect of HCG and perform the experiment when herd fertility would be higher (cooler period of year).

TABLE 2.[a] PREGNANCY PERCENT IN 206 HEIFERS TREATED WITH HCG OR SALINE AT DAY 15 POSTINSEMINATION: TRIAL II.

GROUP	ALL SERVICES	SERVICE	
		1	2-5 (POOLED)
OVERALL MEAN	61.2%	68.8%	36.7%
HCG	54.8% (57/104)	63.3% (50/79)	29.0% (7/25)
SALINE	67.6% (69/102)	74.4% (58/78)	45.8% (11/24)
DIFFERENCE	-12.8%	-11.1%	-17.8%

[a] 3,300 International Units (IU) given intravenously.

As described in Table II, 206 heifers were treated randomly with either HCG or saline. Overall pregnancy rate was 61.2%, and a clear difference in pregnancy rate was detected between first service (68.8%) and services 2-5 (36.7%). Results of Experiment V indicated that HCG decreased overall pregnancy rate by 12.8% compared to the control group (54.8% < 67.5%). Furthermore, this decrease due to HCG was expressed uniformly in groups of heifers presented for first service (-11.1%) or 2 to 5 services (-17.8%).

The contrasting results of both experiments were unexpected but quite interesting. A pooled statistical analysis with 410 heifers indicated that their was a highly significant "Experiment by Treatment" interaction. Biologically this means that HCG had affects on fertility in both experiments, but the effects differed (positive in Experiment IV and negative in Experiment V) between experiments. It is also important to recognize that both experiments represent sensitive systems for testing a treatment effect on pregnancy rate. The experiments were: conducted on one farm, completed over a short period of time (7 to 20 days), inseminations were made by essentially one inseminator who was the same for both experiments, and heifers were uniform in quality and assigned randomly to treatments. Thus, why were the treatment effects different between the two trials? There may have been a true effect of environment between the two experiments. Fertility was lower in Experiment IV. If this was due to a heat stress than conceptuses that survive may benefit from a delay in luteal regression which will permit them an additional period for elongation of extraembryonic membranes. Such a benefit may account for the 10.9% increase in fertility. In contrast, heifers in Experiment V did not have to cope with possible thermal stress effects and pregnancy rate was indeed higher (saline group of Experiment V, 67.6% versus 41.0% for saline group of Experiment IV). In fact, animal ovarian responsiveness was different between the two experiments. The frequency of double corpora lutea between pregnant heifers, treated with HCG versus saline, for Experiment IV (HCG, 7 of 54 [13.0%] versus saline, 0 of 41 [0%]) and

Experiment V (HCG, 17/56 [30.4%] versus saline, 3 of 69 [4.4%]) were different. Ovaries appeared to be more responsive to HCG in Experiment V, and this increased ovarian sensitivity to HCG may exert an overall detrimental effect when embryo survival or fertility is high. Although reasons for the differential response of pregnancy rate to HCG are not now apparent, the potential benefit in periods of lower fertility, associated with a possible heat stress, justifies a third replicate during the intense summer months (e.g. July) of thermal stress. It is not a surprise to Florida dairymen that factors governing fertility during summer and early fall (heat stress periods) differ from those of late fall to early spring. Consequently, a seasonal difference of HCG effects on pregnancy rate may not be surprising. We all look forward to results of Experiment VI to be replicated in July, 1984 during the heat stress period of South Florida.