

## ***Twin Calving Induced by the Control of Numbers of Ovulations using an Ultrasound Scanning Scope and Aspiration of Follicles***

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**Abstract:** The aspiration of excess numbers of follicles was examined as a method for inducing twin pregnancy in cattle. Seven cattle were administered follicle stimulating hormone (FSH) for superovulation. The number of follicles was measured by an ultrasound scanning scope and excess numbers of follicles were punctured and aspirated to decrease the number of ovulations. One or two follicles were left intact in each ovary. Two of six cattle responding to FSH bore twins and one pair of twin calves was born.

**Key words:** Twin, Ultrasound, Follicles, FSH, Bovine.

Twinning is being viewed as a means of decreasing feed costs of beef cattle. There are several methods to induce the production of twins in cattle: genetic selection, treatment with exogenous gonadotropin, immunization against steroids and embryo transfer [1, 2]. Of these embryo transfer is the only approach currently yielding a twin-calving rate significantly higher than the normal incidence in cattle. But at present the costs of embryo transfer make it unfeasible for the production of feeder beef cattle.

Many experiments [3] have been conducted on the use of gonadotropin in the induction of twin-pregnancy in the cow, because of its easiness and the relative cheapness of the treatment. At present, consistent induction of twin pregnancy has not been established, although prostaglandin has been widely used to regress the corpus luteum since the late 1970's and superovulation treatment has become very easy.

The great problem with superovulation is that the accurate control of responses to gonadotropin is very difficult. Consequently, many studies of multiple births from exogenous gonadotropins have reported the production of many single calves as well as twins, triplets and occasionally quadruplets and quintuplets [3]. There is a high mortality associated with litters of three or more calves, and there is, therefore a need for the development of a reliable method for controlling litter sizes to two.

It has been previously reported that twins occur naturally at a higher frequency when a corpus luteum is present in each ovary rather than when two corpora lutea are present in a single ovary [3]. It might therefore be useful to explore the possibility of preventing multiple-pregnancy over twins by aspiration of mature Graafian follicles in excess of two or three at the time of insemination to superovulation-treated cattle.

The recent advances of medical electronics have paved the way to new approaches to animal reproduction. Especially, ultrasound scanning equipment makes it possible to diagnose the correct number of follicles in a superovulation-treated cow, and very early pregnancy [4].

Here we attempted the control of ovulation, by removing mature Graafian follicles in excess of two or three before insemination using ultrasound scanning and a special but simple aspirator, to induce twinning.

### **Materials and Methods**

Multiparous non-lactating beef cows and Holstein heifers were administered follicle stimulating hormone (Antorin, Denka Pharmaceutical Co., Ltd., Kawasaki, Japan) twice daily in a declining dose over four days (22 mg total/animal). Prostaglandin F2 $\alpha$  analogue (ONO1052,

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Ono Medicine Industry Co., Ltd., Osaka, Japan) was administered on the 3rd day of FSH administration in equal two divided doses (1000 ug/animal).

FSH treated cattle were submitted to the following procedure in their middle estrous period, i.e. 52 hr after the first prostaglandin injection. The animals were placed in a narrow stanchion. First, the number and size of large follicles (diameter, over 10 mm) were determined by means of a rectal probe (5 Mhz) connected to an echo camera, Aloka SSD 246V (UST 588-5, Aloka, Tokyo, Japan). Then, the excess number of follicles were aspirated by a syringe-type aspirator (improved IF-injector, Fujihira Industry Co., Ltd., Tokyo, Japan) leaving two or three follicles, after an epidural injection of 6 to 10 ml of 2% procaine. The aspirator was introduced into the vagina and an aspirating needle was transvaginally pushed into the follicles, while the ovary was held by hand per rectum. An assistant pulled the piston of the syringe for aspiration of the follicles. One or two large follicles were left intact in each ovary. After aspiration the ovaries were observed again to determine whether the aspiration had been properly performed, and then the animal was inseminated artificially.

The aspirated fluid was examined for oocytes using a stereomicroscope.

Ultrasound diagnosis for pregnancy and the number of fetus was performed 30, 45 and 60 days after the insemination.

## Results

Six of seven cattle responded to FSH, having 4 to 12 large follicles diagnosed by the scanner. The aspiration was completed and the number of follicles was reduced, and when the aspirated fluid was examined, ten oocytes from 31 aspirated follicles were found (Table 1).

The mature follicles of four cattle were aspirated in excess of three, and one pair of twin was born healthily (B130), a single calf died soon after birth (H285) and a triplet-pregnancy was ended by abortion at 124 days of gestation. In two animals one mature Graffian follicle was left in each ovary. One of them bore twins but they were aborted at 240 days of gestation period (H307).

## Discussion

Four of six animals responding to FSH became pregnant and two bore twins and one had three fetus until 124 days of gestation. It seems that the ovulation was controlled by removing the excess follicles and that the aspiration procedure did not prevent pregnancy.

Philipsen [1956, cited in 5] has reported successful prevention of twin pregnancy by rupturing one mature follicle in animals possessing two prior to ovulation; forty seven cows underwent pregnancy and produced single calves. However, in the case of superovulated animals Lamnd and Clark [1960, cited in 5] considered that to destroy physically some follicles as they develop was a difficult process. The recent advances of medical electronics may change their consideration since the pregnancy rate of this report was good.

It is reported that it is difficult to predict the level of hormone that should be administered to produce the growth of only two follicles. However, using the method presented in this study it will be possible to ovulate one oocyte in each ovary. Twin calving is associated with many problem; abortion, dystocia, calf mortality and retained placenta [2]. In our experiment there were two cases of perinatal death including abortion and stillbirth. If these problems were resolved our method may provide a good means of increasing the efficiency of beef cattle production. A new system for oocyte pick-up by ultrasound guided instruments [6] is being used for *in*

**Table 1.** Response to FSH and fertility after the aspiration of excess follicles

Animal	No. of follicles			No. of fetus		Results
	Total	Aspirated (oocytes)	Intact	R	L	
H310	12	9 (5)	3	2	1	Aborted (124-d*)
H285	4	1 (0)	3	1	1	35 kg (Dead)
B130	7	4 (3)	3	1	1	23, 24 kg
B599	7	4 (1)	3	1	1	-(estrus, 40-d)
H307	6	4 (0)	2	1	1	21, 23 kg (240-d)
H309	11	9 (1)	2	—	—	
H305	1	—	1	—	—	
Mean	6.86	5.17 (1.67)	2.43	—	—	

\*124-d: 124-days after insemination.



*vitro* fertilization of cattle oocytes, and it also shows promise for the induction of twinnings.

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