Date of Approval: July 13, 2014

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-431

FOLLTROPIN

Porcine pituitary-derived follicle stimulating hormone for injection

Solution for Injection

Cattle/beef and dairy heifers and cows

For the induction of superovulation in beef and dairy heifers and cows

Sponsored by:

Bioniche Animal Health USA, Inc.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 141-431

B. Sponsor

Bioniche Animal Health USA, Inc.
119 Rowe Rd.
Athens, GA 30601

Drug Labeler Code: 064847

C. Proprietary Name

FOLLTROPIN

D. Established Name

Porcine pituitary-derived follicle stimulating hormone for injection

E. Pharmacological Category

Reproductive hormone

F. Dosage Form:

Solution for injection

G. Amount of Active Ingredient

700 IU FSH/vial (equivalent to 400 mg NIH-FSH-P1); when reconstituted, the final solution contains 35 IU/mL FSH

H. How Supplied

20 mL vial containing FSH and 20 mL vial containing diluent

I. Dispensing Status

Rx

J. Dosage Regimen

Administer 2.5 mL (87.5 IU) intramuscularly, twice daily at 12 hour intervals, for 4 consecutive days. In conjunction with the 6^{th} dose of FOLLTROPIN, administer an FDA-approved prostaglandin product (cloprostenol sodium or dinoprost tromethamine) for cattle, using the labeled dosage and administration instructions to cause luteolysis and induce estrus.

K. Route of Administration

Intramuscular injection

L. Species/Class

Cattle/beef and dairy heifers and cows

M. Indication

For the induction of superovulation in beef and dairy heifers and cows

II. EFFECTIVENESS

A. Dosage Characterization

Follicle stimulating hormone (FSH) is a glycoprotein hormone produced by the anterior pituitary gland which acts to initiate ovarian follicular growth. Following ovulation in cattle, groups of ovarian follicles emerge in waves multiple (usually two or three) times during the estrous cycle. These follicular waves are under the influence of FSH. In a normal estrous cycle, generally only one follicle becomes dominant, fully develops, and is ovulated, and other smaller follicles regress during each follicular wave. The administration of exogenous FSH at the time of the emergence of ovarian follicles allows for additional follicles to develop and ovulate. Treatment initiated between 8 and 10 days after estrus is based on the timing of the emergence of the second follicular wave in the estrous cycle.

The proposed dose of FOLLTROPIN (700 IU total dose) was selected based on widespread clinical use experience and published literature. Due to the short half-life (approximately five hours) of FSH in cattle following intramuscular injection (Demoustier et al., 1988), twice daily injections are recommended to induce superovulation.

The administration of prostaglandin F2α or a prostaglandin F2α analogue is used to induce regression of the corpus luteum (luteolysis) and allow for the precise timing of estrus and ovulation for breeding purposes. The administration of dinoprost tromethamine or cloprostenol sodium in conjunction with the sixth dose of FSH is consistent with the biologically appropriate time for the induction of luteolysis.

A target dosage regimen of 87.5 IU of FOLLTROPIN, given as an intramuscular injection twice daily for four consecutive days (total dose of 700 IU), beginning 8 to 10 days following observed estrus, for the superovulation of beef and dairy heifers and cows, was selected for further evaluation through the use of a systematic review and meta-analysis.

B. Substantial Evidence

1. Systematic Review and Meta-analysis

a. Title: "The effectiveness of FOLLTROPIN for the induction of superovulation in beef and dairy heifers and cows: systematic review and meta-analysis."

b. Study Methods:

(1) Objective: To evaluate the effectiveness of FOLLTROPIN for the induction of superovulation in beef and dairy heifers and cows based on the number of transferable embryos.

(2) Eligibility criteria: All peer-reviewed complete original research reports and sponsor conducted studies evaluating the effectiveness of FOLLTROPIN for the induction of superovulation were considered. Abstracts and review articles were excluded. Language and publication dates were not restricted; however, no reports required translation.

(3) Information sources: Peer reviewed original research reports were identified by searching electronic databases and the reference lists in the reports independent of the publication date. The databases searched included Scirus, Science.gov, World Wide Web Science.org, ScienceDirect and NCBI (PubMed). The search results from PubMed encompassed the articles found in the other search engines. A final search of PubMed was completed on February 3, 2012.

(4) Search: The following search terms were utilized:

Folltropin
FOLLTROPIN
FSH and cattle and superovulation
Follitropin and cattle and superovulation
pFSH
Porcine FSH and cattle
Ovarian stimulation and cattle and FSH
Ovarian stimulation and cattle and Folltropin
Superovulation and cattle
Superovulation and cow

(5) Study selection and data collection process: The eligibility assessment was performed independently in an unmasked and standardized manner by a panel of experts. Disagreements were resolved by consensus. Where possible, study authors were contacted to obtain missing information critical to the eligibility assessment. Treatment arms (i.e., treatment groups) from studies were selected for inclusion in the systematic review and meta-analysis if they met the following criteria:

- The use of the final formulation of FOLLTROPIN;
- The use of the proposed route of administration (intramuscular);
- The use of a dose equal to or less than the proposed dose (total dose of 700 IU) given once or twice daily over multiple days (studies that used a single injection were excluded) beginning mid-way through the estrous cycle;
- Complete internal reports from sponsor or peer reviewed original research reports (excluded abstracts and review articles);
- The use of heifers and cows representative of North American beef and dairy cattle;
- No hormonal treatments overlapping the FSH treatment other than properly timed prostaglandin injection(s);
- Estrus synchronization prior to the superovulation treatment utilized

FDA approved regimens or regimens using FDA approved drugs; and

- Study designed to evaluate the use of FSH on the number of transferable embryos

The above inclusion criteria were selected for the purpose of reducing sources of variability that would be expected to have a significant impact on the evaluation of the effectiveness of FOLLTROPIN for the induction of superovulation, while allowing for the inclusion of studies which reflected the conditions of use.

(6) Data items: The information extracted from each study included the geographic location of the study; the number of times individual cattle were superovulated within the study; the total dose of FOLLTROPIN; the total days of treatment dosing; the frequency of treatment administration (once or twice daily); the breed and class (beef or dairy or both) of study animals; the age of study animals (cow or heifer or both); the type of prostaglandin (dinoprost tromethamine or cloprostenol sodium), total dose, and number of injections of prostaglandin given for luteolysis; the number of superovulation events in the treatment arm; the number of cattle and the number of superovulation events used in the effect size (number of transferable embryos) calculations for each treatment arm; the transferable embryo value and the units (mean or least square mean); the measure and type of variability of the transferable embryo value; the statistical method used to estimate the effect; and the percent missing data in the treatment arm.

(7) Risk of bias in individual studies: Individual studies were evaluated in an unmasked manner to determine whether they used appropriate scientific methods to control bias and evaluate the primary effectiveness endpoint (number of transferable embryos).

(8) Summary measure: The mean number of transferable embryos was the primary summary measure of the effectiveness of treatment. Embryos were defined as transferable in accordance with the quality codes established by the International Embryo Transfer Society [International Embryo Transfer Society Manual (IETS), 4th edition, 2010].

c. Analysis:

A funnel plot was used to detect bias and heterogeneity across treatment arms visually. A stratified analysis by study size was used to assess the publication bias and an Egger's regression test was used to assess symmetry of the funnel plot quantitatively. A Q statistic test was used to test the heterogeneity among the treatment arms and the I^2 statistic was used to quantify heterogeneity.

The overall effect size (mean number of transferable embryos and the upper and lower bounds of the 95% confidence intervals) was estimated using a random effects model because of the significant heterogeneity ($P < 0.0001$) between treatment arms.

Sensitivity analyses were performed to investigate how sensitive the estimates of the overall effect size were to the method of analysis and changes in the dataset; to determine the impact of studies characterized as "high risk" and/or exhibiting extreme variation in the funnel plot; and the impact of confounding factors within the dataset.

d. Results:

(1) Study selection: Treatment groups within 21 studies met the inclusion criteria, were included in the systematic review, and were used in the estimation of the effect size in the meta-analysis. Table II.B.1 summarizes the studies included in the meta-analysis.

Table II.B.1: List of study reports included in the meta-analysis

Study Identification:
Adams GP, et al (1994). Superovulatory response of ovarian follicles of wave 1 versus wave 2 in heifers. *Theriogenology*, 42:1103-1113.
Amiridis GS, et al (2006). Follicle ablation improves the ovarian response and the number of collected embryos in superovulated cows during the early stages of lactation. *Reproduction in Domestic Animals,* 41:402-407.
Bennett-Steward K (2002). A retrospective study to confirm the reproductive and embryological safety of FOLLTROPIN®-V use in dairy cattle. Bioniche internal document, Study BF-20.
Bo GA, et al (1994). Superovulatory response to a single subcutaneous injection of FOLLTROPIN-V in beef cattle. *Theriogenology*, 42:963-975.
Bo GA, et al (1996). Effect of progestogen plus estradiol-17β treatment on superovulatory response in beef cattle. Theriogenology, 45:897-910.
Boland MP (1999). Safety and efficacy of FOLLTROPIN for the induction of superovulation in cattle. Bioniche internal document, Study BF-9.
Bungartz L & Niemann H (1994). Assessment of the presence of a dominant follicle and selection of dairy cows suitable for superovulation by a single ultrasound examination. *Journal of Reproduction and Fertility*, 101:583-591.

Study Identification:
Callejas S, et al (2008). Effect of progesterone administration on the ovarian response to superovulatory treatments in cattle. *Animal Reproduction Science*, 107:9-19.
Childs S, et al (2008). Embryo yield and quality following dietary supplementation of beef heifers with *n*-3 polyunsaturated fatty acids (PUFA). *Theriogenology*, 70:992-1003.
Clements P (1999). Efficacy study in cattle to determine the superovulatory response to a porcine pituitary follitropin extract (Folltropin®-V) under field conditions; United Kingdom; Bioniche internal document, Study FOL-UK.
de Feu MA, et al (2008). The effect of strain of Holstein-Friesian cow on size of ovarian structures, periovulatory circulating steroid concentrations, and embryo quality following superovulation. *Theriogenology*, 70:1101-1110.
DeGrofft D (1992). A comparison of duration of FOLLTROPIN®-V administration in the cow to optimize the superovulatory response. Bioniche internal document, Study FOL-US5.
Ireland JJ, et al (2007). Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. *Human Reproduction*, 22:1687-1695.
Kelly P, et al (1997). Superovulation in cattle: effect of FSH type and method of administration on follicular growth, ovulatory response and endocrine patterns. *Animal Reproduction Science*, 46:1-14.
Larson JE, et al (2010). Embryo production in superovulated Angus cows inseminated four times with sexed-sorted or conventional, frozen-thawed semen. *Theriogenology*, 73:698-703.
Lima WM, et al (2007). Improved superovulatory response in beef cattle following ovarian follicular ablation using a simple transvaginal device. *Animal Reproduction Science*, 100:364-370.
Mapletoft RJ (1994). Superovulation with Vetrepharm FSH (Folltropin): Dose-response trial – dosage regimen. Bioniche internal document.

Study Identification:
Rajamahendran R & Calder MD (1993). Superovulatory responses in dairy cows following ovulation of the dominant follicle of the first wave. *Theriogenology*, 40:99-109.
Schenk JL, et al (2006). Embryo production from superovulated cattle following insemination of sexed sperm. *Theriogenology*, 65:299-307.
Staigmiller RB, et al (1995). The effect of estrus synchronization scheme, injection protocol and large ovarian follicle on response to superovulation in beef heifers. *Theriogenology*, 43:823-834.
Walsh JH, et al (1993). The effects of once or twice daily injections of pFSH on superovulatory response in heifers. *Theriogenology*, 40:313-321.

(2) Study characteristics: The studies included 20 clinical studies and one retrospective study. Fifty treatment groups within the 21 studies were included in the meta-analysis. There were 2177 study animals and 2685 superovulation records used in the effect size calculation. Table II.B.2 provides a summary of the treatment groups (treatment arms) used from each study including the sample size, effect size, and 95% confidence interval.

Table II.B.2: Treatment groups included in the meta-analysis

Treatment Arms[1]	Sample Size[2]	Effect Size[3]	95% Lower Limit	95% Upper Limit
Adams et al (1994); Exp 2 wave 2 group	21	3.00	1.82	4.18
Amiridis et al (2006); Trial 1, Grp 1A	18	1.67	0.98	2.36
Amiridis et al (2006); Trial 1, Grp 1B	9	3.00	-0.49	6.49
Bo et al (1994); Exp 1, Grp I	7	4.80	0.10	9.50
Bo et al (1994); Exp 2, Grp I	9	2.80	0.06	5.54
Bo et al (1996); Exp 1, Control grp	16	5.50	2.17	8.83
Bungartz & Neimann (1994); Exp 1+, dominant follicle present	9	2.10	0.34	3.86
Bungartz & Neimann (1994); Exp 1-, dominant follicle absent	17	5.00	3.04	6.96
Bungartz & Neimann (1994); Exp 2+, dominant follicle present	12	0.30	-0.09	0.69
Bungartz & Neimann (1994); Exp 2-, dominant follicle absent	14	7.80	2.90	12.70
Bungartz & Neimann (1994); Exp 4+, dominant follicle present	11	1.00	0.02	1.98
Bungartz & Neimann (1994); Exp 4-, dominant follicle absent	14	10.30	5.99	14.61
Bungartz & Neimann (1994); Exp 4-,aspiration	13	10.10	7.16	13.04
Callejas et al (2008); Control group	12	2.80	0.64	4.96
Childs et al (2008); Control (standard diet)	16	6.60	4.93	8.27
Childs et al (2008); n-3 PUFA dietary group	17	5.88	4.21	7.55
deFeu et al (2008); NZ Holstein	15	4.20	3.55	4.85
deFeu et al (2008); NA Holstein	14	3.00	2.11	3.89
Ireland et al (2007); Exp 3, high follicle # grp	19	5.40	2.85	7.95
Ireland et al (2007); Exp 3, low follicle # grp	21	3.80	2.23	5.37
Kelly et al (1997); Exp 1, MF grp	22	5.50	3.15	7.85
Larson et al (2010); Conventional semen, period 1 & 2 combined	32	5.90	3.94	7.86
Larson et al (2010); Sexed semen, period 1 & 2 combined	32	3.80	2.04	5.56
Lima et al (2007); Grp 1, non-palpable follicle	106	8.10	7.10	9.10
Lima et al (2007); Grp 2, follicle left intact	62	6.40	5.09	7.71
Lima et al (2007); ablation 0 h	31	10.30	8.46	12.14
Lima et al (2007); ablation 24 h	35	10.90	9.16	12.64
Lima et al (2007); ablation 48 h	10	7.30	4.05	10.55
Rajamahendran & Calder (1993); Control grp	10	3.10	0.36	5.84
Schenk et al (2006); Trial 1, Control grp, nonsexed semen	29	8.70	6.94	10.46
Schenk et al (2006); Trial 1, $10x10^6$ sexed semen	30	4.10	2.34	5.86

Treatment Arms[1]	Sample Size[2]	Effect Size[3]	95% Lower Limit	95% Upper Limit
Schenk et al (2006); Trial 1, $2x10^6$ sexed semen	30	3.30	1.54	5.06
Staigmiller et al (1995); Trial II, MGA 8	5	11.60	7.11	16.09
Staigmiller et al (1995); Trial II, PGF 8	5	10.80	6.31	15.29
Walsh et al (1993); Exp 2, grp T2	40	4.70	3.33	6.07
Bennett-Steward BF-20 study (2002)	1164	7.52	7.15	7.89
Boland BF-9 study (1999); Folltropin grp	13	4.55	3.22	5.88
Clements FOL-UK study (1999); Folltropin-V data	639	5.20	4.86	5.54
DeGrofft, FOL-US5 study (1992); Grp A-40	12	6.70	2.98	10.42
DeGrofft, FOL-US5 study (1992); Grp B-80	11	2.80	1.23	4.37
DeGrofft, FOL-US5 study (1992); Grp C-50	11	4.60	2.25	6.95
DeGrofft, FOL-US5 study (1992); Grp D-100	11	2.70	1.52	3.88
DeGrofft, FOL-US5 study (1992); Grp E-66.5	9	6.40	3.07	9.73
DeGrofft, FOL-US5 study (1992); Grp F-133	10	5.20	0.69	9.71
DeGrofft, FOL-US5 study (1992); Grp G-100	7	7.00	2.49	11.51
DeGrofft, FOL-US5 study (1992); Grp H-200	9	4.90	1.18	8.62
Mapletoft dosage regimen study (1994); Grp A, bid, 4d, constant	6	6.30	2.58	10.02
Mapletoft dosage regimen study (1994); Grp B, bid, 5d, constant	6	3.30	1.14	5.46
Mapletoft dosage regimen study (1994); Grp C, bid, 4d, decrsg	7	2.10	0.14	4.06
Mapletoft dosage regimen study (1994); Grp D, bid, 5d, decrsg	7	1.10	-0.27	2.47
Boland BF-9 study control (1999); Placebo grp[4]	14	1.66	0.07	3.25

[1]Treatment Arms: Treatment groups within studies that are included in the meta-analysis

[2]Sample size: The number of superovulation events used in the effect size calculations for each treatment arm

[3]Effect size: The mean number of transferable embryos within the treatment arm

[4]Boland BF-9 placebo control: The mean number of transferable embryos (1.66) in 14 cows given the sterile diluent for FOLLTROPIN within the Boland (1999) study.

(3) Bias and heterogeneity assessment: A funnel plot of the effect size (number of transferable embryos) versus the standard error was used to detect heterogeneity and bias, including publication bias. A slightly asymmetric funnel plot suggested potential publication bias within the studies included in the meta-analysis. However, a stratified analysis by study size did not support the existence of potential publication bias, and an Egger's regression test showed that the potential publication bias was within an acceptable range given the power of the test.

A Q statistic test revealed that the heterogeneity among treatment

arms was significant ($P < 0.0001$). The I^2 statistic was used to quantify the heterogeneity. The percentage of total variability due to between-treatment group variability was 95.5%. A two-level mixed effects model was chosen to estimate the overall effect size because it accounted for both between-study and between-treatment group variability.

(4) Synthesis of results: The overall effect size results are summarized in Table II.B.3

Table II.B.3: Effect size estimation

Estimate[1]	Standard Error	DF[2]	t Value[3]	Pr > |t|	Alpha[4]	Lower[5]	Upper[5]
4.5	0.49	20	9.29	<0.0001	0.05	3.5	5.6

[1] Estimate of overall mean of number of transferable embryos
[2] Degree of freedom
[3] Value of T statistics
[4] Level of significance
[5] Lower and Upper limit of 95% confidence interval

Table II.B.3 shows that cows treated with FOLLTROPIN produce 4.5 transferable embryos on average. The estimated mean number of transferable embryos (4.5) is a biologically meaningful superovulatory response. In contrast, the control group not given exogenous FSH in the Boland (1999) study produced 1.66 transferable embryos on average, which is consistent with the historical observation that the majority of cattle experience single ovulations (Hunter et al., 2004).

(5) Sensitivity analyses: Sensitivity analyses demonstrated that the two-level mixed effect model provides the most conservative estimate of the lower bound of the 95% confidence interval. Sub-group analyses were not substantially different from the estimate of the estimated effect size based on the complete dataset. However, the sub-set analyses were limited by small sample sizes. Sensitivity analyses conducted without studies which were characterized as "high risk", or exhibiting extreme variation in the funnel plot, did not result in a clinically relevant difference in the estimated effect size.

The sensitivity analyses confirmed the validity of the chosen model and confirmed that uncontrolled sources of variability between treatment groups did not have a significant impact on the estimated effect size.

e. Conclusions: The effectiveness of FOLLTROPIN for the induction of superovulation of dairy and beef heifers and cows is substantiated by the systematic review and meta-analysis for the following use conditions: administration of FOLLTROPIN as an intramuscular injection of 87.5 IU, given twice daily, at 12 hour intervals for four consecutive days (total dose of 700 IU), with dinoprost tromethamine or cloprostenol sodium administered in conjunction with the 6th injection of FOLLTROPIN.

References:

1. Demoustier MM, Beckers JF, Van Der Zwalmen P, Closset J, Gillard JL, and Ectors F (1988). Determination of porcine plasma follitropin levels during superovulation treatment in cows. *Theriogenology*, 30:379-386.

2. Hunter MG, Robinson RS, Mann GE, and Webb R (2004). Endocrine and paracrine control of follicular development and ovulation rate in farm species. *Animal Reproduction Science*, 82-83:461-477.

3. International Embryo Transfer Society Manual (IETS), 4th edition, 2010 – Chapter 9, Certification and identification of the embryo (by, Irma Robertson & Richard E. Nelson).

III. TARGET ANIMAL SAFETY:

The clinical effects of FOLLTROPIN (porcine pituitary-derived follicle stimulating hormone for injection) were evaluated using a pharmacologic and toxicologic characterization, a study designed to demonstrate injection site safety and the safety of FOLLTROPIN under conditions of use, a retrospective evaluation of reproductive safety including safety after multiple sequential superovulation procedures, and supportive target animal safety information. A traditional margin of safety study was not required because of the existing body of knowledge of the pharmacology and toxicology of FOLLTROPIN (porcine pituitary-derived follicle stimulating hormone for injection) and its widespread clinical use in cattle.

A. Pharmacologic and Toxicologic Characterization

A literature review was submitted to characterize the pharmacologic and toxicologic properties of FOLLTROPIN and safety under clinical conditions of use. The proposed total dose of 700 IU of FOLLTROPIN, given as an intramuscular injection twice-a-day over four days (87.5 IU twice daily for 4 days) is equivalent to 400 mg NIH-FSH-P1 (National Institute of Health porcine FSH standard) and 18 mg PPFE (purified porcine folltropin extract) which are units of measurement frequently cited in the literature.

Pharmacokinetics of FSH in non-target and target species

Laster (1972) studied the pharmacokinetics of radioactively labeled FSH (^{125}I-FSH) in the blood of the rat, rabbit, ewe, and cow after a single intramuscular or subcutaneous injection. There was a difference in the rate of disappearance among the four species, but the shapes of the curves were similar in all species. Intramuscular injections resulted in higher ^{125}I-FSH blood levels than subcutaneous injections for all species. The disappearance rate was faster ($P<0.01$) during the follicular phase than during the luteal phase of the estrous cycle in the ewe and cow. The half-life calculated from the total decay curve (1 to 256 min) was 268+/-18 min in ewes and 252+/-27 min in cows during the follicular phase, compared with 393+/-15 min in ewes and 299+/-21 min in cows during the luteal phase.

Studies conducted in humans (Ben-Rafael et al., 1995) indicate that follicle stimulating hormone (FSH) is subject to hepatic metabolism and renal excretion. In humans, FSH is metabolized into constituent peptides, amino acids and

saccharides, which then follow normal excretion pathways. The metabolism of FOLLTROPIN in cattle is expected to be similar.

Pharmacokinetics of FSH in cattle

Demoustier et al. (1988) superovulated cows with intramuscular injections of 32 mg PPFE over four days in eight equal doses at 12-hour intervals. FSH levels were measured in plasma by radioimmunoassay. The study demonstrated that the FSH of porcine origin was rapidly absorbed from the site of injection, had a terminal elimination half-life in the cow of approximately five hours, and could not be detected in the blood twelve hours after injection.

Toxicology information from non-target species

Several human studies (Ben-Rafael et al., 2000; Merino et al., 1996; and Arnaldi et al., 2000) showed that there were no negative effects on fertility rates, sperm morphology, and/or semen parameters in men with oligoteratoasthenozoospermia treated with doses of 75 IU to 150 IU of FSH for up to six months.

Closset and Hennen (1989) reported that hypophysectomized male rats treated daily for seven days with a purified form of porcine FSH showed dose-related increases in testicular weight compared to hypophysectomized control animals.

Armstrong MA and Opavsky MA (1986) published data to demonstrate that an increased ratio of luteinizing hormone to follicle stimulating hormone in a porcine derived FSH preparation had a detrimental effect on the superovulatory response in rats.

Bussi et al. (1995) evaluated the potential adverse effects of subcutaneous injections of recombinant human follicle stimulating hormone (r-hFSH) on fertility and early embryogenesis; teratogenesis; and peri- and postnatal development in rats and rabbits. The study design and results are summarized below. In all phases, animals were treated with r-hFSH at doses of 5, 40, or 320 IU/kg/day. Control animals were treated with sterile saline.

- Phase I (fertility and embryogenesis): Male rats were treated daily from 60 days prior to and throughout mating and were sacrificed after mating. Female rats were treated daily from 14 days prior to mating until Day 7 of pregnancy and were sacrificed on Day 20 of pregnancy (fetuses removed via cesarean section). Treated males were mated with treated females.

  No systemic clinical signs or deaths were seen in any experimental group. Female rats treated with 40 IU/kg/day and 320 IU/kg/day had dose related increases in the incidence of irregular estrous cycles and decreases in the fertility index [(number of pregnant females/number of females with successful copulation) x 100]. There were increased numbers of corpora lutea and early resorptions and lower numbers of live fetuses in the females treated with 40 IU/kg/day and 320 IU/kg/day. A slight decrease in testis weight was observed in the male rats treated with 320 IU/kg/day but the decreased testis weight was not accompanied by histological changes. No dose related changes in skeletal and visceral anomalies or variants were found in fetuses.

- Phase II (teratogenesis):

  Pregnant female rats were treated daily from Day 6 through Day 17 of pregnancy. Two-thirds of the rats were sacrificed on Day 20 of pregnancy (fetuses removed via cesarean section) and one-third was sacrificed on Day 21 of lactation.

  No systemic clinical signs or local reactions were observed in the female rats during gestation. A dose related increase in the mean number of corpora lutea and ovarian weights was found in the female rats treated with 40 IU/kg/day and 320 IU/kg/day. The female rats treated with 320 IU/kg/day and sacrificed on Day 20 of pregnancy had significantly increased early resorptions and lower fetal weights. For rats allowed to litter, dystocia was observed in three of eight females treated with 40 IU/kg/day and four of thirteen females treated with 320 IU/kg/day, leading to death of one dam in the 320 IU/kg/day treatment group. The mean values per litter of stillbirths and post-implantation losses were higher and postnatal survival of pups was lower for the females treated with 40 IU/kg/day and 320 IU/kg/day. The frequencies of malformed fetuses were not different between the treated and control groups.

  Pregnant female rabbits were treated from Day 6 through Day 18 of pregnancy and were sacrificed on Day 29 of pregnancy.

  Female rabbits were more sensitive than the female rats; all the female rabbits treated with 40 IU/kg/day and 320 IU/kg/day and 14 of 16 females treated with 5 IU/kg/day had 100% early resorptions and no live fetuses. Two females in the 5 IU/kg group had viable fetuses and in these two females, the live fetuses (a total of 17 from both litters) were normal.

- Phase III (peri- and postnatal development): Pregnant female rats were treated from Day 15 of pregnancy until Day 21 of lactation and were sacrificed on Day 29 of lactation.

  No clinical signs or local reactions were observed during gestation. However, all pregnant female rats treated with doses of 320 IU/kg/day experienced dystocia (with some deaths of dams) and a high mortality rate of fetuses/pups. There were no deaths or dystocia in the 5 IU/kg/day or 40 IU/kg/day dose groups. The postnatal survival and maturation of pups was unaffected at doses of up to 40 IU/kg/day.

Bussi et al. (1995) concluded that the embryo/fetotoxicity and decreased fertility noted in the study resulted from high levels of FSH which may have caused hormonal imbalances resulting in disruptions in the ovulation process, abnormalities in the timing and quality of the luteinizing hormone surge, an over-production of estrogens by the ovaries, and abnormally high levels of progesterone.

The studies in rodents and rabbits indicate that adverse effects observed at doses as low as 5 IU/kg/day are limited to the reproductive system. This dose is significantly higher than the proposed dose in cattle (175 IU/cow/day).

Toxicology information in cattle

Casida et al. (1943) reported the results of a series of studies in cattle given several different preparations of extracted sheep pituitary gland (unfractionated, follicle stimulating, and/or luteinizing) at several doses, by subcutaneous and/or intravenous injection, at different stages of the reproductive cycle (including pregnancy). The majority of cattle were treated daily for 3 to 6 days, and there were no effects on any body system other than the reproductive system in these cows. Two cows given 3 gram equivalents of unfractionated extracts of sheep pituitary daily for five to six weeks eventually went off feed after twenty-four to thirty-seven days of daily treatment and one developed ascites. The ovaries from these two cows were grossly enlarged and contained many cysts and partially luteinized cysts.

Donaldson (1994) demonstrated that a total dose of 750 mg of a purified porcine FSH preparation (based on NIH-FSH-S1, National Institute of Health sheep FSH standard) given over a four day period at 12 hour intervals to three cows resulted in no changes in physical examination findings, body weights, feed consumption, clinical chemistry or hematological test results, or gross necropsy findings other than the expected physiologic effects on the ovaries. The three cows were necropsied approximately 4, 10, and 21 days following the last treatment.

Murphy et al. (1984), Donaldson (1994), and Gonzalez et al. (1990) published data to suggest that FSH preparations with high levels of luteinizing hormone have a deleterious effect on the superovulatory response, fertilization rates, and embryo quality.

Wu et al. (1988) reported that the optimal dose of an early formulation of FOLLTROPIN was 20 mg PPFE, equal to 778 IU, given over 4 days, based on the number of transferable embryos recovered. A dose of 25 mg produced a slight, but insignificant, decrease in the number of transferable embryos. Gonzalez et al. (1990) reported that when an early formulation of FOLLTROPIN was given subcutaneously (twice daily dosing over four days), the greatest number of transferable embryos were obtained with a dose of 10 mg (389 IU) to 20 mg PPFE (778 IU). Doses of 30 mg PPFE (1167 IU) and 40 mg PPFE (1556 IU) produced lower numbers of transferable embryos than 20 mg but the differences were not statistically significant. Similarly, Bo et al. (1994) reported that when an early formulation of FOLLTROPIN was given subcutaneously as a single dose of 200 mg, 400 mg, 600 mg, or 800 mg NIH-FSH-P1, the dose of 400 mg produced the greatest number of transferable embryos (9.5 +/- 2.3). Doses of 600 mg and 800 mg produced significantly more large follicles (greater than 10 mm) than the lower doses.

Immunogenicity of FSH

Literature reports document that following repeated superovulation with pregnant mare serum gonadotropin (PMSG), the superovulatory response (as measured by corpora lutea or ovulation rate) was highly variable, with many cows showing a decreased response with successive treatments (Willett et al., 1953 and Chupin and Saumande, 1979). A third study demonstrated that a reduced superovulatory effect (as measured by the number of corpora lutea) after repeated injections of PMSG was associated with antigonadotropin antibodies (Jainudeen et al., 1966).

Remy et al. (1991) evaluated the immunogenicity of porcine FSH (pFSH) and its relation to subsequent fertility or responses to superovulatory treatments in goats and cattle. The authors demonstrated a high degree of correlation between the lack of superovulatory response to treatment (based on the number of corpora lutea) and anti-pFSH antibody levels in goats after multiple treatments. However, the same immunologic response was not seen in more than 100 cows receiving pFSH to induce superovulation (many after two to ten superovulatory treatments). The authors proposed a lack of immunogenicity of pFSH in cattle due to the extremely high sequence homology of bovine and porcine FSH alpha and beta subunits.

Conclusions

The pharmacologic and toxicologic information provided in the literature review demonstrates that FOLLTROPIN, at a dose of 87.5 IU given twice daily for four consecutive days (total dose 700 IU), is not systemically toxic to the cow and the targets of toxicity are limited to the reproductive organs and functions.

References:

1. Arnaldi G, Balercia G, Barbatelli G, and Mantero F (2000). Effects of long-term treatment with human pure follicle-stimulating hormone on semen parameters and sperm-cell ultrastructure in idiopathic oligoteratoasthenozoospermia. *Andrologia*, 32:155-161.

2. Armstrong MA and Opavsky MA (1986). Biological characterization of a pituitary FSH preparation with reduced LH activity. *Theriogenology*, 25:135.

3. Ben-Rafael Z, Levy T, and Schoemaker J (1995). Pharmacokinetics of follicle-stimulating hormone: clinical significance. *Fertility and Sterility*, 63:689-700.

4. Ben-Rafael Z, Farhi J, Feldberg D, Bartoov B, Kovo M, Eltes F, and Ashkenazi J (2000). Follicle stimulating hormone treatment for men with idiopathic oligoteratoasthenozoospermia before in vitro fertilization: the impact on sperm microstructure and fertilization potential. *Fertility and Sterility*, 73:24-30.

5. Bo GA, Hockley DK, Nasser LF, and Mapletoft RF (1994). Superovulatory response to a single subcutaneous injection of FOLLTROPIN-V in beef cattle. *Theriogenology*, 42:963-975.

6. Bussi R, Ciampolillo C, Comotto L, Maraschin R, and Komatsu S (1995). Reproductive and developmental toxicity studies of SJ-0021 (r-hFSH). *Japanese Pharmacology and Therapeutics*, 23:115-139.

7. Casida et al (1943). Effects of pituitary gonadotropins on the ovaries and the induction of superfecundity in cattle. *American Journal of Veterinary Research*, 4:76-94.

8. Chupin D and Saumande J (1979). New attempts to decrease the variability of ovarian response to PMSG in cattle. *Annales de Biologie Animale Biochimie Biophysique*, 19:1489-1498.

9. Closset J and Hennen G (1989). Biopotency of highly purified porcine FSH and human LH on gonadal function. *Journal of Endocrinology*, 120:89-96.

10. Demoustier MM, Beckers JF, Van Der Zwalmen P, Closset J, Gillard JL, and Ectors F (1988). Determination of porcine plasma follitropin levels during superovulation treatment in cows. *Theriogenology*, 30:379-386.

11. Donaldson LE (1994). The development of Super-OV® from FSH-P. *Embryo Transfer Newsletter*, 12:17-25.

12. Gonzalez A, Lussier JG, Carruthers TD, Murphy BD, and Mapletoft RJ (1990). Superovulation of beef heifers with Folltropin: A new FSH preparation containing reduced LH activity. *Theriogenology*, 33:519-529.

13. Jainudeen, MR, Hafez ESE, Gollnick PD, and Moustafa LA (1966). Antigonadotropins in the serum of cows following repeated therapeutic pregnant mare serum injections. *American Journal of Veterinary Research*, 27:669-675.

14. Laster DB (1972). Disappearance and uptake of [^{125}I] FSH in the rat, rabbit, ewe, and cow. *Journal of Reproduction and Fertility*, 30:407-415.

15. Murphy BD, Mapletoft RJ, Manns J, and Humphrey, WD (1984). Variability in gonadotrophin preparations as a factor in the superovulatory response. *Theriogenology*, 21:117-125.

16. Remy B, Baril G, Vallet JC, Dufour R, Chouvet C, Saumande J, Chupin D, and Beckers JF (1991). Are antibodies responsible for a decreased superovulatory response in goats which have been treated repeatedly with porcine follicle-stimulating hormone? *Theriogenology*, 36:389-399.

17. Merino G, Carranza-Lira S, Martinez-Chequer JC, Barahona E, Moran C, and Bermudez JA (1996). Sperm characteristics and hormonal profile before and after treatment with follicle-stimulating hormone in infertile patients. *Archives of Andrology*, 37:197-200

18. Willett EL, Buckner PJ, and McShan WH (1953). Refractoriness of cows repeatedly superovulated with gonadotrophins. *Journal of Dairy Science*, 36:1083-1088.

19. Wu M, Wang H, Murphy BD, and Mapletoft RJ (1988). Superovulation of beef cows with Folltropin: A dose trial. *Theriogenology*, 29:332.

B. Injection Site Safety and Safety under Conditions of Use

1. Field and Injection Site Safety Study

Title: "Safety and Efficacy of FOLLTROPIN®-V for the induction of superovulation in cattle" (Study BF-9, 1999)

Investigator: Maurice Boland, MAgr Sci, PhD, DSc
Dublin, Ireland

Study Design:

a. *Objective:* To confirm the efficacy and safety of FOLLTROPIN for the induction of superovulation in sexually mature cattle

b. *Study Animals:* 32 crossbred beef heifers (Charolais, Limousin, Hereford, Simmental, and Piedmontese crosses), 2 to 3 years old, and weighing 511 to 712 kg, were blocked by weight and randomly allocated to one of two treatment groups for a total of 16 animals per group. Animals were housed in four pens.

c. *Treatment Groups:* Two treatment groups were included in the study: Group 1 was treated with FOLLTROPIN and Group 2 was treated with the commercial diluent for FOLLTROPIN.

d. *Drug Administration:* 50 mg (2.5 mL) FOLLTROPIN or 2.5 mL diluent was administered via intramuscular injection twice daily for four consecutive days (Days 9, 10, 11, and 12) after the start of estrus (Day 0). Injections were given in the neck dorsal to the jugular groove. Estrus was induced using an injection of 500 mcg ESTRUMATE (cloprostenol sodium) in conjunction with the sixth injection of FOLLTROPIN (or diluent) in all animals.

e. *Superovulation procedure:* Artificial insemination (AI) was performed approximately 56, 72, and 84 hours after the cloprostenol sodium injection on Day 11 (+/- 1 day). Embryos were collected using a non-surgical flushing technique approximately six days after the first AI was performed (Day 20). The flushing was performed by one of two qualified personnel (referenced as operators in the statistical analysis).

f. *Measurements and Observations:*

Physical examinations were conducted by a veterinarian on all animals prior to enrollment in the study.

General health observations were conducted on all animals prior to the start of treatment, three days following the last treatment, and at the end of the study. Health observations included an evaluation for the following abnormalities: abnormal gait, tremors, collapse, excess salivation, dyspnea/hyperpnea, diarrhea, abnormal ocular/nasal discharge, abnormal vulvar discharge, and lesions at the injection site. Health observations were also conducted twice daily after the administration of test or control article.

An ovarian assessment was conducted on Day 20 (+/- 1 day) by manual rectal palpation and ultrasound to determine the number of corpora lutea and the number of large follicles (>10 mm diameter).

Ova/embryo assessment: After non-surgical flushing, the following endpoints were reported: number of recovered ova/embryos, number of fertilized ova, number of transferable embryos, and the number of non-transferable embryos. A normal transferable embryo was defined as an embryo with a cleavage state compatible with the day of recovery, which

showed no evidence of morphological abnormality or blastomere degeneration, consistent with the International Embryo Transfer Society quality codes of Grade 1 and Grade 2.

Statistical Analysis:

The number of recovered ova/embryos, number of fertilized ova/embryos, the number of transferable embryos, and the number of large follicles was analyzed using a mixed effect model with the blocking factor of weight as a random effect and plane of nutrition, treatment, and operator as fixed effects. The number of corpora lutea was analyzed using the same model without the fixed effect of operator because there was only one operator involved in the evaluation.

Results:

No adverse events or mortalities occurred during the study and no injection site reactions were noted after the administration of eight injections of FOLLTROPIN or diluent per animal.

Four animals did not complete the study for the following reasons:

- Two animals (one from each treatment group) could not be flushed because the cervix could not be passed (one because of unsuccessful epidural anesthesia).
- One FOLLTROPIN treated animal could not be successfully flushed because the reproductive tract was too large.
- One control animal was not flushed because ovarian examination revealed a follicular cyst.

Table III.B.1: Superovulation results

Parameter	FOLLTROPIN treated group, least square means	Diluent treated group, least square means	P value
Corpora lutea	13.2	1.04	<0.001
Recovered ova/embryos	7.1	2.18	P=0.0021
Fertilized ova	5.95	1.87	P=0.0107
Transferable embryos	4.55	1.66	P=0.0199
Large follicles	2.11	1.15	P=0.0090

Conclusions:

Based on the general health and injection site observations made during the study, FOLLTROPIN was well-tolerated in beef heifers at a dose of 50 mg (2.5 mL) administered twice daily for four days.

2. Supportive Field Safety Information

The following uncontrolled study was conducted by the sponsor and submitted to support the evaluation of target animal safety under conditions of use: "Efficacy Study in Cattle to Determine the Superovulatory Response to a Porcine Pituitary Follitropin Extract (FOLLTROPIN) under Field Conditions" (Study FOL-UK, 1992).

Three veterinarians, experienced in the field of embryo transfer, enrolled cases in the study. The final data set included 639 reproductively healthy and mature female beef and dairy cattle of various ages (2 to 17 years, where specified), representing 9 different breeds of beef cattle (Belgian Blue, Charolais, Saler, Blonde, Simmental, Limousin, Piedmontese, Welsh Black) and 3 breeds of dairy cattle (Fresian, Holstein, Ayrshire). The final dataset included 108 dairy heifers, 327 dairy cows, 112 beef cows, 23 beef heifers, and 69 females of unspecified age or breed.

FOLLTROPIN was administered at a dose of 50 mg (2.5 mL) FOLLTROPIN via intramuscular injection twice daily for four consecutive days on Days 9, 10, 11, and 12 after the start of estrus. Estrus was induced using an injection of 500 mcg cloprostenol sodium in conjunction with the sixth injection of FOLLTROPIN in all animals. Following treatment, those animals observed in estrus were inseminated twice about 8 to 16 hours apart in the first 24 hours after the onset of estrus. If signs of estrus persisted for longer than 24 hours, additional inseminations were performed at 8 to 16 hour intervals until estrus was no longer observed.

The investigators reported that there were no injection site reactions or other adverse events noted during the study.

C. Reproductive Safety

1. Retrospective reproductive safety study

Title: "A retrospective study to confirm the reproductive and embryological safety of FOLLTROPIN use in dairy cattle"
(Study BF-20, December 1, 2001 to March 28, 2002; superovulation events occurred between January 4, 1999, and September 29, 2000)

Study Director: Kristina Bennett-Steward BSc, DVM, MRCVS

Study Veterinarians: Data was sourced from clinical records of the following veterinarians or practices that specialize in dairy cow reproduction and embryo transfer.

Macintosh Embryo Transfer Embro, ON	Tom Wheal Ingersoll, ON	John Chesney Tavistock, ON
Marc Dery St-Foy, QC	Pierre Clavel St-Foy, QC	Louis Picard St-Foy, QC

Study Design:

a. *Objective:* To confirm the reproductive and embryological safety of FOLLTROPIN in dairy cattle through the use of retrospective data from cows that were superovulated using FOLLTROPIN and the use of data from second and third generation cows which were themselves a result of superovulation and embryo transfer using FOLLTROPIN.

b. *Study Animals:* 682 dairy cows (primarily Holstein breed), from commercial farm operations in Canada. All animals within the time period, regardless of treatment success, were included in the dataset. Both donor and recipient cows were reproductively mature and determined to be suitable for superovulation and embryo transfer. Information including individual cow registration numbers, generational relationships between cows, documentation regarding whether cows were the result of embryo transfer, and the dates of normal calvings for individual donor cows was retrieved from the Holstein Canada database.

c. *Treatment Groups:* Cow data were separated into the following groups:

   1. Records of 1164 superovulation procedures from 682 dairy cows (primarily Holstein breed) where FOLLTROPIN was used

   2. Records of 316 superovulation events where FOLLTROPIN was used in 153 cows that were related (grandmother, mother, and daughters)

d. *Drug Administration:* Cows were treated with FOLLTROPIN at a dose of 50 mg twice daily for 4 days (total dose of 400 mg or 700 IU). The type, administration, and dose of prostaglandin used to induce luteolysis were not reported consistently.

e. *Measurements and Observations:*

   The safety of FOLLTROPIN after multiple superovulation cycles was evaluated using the number of transferable embryos flushed from cows in which repeated treatments were spaced less than three months apart (<93 days). Treatment number was reset to 1 for an individual cow if the interval between repeated treatments with FOLLTROPIN exceeded 93 days. The 93-day maximum treatment interval was selected because beyond this time, residual effects of one superovulation event are not expected to impact a subsequent superovulation event. Each time the treatment number was reset to one for an individual cow, a new sequential period was started for the purposes of the statistical analysis. For example, if a cow was superovulated a total of seven times, with greater than 93 days between superovulatory treatments three and four, treatments four, five, six, and seven would be recoded as one, two, three, and four. The first three treatments would be assigned to period 1 and the second four treatments to period 2.

   The dataset reported the number of live calves born to embryo transfer recipient cows using embryos that were the result of superovulation with FOLLTROPIN for each individual cow and the number of live calves produced by each cow that had been superovulated with FOLLTROPIN.

Statistical Analysis:

Effect of repeated sequential treatments on the number of transferable embryos:

The entire treatment history for each cow was classified into periods containing repeated treatments at intervals of 93 days or less and were labeled sequentially as 1, 2, …, in the analysis. The number of sequential treatments received by each cow within each period, the sequential period, and their interaction were considered fixed effects in the mixed effect model to test the long term and short term repeated treatment effects respectively. Each subject with unstructured correlations within the periods and the repeated treatments was considered as the random effect in the model. Observations with periods of > 3 and treatment numbers of > 4 were not included in the statistical analysis due to the sparse observations at these categories.

Effect of repeated sequential treatments on the number of transferable embryos within generation (grandmother, mother, and daughter relationships)

To further test the long term and short term repeated treatment effects within each generation, period, treatment number, treatment number by generation, and treatment number by period and generation by period effects were considered as the fixed effects in the mixed effect model. Each subject with unstructured correlations within the periods and the repeated treatments was considered as the random effect in the model. Observations with periods of > 3 and treatment numbers of > 4 were not included in the statistical analysis due to the sparse observations at these categories.

Results:

Repeated use in individual animals:

There were no significant repeated treatment effects on the number of transferable embryos across the sequential periods and within each period when cattle were superovulated repeatedly at intervals of less than 93 days.

Table III.C.1. Summary of the number of transferable embryos from repeated superovulations

Period of treatment[1]	Treatment number[2]	Number of observations[3]	Minimum # total transferable embryos (TTE)[4]	Maximum # TTE[5]	Mean # TTE[6]	Standard deviation[7]
1	1	682	0	42	7.62	6.63
1	2	157	0	33	7.34	6.20
1	3	51	0	31	8.49	7.20
1	4	23	0	13	4.70	3.60
1	5	6	1	10	3.50	3.39
1	6	2	2	4	3.00	1.41
2	1	149	0	33	7.50	6.76
2	2	40	1	25	7.20	6.74
2	3	9	1	20	7.89	6.55
2	4	4	1	19	8.75	7.76
2	5	1	10	10	10.00	n/a
3	1	21	1	25	8.57	5.98
3	2	6	3	18	10.83	4.92
3	3	5	2	14	4.80	5.22
3	4	3	2	9	5.33	3.51
4	1	4	1	17	6.25	7.27
4	2	1	2	2	2.00	n/a

1: A single period includes sequential treatments at <93 day intervals; when the interval between sequential treatments was >93 days for an individual cow, the treatment number was reset to one and the sequence moved to the next period.

2: The treatment number within a sequence of treatments at <93 day intervals

3: The number of cows with data within the defined period and treatment number

4: The minimum number of total transferable embryos recorded for an individual cow within the period and treatment number

5: The maximum number of total transferable embryos recorded for an individual cow within the period and treatment number

6: The arithmetic mean of the total transferable embryos recorded for individual cows within the period and treatment number

7: The standardized deviation of total transferable embryos recorded from the arithmetic mean for individual cows with the period and treatment number

Repeated use between generations

When data on the number of transferable embryos were sorted by generation (mother, daughter, and grandmothers), there were no significant long and short term repeated treatments, or generation effects on the number of transferable embryos recovered.

Relationship between FOLLTROPIN use and future reproductive function

Of the 682 cows included in the analysis, 214 cows produced one or more normal calves following treatment with FOLLTROPIN, and 360 cows produced one or more calves through embryo transfer. The data on the

number of calves born to superovulated cows is confounded because only female calves were reported to the Holstein Canada database.

Data on post-treatment conception rates and the interval to first estrus could not be determined because the commercial interests of animal owners determined the interval between embryo collections and the decision to breed cows following superovulation.

Conclusions:

This study demonstrates that FOLLTROPIN when administered repeatedly at intervals of less than three months does not have a detrimental effect on the number of transferable embryos recovered at flushing. This conclusion also applies to cows born as a result of embryo transfer following superovulation with FOLLTROPIN.

Cows within the superovulation program produced embryos that were successfully transferred into recipient cows and were able to themselves conceive and deliver normal calves.

2. Supportive Reproductive Safety Information

The sponsor submitted a pilot study with raw data (Hackett, 1989, Study FOL-2), in which 13 reproductively mature dairy cows were superovulated three consecutive times with one estrus cycle between superovulation procedures. In this study, 87.5 IU of FOLLTROPIN was administered intramuscularly twice daily for four days (total dose 700 IU). After the third superovulation procedure, the animals were bred at the second observed heat using typical breeding practices. One cow was not bred due to a punctured uterus at the last flush. Of the twelve remaining cows, eight were confirmed pregnant by the third breeding after the last superovulation treatment. Among the other four cows, one was confirmed not pregnant, two were slaughtered before the third breeding, and one was lost to follow-up. Only ten of the 34 flushes performed during the study yielded embryos, and the study demonstrated a trend towards decreasing embryo yield with each successive treatment. Ovarian cysts were noted pre-treatment in four of 13 cows given FOLLTROPIN treatment. Three cows had no cysts pre-study but developed cysts on their ovaries during the course of treatment with FOLLTROPIN. Two of these animals were bred and were pregnant by the first or second breeding following the third superovulation cycle. The study demonstrated that cows treated with FOLLTROPIN for three consecutive superovulation procedures could be successfully bred. A direct correlation between the use of FOLLTROPIN and the development of cystic ovaries cannot be made because of the small number of study animals, the presence of cystic ovaries in some animals prior to treatment, and the lack of a control group.

Published studies conducted with unidentified formulations of porcine pituitary-derived follicle stimulating hormone have reported conflicting results regarding the effect of repeated superovulation on the recovery of transferable embryos. Hasler et al. (1983) and Wooliams et al. (1995) reported a potential for a decrease in the number of transferable embryos

after repeated superovulatory treatments with porcine-pituitary derived follicle stimulating hormone based on retrospective data.

A comprehensive literature review did not reveal any reports of adverse events, impairment of future fertility, or significant reductions in the number of transferable embryos following repeated superovulatory treatments with FOLLTROPIN. Two publications [Yang WC, et al. (2010) and Larson JE, et al. (2010)] specifically reported the number of transferable embryos for sequential superovulation events using FOLLTROPIN.

Yang WC, et al. (2010) evaluated the effect of repeated sequential superovulation with FOLLTROPIN in Chinese Holstein cows. In the study, cows superovulated two to four times at approximately one month intervals produced an average of 3.85+/- 0.357 (118 cows), 4.12 +/-.406 (118 cows), 4.2 +/- .437 (92 cows), and 3.91 +/-.456 (71 cows) transferable embryos, at the first, second, third or fourth superovulation treatment, respectively, using a total dose of 546 IU of FOLLTROPIN. There were no significant differences in the number of transferable embryos between groups.

Larson JE, et al. (2010) reported similar results following the superovulation of 32 Angus cows two times, 30 days apart, with a total of approximately 501 IU of FOLLTROPIN. The study was designed as a cross over design intended to evaluate the difference in superovulation rates between sexed and conventional semen. There were no significant differences noted between sequential treatments for the number of transferable embryos; however, the percentage of transferable embryos decreased over the two treatments. The decreased percentages of transferable embryos were attributed to weather conditions and individual animal variation.

References:

a. Hasler JF, McCauley AD, Schermerhorn EC, and Foote RH. (1983) Superovulatory responses of Holstein cows. *Theriogenology*, 19:83-99.

b. Larson JE, Lamb GC, Funnell BJ, Bird S, Martins A, Rodgers JC. (2010) Embryo production in superovulated Angus cows inseminated four times with sexed-sorted or conventional, frozen-thawed semen. *Theriogenology*, 73:698-703.

c. Woolliams JA, Luo ZW, Villanueva B, Waddington D, Broadbent PJ, McKelvey WAC, and Robinson JJ. (1995) Analysis of factors affecting superovulatory response in ruminants. *Journal of Agricultural Science*, 124:61-70.

d. Yang WC, Li SJ, Tang KQ, Hua GH, Zhang CY, Yu JN, Han L, Yang LG. (2010) Polymorphisms in the 5' upstream region of the FSH receptor gene, and their association with superovulation traits in Chinese Holstein cows. *Animal Reproduction Science*, 119:172-177.

D. Other Safety Information

In foreign, post-market pharmacovigilance data for FOLLTROPIN collected by Bioniche Animal Health, the most common adverse reaction was hypersensitivity

reactions. Worldwide pharmacovigilance reports show that hypersensitivity reactions occurred in approximately 16 cattle out of more than 8,000,000 doses sold worldwide. All cows recovered after treatment with anti-histamines and/or epinephrine.

IV. HUMAN FOOD SAFETY:

A. Antimicrobial Resistance:

Porcine pituitary-derived follicle stimulating hormone is not known to have antimicrobial properties, and has not been shown or been reported to affect antimicrobial resistance among bacteria of public health concern; therefore, evaluation of microbial food safety (antimicrobial resistance) for this use of porcine pituitary-derived follicle stimulating hormone in beef and dairy cattle is not warranted at this time.

B. Impact of Residues on Human Intestinal Flora:

Residues and metabolites of porcine pituitary-derived follicle stimulating hormone in or on the edible tissues of treated animals are not known to have antimicrobial properties, and have not been shown or been reported to have antimicrobial effects on the intestinal bacteria of human consumers; therefore, evaluation of microbial food safety (impact on intestinal flora) for this use of porcine pituitary-derived follicle stimulating hormone in beef and dairy cattle is not warranted at this time.

C. Toxicology and Residue Chemistry:

Evaluation:

The Agency has determined that the traditional paradigm of separately evaluating the toxicology and residue chemistry components of the Human Food Safety Technical Section is not suitable for the approval of FOLLTROPIN for cattle. The Agency evaluated the toxicology and residue chemistry components using a risk-based approach that collectively considered hazard identification, hazard characterization, and potential human exposure to FOLLTROPIN residues. The individual residue chemistry studies submitted by the sponsor were reviewed and used by the Agency as non-pivotal supporting information for this approval.

The Agency has determined that the traditional series of toxicological studies for identifying and characterizing the toxicology concern for FOLLTROPIN are not needed. FOLLTROPIN is a purified, lyophilized porcine pituitary gland extract containing follicle stimulating hormone (FSH) and luteinizing hormone (LH) at a low LH:FSH ratio. FOLLTROPIN is reconstituted in a solution containing sodium chloride (9 mg/mL), benzyl alcohol (18 mg/mL), and water (q.s. to 1 mL) for use as an injectable product.

The FSH in FOLLTROPIN is a glycoprotein extracted from porcine pituitary glands and has not been structurally modified. Therefore, it is biochemically identical to porcine pituitary FSH. Because passage of FOLLTROPIN residues through the human gastrointestinal system renders pituitary FSH mostly inactive, and absorption of FSH by the human gastrointestinal system is limited and negligible,

the oral bioavailability of FSH to human consumers is insignificant[1]. This rationale also applies to the safety assessment of LH, which is present at a much lower concentration than FSH in FOLLTROPIN.

The Agency evaluated two residue chemistry studies submitted by the sponsor and used them as supporting information. The two studies examined FSH residues at the injection site (Bioniche Internal Study, BF-13 A, B and C, 2001) and in milk (Bioniche Internal Study, BF-16 A, B and C, 2001) from cattle treated with FOLLTROPIN or a placebo. The studies revealed that the FSH concentrations at the injection site and in milk from FOLLTROPIN-treated animals and placebo-treated animals were comparable.

The Agency has determined that additional toxicology and residue chemistry studies are not required for the use of FOLLTROPIN with dinoprost tromethamine or cloprostenol sodium drug products that are FDA-approved for cattle, as directed in the product labeling for FOLLTROPIN. This is because the dinoprost tromethamine and cloprostenol sodium drug products have been approved for cattle with no withdrawal period or milk discard time requirement, and their residues are not likely to interfere with depletion of FOLLTROPIN residues, and vice versa.

In addition, the Agency has evaluated the human food safety of the excipients (sodium chloride and benzyl alcohol) in the FOLLTROPIN product formulation and found that they do not cause human food safety concerns for their intended use.

Conclusion:

The Agency has concluded that the use of FOLLTROPIN in cattle does not cause toxicology or residue chemistry concern.

D. Human Food Safety Parameters:

1. Acceptable Daily Intake (ADI)

   An ADI for residues of FOLLTROPIN does not need to be established.

2. Target Tissue and Marker Residue

   A target tissue and a marker residue for FSH residues in cattle are not needed.

3. Tolerances

   Tolerances for FSH in the edible tissues or milk of cattle are not required.

4. Withdrawal Period and Milk Discard Time

   The use of FOLLTROPIN in cattle does not require a withdrawal period or a milk discard time.

[1] Waller DG, Renwick AG, Hillier K (2005). Medical Pharmacology and Therapeutics. 2nd edition, Oxford, UK, Saunders (W.B.) Co Ltd, 788 p.

E. Analytical Method for Residues:

A regulatory method for monitoring FSH residues in cattle is not required.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to FOLLTROPIN:

FOLLTROPIN labeling: "Not for use in humans. Keep out of reach of children. Care should be taken when handling the product to avoid accidental self-injection. Accidental self-injection may cause biological effects in women and, if pregnant, to the unborn child. In the event of accidental self-injection seek medical attention immediately by consulting a physician/health professional, particularly in women who are pregnant, or whose pregnancy status is unknown. To obtain a Material Safety Data Sheet, contact 1-888-549-4503 and make this number available to the physician/health professional."

Diluent labeling: "User Safety Warnings: Not for use in humans, keep out of reach of children. In the event of accidental self-injection seek medical attention immediately."

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that FOLLTROPIN, when used according to the label, is safe and effective for the induction of superovulation in beef and dairy heifers and cows. Additionally, data demonstrate that residues in food products derived from species treated with FOLLTROPIN will not represent a public health concern when the product is used according to the label.

A. Marketing Status:

This product may be dispensed only by or on the lawful order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because (a) the process of superovulation should be undertaken only by those trained and qualified to perform embryo recovery and possible transfer, and (b) the manipulation of the cow for superovulation requires an extensive knowledge of anatomy, physiology, pharmacology (including the use of other prescription drugs), biochemistry, endocrinology, and the use of aseptic technique, and this knowledge base is part of the training of the licensed veterinarian.

B. Exclusivity:

FOLLTROPIN, as approved in our approval letter qualifies for THREE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(ii) of the Federal Food, Drug, and Cosmetic Act because the sponsor submitted an original NADA that contains new studies that demonstrate safety and effectiveness of FOLLTROPIN.

C. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.

VII. ATTACHMENTS:

None