The Effect of Exogenous Progesterone on the Quality of in vitro Produced Bovine Embryos

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Introduction

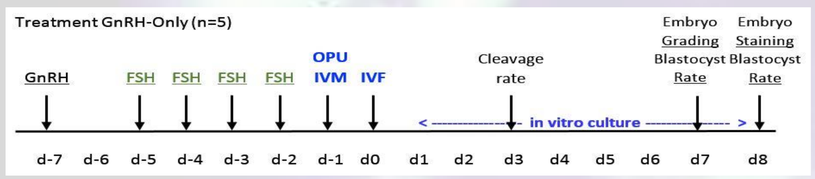
Maternal genetics can be advanced with the implementation of in vitro fertilization (IVF) in the beef and dairy industries. The efficiency of IVF-derived pregnancies is impacted by embryo quality which can be determined by cell counts.

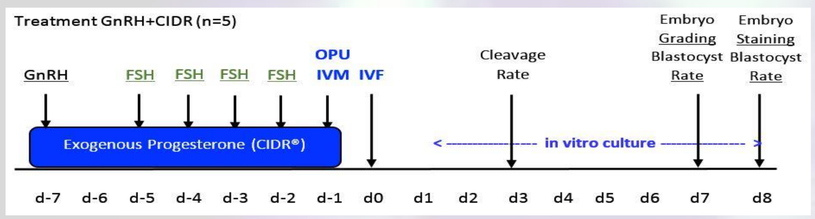
Objective

To determine what effect exogenous progesterone has on the quality of in vitro derived beef embryos

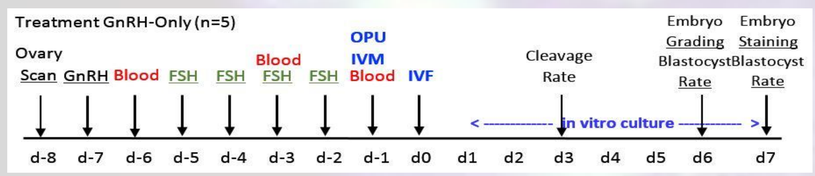
Experimental Design

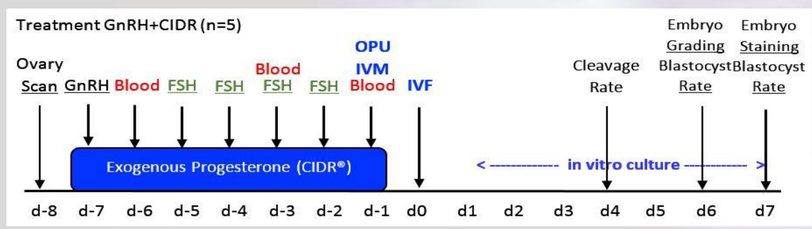
Year-1





Year-2





Methods and Materials

The Kansas State University Purebred Teaching Unit provided 10 head of Angus, Hereford, Simmental crossed mature cows. The cows were evenly divided into two treatment groups (GnRH+CIDR and GnRH-Only) with five animals per treatment. On Day -7, at 3:00 pm all cows received 100 µg of GnRH (Cystorelin®). Cows in the GnRH+CIDR treatment received exogenous progesterone (Eazi-Breed CIDR®). On Day -5, all cows received 70 I.U. of FSH (Follitropin®) administered intramuscularly at 4:30 p.m. On Day -4, all cows received 56 I.U. of FSH administered intramuscularly at 7:30 am and at 4:30 p.m. On Day -3, all cows received 56 I.U. of FSH administered intramuscularly at 7:00 a.m. and the same dosage was administered at 4:30 p.m. On Day -2, at 7:00 a.m., 56 I.U. of FSH was administered intramuscularly to both treatment groups.

On Day -1, ovum pick up (OPU) was performed. Follicular aspiration was completed via an ultrasound-guided transvaginal probe. Oocytes were transferred into pre-equilibrated maturation media and placed in a transport incubator at 38.5˚ Celsius. Oocytes were moved after ~ 3 hours to a 6 % CO2 incubator at 38.5˚ C. Oocytes underwent in vitro maturation (IVM) for 24 hours.

On Day 0, oocytes were added to the in vitro fertilization (IVF) media. Sperm was prepped and then added to the oocytes. On Day 1, 18-22 hours after fertilization, presumptive zygotes were transferred into in vitro culture (IVC) media. Culture took place in a tri-gas Incubator. On Day 3, cleavage rates, (single cell division) were evaluated. On Day 7, embryos were graded and blastocyst (≥ Stage 5) rate was recorded.

On Day 8, embryos were stained using the following procedures. Microdrops of 45 µL that contained embryos had 5 µL of RNase solution added directly to them. These drops were incubated at 38.5˚ C for 1 hour. A gridded petri dish was divided into four quadrants. One quadrant, labeled PI, contained a 50 µL drop of PI solution. The remaining three quadrants, labeled PBS/PVP, contained a 50 µL drop of PBS/PVP drop. The PI solution in the petri dish was warmed for 5-10 minutes prior to staining on a slide warmer. A Drummond was used to remove blastocysts from culture. They were then pipetted into the Pi solution for 30 seconds. After the 30 seconds, blastocysts were washed through the 3 drops of PBS/PVP. A small drop of glycerol was placed onto microscope slides and embryos were placed into a small volume of solution onto the glycerol drop. The coverslip was then mounted. Embryos were viewed using an epifluorescent microscope with blue filters. Pictures were taken, and cells were counted.

Data were analyzed using the GLIMMIX procedure of SAS v.9.4 (Cary, NC) with cow as the experimental unit.

The procedures from Year-1 were followed for Year-2 but the following adjustments were made. On Day -8, ultrasonography was conducted to determine the presence of a corpus luteum (CL). The information collected was then used to assign cows to treatment. On Day -6, -3, and -1 blood samples were collected and used to test progesterone levels. Embryos were graded, and blastocyst rate was checked on day 6 instead of 7 and then stained on Day 7 instead of Day 8.

Figure 1. Stained Embryos with cells highlighted.

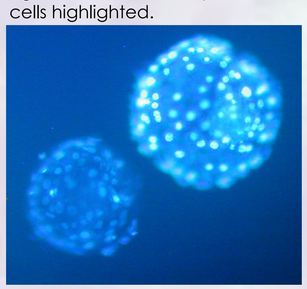


Figure 2. Example of how cells were counted.

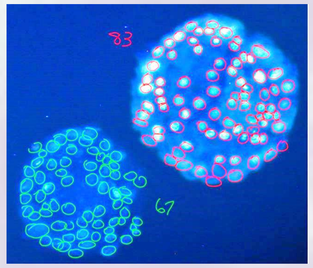
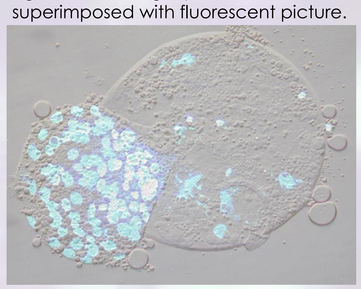


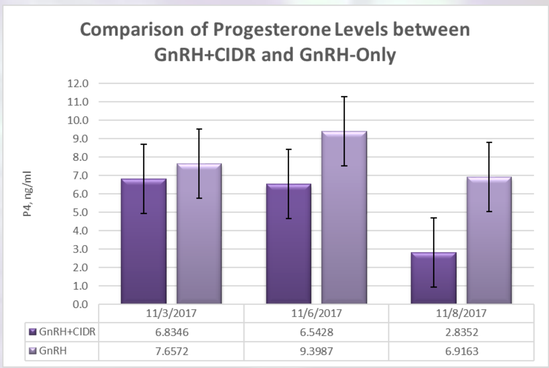
Figure 3. White light photo superimposed with fluorescent picture.

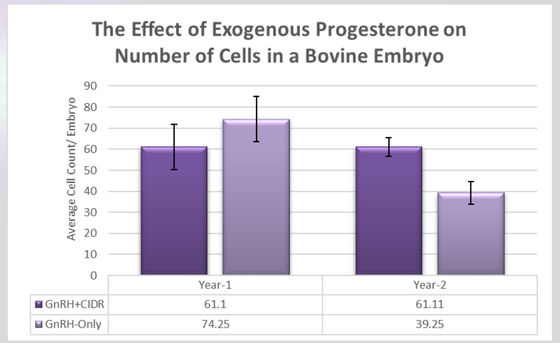


Results

GnRH+CIDR						GnRH-Only					
Cow ID	Cleavage[1]		Blastocyst[2]		Average Cell Count	Cow ID	Cleavage[1]		Blastocyst[2]		Average Cell Count
	#	%	#	%			#	%	#	%	
Year-1											
7136	2/9	22	2/9	22	88	6133	9/11	80	2/11	18	106
12T	6/6	100	6/6	100	61	918	3/7	43	3/7	43	75
14S	8/9	89	4/9	44	53	5204	8/12	67	4/12	33	65
6157	7/9	78	4/9	44	42	4105	7/12	58	2/12	17	51
8179	0/0	0	0/0	0	0	82P	0/0	0	0/0	0	0
Year-2											
0304	12/31	38	3/31	10	70	61C	10/13	77	4/13	31	44
5225	4/7	57	3/7	43	58	N594	12/15	80	4/15	27	34
4329	15/21	71	3/21	14	56	5104	2/4	50	0/4	0	0
3157	10/21	47	0/20	0	0	513	7/10	70	0/10	0	0
5331	3/9	33	0/9	0	0	507	14/24	58	0/24	0	0

[1]Single Cell Division [2]≥Stage 5





Conclusions

- Exogenous progesterone did not affect the quality of in vitro produced embryos.
- The results of this experiment suggest that further research that includes more experimental units would be beneficial in determining what influence progesterone has on oocyte and embryo quality.

Future Research

- Is there a threshold on progesterone for embryo quality?
- Does progesterone directly affect the quality of the oocyte?
- Does progesterone have an indirect affect by manipulating the stage of the cycle so that the cow is set up to have a new follicular wave?
  - Does this indicate that the use of a pre-synchronization protocol may be influential.