

## Implementation of AI (Artificial Insemination) and ET( Embryo Transfer) to Produce Twins Pregnancy

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### Original Research Article

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**Abstract:** The aim of this research is to produce twin pregnancies in Simmental cows through AI and Embryo transfer (ET). This study performed AI and ET of an additional embryo on the 7th day after insemination using 16 Simmental cows at. Andalas University Animal Husbandry and Breeding Center (BPTU-HPT). Of the 16 cows were treated using a combination of AI and ET resulting of a pregnancy rate (Conception Rate) of 37.5 %. Half the Simmental cattle to AI and ET had single offspring, 50%, 16.6% had a healthy twin birth, 16.6% an abortion and the remaining 16.6% had twins that died soon after birth. Only one of 6 cows who became pregnant was successful in producing healthy twins.

**Keywords:** Estrus, Corpus Luteum, AI (Artificial Insemination) and ET (Embryo Transfer), Twin Birth.

### INTRODUCTION

Beef is a nutritious food source providing people with valuable animal protein and so is important in supporting the development of human resources in Indonesia. Along with population growth and the improvement of living standards in Indonesia, the need for food is increasing, as well as the demand for animal protein. The demand for beef in Indonesia increases year on year, it is influenced by the tastes of a growing middle class. To reduce the quantity of imported beef and to achieve self-sufficiency a more drastic increase in domestic production is needed. This will require reducing the productive female slaughter rate and increasing birth rate.

In order to increase the livestock population from a relatively small female population, it is necessary to find a way to increase the percentage of twin births. Artificial insemination technology needs to be developed by combining Artificial Insemination (AI) and Embryo Transfer (ET) to raise the number of twin births. The birth of twins can be achieved with AI followed by ET of an additional one embryo on the 7th day after the AI. Births of more than two calves to one mother are usually difficult to maintain. The birth of twins can also result in a fertility problem in the next generation if the twins consist of a male and a female. A female born with a male twin is generally sterile as the reproductive organs are not developed. These infertile cow calves are called freemartin [1]. So research needs to be done to discover if the birth of twins engineered through AI and ET can improve the reproduction performance of cattle.

### MATERIALS AND METHODS

#### Time and Place of Research

This research was conducted at the Technical Implementation Unit (UPT) of the Faculty of Animal Husbandry Andalas University Campus Limau Manis and at the Superior Cattle Breeding Center (BPTU-HPT) Padang. The study was conducted from July 2016

to October 2016 at UPT Faculty of Animal Husbandry Andalas, and August 2016 until November 2016 at BPTU Beef unit Padang.

#### This research was conducted, namely:

Application of AI and ET to create pregnancies of twins. This stage used purposive sampling using 16 cows. Every day the cows were monitored in the field to pinpoint oestrus. If a cow was in oestrus it was tagged to be used as part of the research sample until 16 such cows had been identified. These cows were subjected to AI and then seven days later ET was performed using a single embryo. The cows were tested for pregnancy on the 30th and 60th days after the ET. The indications for AI and ET treatment were as follows:

- For AI the cow must be in oestrus.
- For ET Corpus Luteum must be evident.

#### Variables

Twin births resulting from AI and ET

- Conception Rate
- Live births from AI and from ET

**RESULTS AND DISCUSSION****Combination of AI and ET treatments**

In the study, AI was administered to Simmental cows during oestrus. At this stage, the de

Graaf follicle has matured and releases an ovum from the ovary. The appearance and location of the observed de Graaf follicles are listed in Table 1.

**Table-1: Existence of de Graaf follicle and Corpus Luteum in right and left ovary on examination before administration of AI.**

No.	No birth (Earteg)	Ovary that Ovulation			
		right		left	
		F(dG/Do)	CL	F(dG/Do)	CL
1	1	+(dG)	+do	-	-
2	2	+do	-	-	+
3	3	-	+	+do	-
4	4	+do	-	-	-
5	9	-	-	-	-
6	10	-	-	+do	+
7	BV130018	+(dG)	-	-	-
8	968	+(dG)	-	-	-
9	7101	+(dG)	-	-	-
10	BV130059	-	+(h)	-	-
11	1625	+(dG)	-	-	-
12	7025	+(dG)	-	-	-
13	7067	-	-	+(do)	-
14	BV160099	+(do)	-	-	-
15	1700	+(do)	-	-	-
16	637	+(dG)	-	-	-

Information; dG: de Graaf follicle; do: dominant follicles; CL: Corpus Luteum; h: hemorrhagicum; +: Evident; -: Absent

Follicles reach maturity via a number of stages termed primary, secondary, tertiary (still in the process of growth) then finally *de Graaf* (fully mature) [2]. The dominant follicle is one which is reaching maturity and contains the ovum that will be released at ovulation. This dominant follicle excretes estrogen that then triggers the symptoms of oestrus. A *corpus hemorrhagicum* forms in the ovary in the place where the de Graaf follicle has just released an ovum. As the ovum leaves the follicle it enters the fallopian tube which carries it to the uterus [3]. The corpus hemorrhagicum develops into a corpus luteum which in turn produces progesterone to prepare the uterine lining to facilitate implantation of a fertilized ovum. In each oestrus cycle, 2 to 3 follicles are formed successively in waves [4]. The clear sign of ovulation and oestrus that signals the appropriate timing for AI is the formation of the de Graaf follicle at the site of the dominant follicle and the formation of a Corpus Luteum which forms in stages after ovulation[3].

If the cervix, uterus, and cornua (the horn-shaped structures between the fallopian tubes and the uterus) appear normal and a corpus luteum is evident then this indicates that a cow that has received AI is ready for an ET. The best recipients for ET are healthy young cows with high fertility who are at a good size to have a healthy birth. The cows in this study who were given AI at oestrus received a single embryo ET 7 days subsequently when the Corpus Luteum was evident in

the ovary as can be seen in Table 2.

The corpus luteum usually forms in the right ovary as in ruminants most of the left side of the abdominal cavity is occupied by digestive organs. This restricts the blood flow and hence nutrition and hormones to the ovary on this side making it less active and less likely to produce ovum or corpus luteum than the better supplied right ovary [5].

**Conception Rate of twins after AI and ET**

The combination AI and ET treatment resulted in a 37.5 % conception rate from 16 cows of which half were single pregnancies. Normally, it would be expected that this treatment would produce a higher number of offspring because of the increase in twin births but it appears that with the use of ET there was a reduced rate of cows with established pregnancies. It may be that many of the embryos died in the uterus. In some cows, the corpus luteum was relatively small. The low pregnancy rate may also be related to the viability of the embryo used for ET due to the storage conditions. Furthermore, with a limited number of embryos available to choose from, lower quality embryos were sometimes used. The condition of the cows along with less than optimal quality and quantity of the available feed may also have played a part particularly in the cows who lost twins late in the pregnancy and soon after birth.

**Table-2: Existence of Corpus Luteum in right and left ovary before ET**

The existence of corpus luteum in the ovary			
No.	No part Earteg)	right CL	left CL
1	1	+	-
2	2	+	-
3	3	+	-
4	4	+	+
5	9	+	-
6	10	+	-
7	BV130018	+	-
8	968	+	-
9	7101	+	-
10	BV130059	+	-
11	1625	-	+
12	7025	+	-
13	7067	+	-
14	BV160099	-	+
15	1700	+	-
16	637	+	-

Information: CL: Corpus Luteum; h : hemorrhagicum; + : Evident; - : Absent

If the feed is deficient in protein the oestrus will be weak, embryos often fail to implant and are aborted [6]. Even if implantation is successful, with twin pregnancies there is a greater rate of embryo death than with single pregnancies because of the reduced space for development and because the two fetuses compete for nutrition. The total amount of nutrition

available to the fetuses will depend on the condition of the cow [7].

#### **Birth rate from AI plus ET Treatment in Simmental Cattle**

Births to the 6 pregnant cattle in the second stage of the study is shown in Table 3.

**Table-3: Births to Simmental cattle given AI and ET**

n	Birth	Percentage (%)
3	single	50%
1	twin	16.6%
1	Abortion	16.6%
1	died after birth	16.6%
Total 6		

Birth occurrence in Simmental cattle in IB and in TE ie, single birth was 50%, twin births 16.6%, abortion 16.6% and twins but died after birth 16.6%. Of the 6 pregnant cows recipients, 2 pregnant recipients in the Livestock Unit (UPT) Animal birth of the IB result and the twin births of IB and TE results but the fetal abortus over six months ( $\geq 6$  months) may be due to less than optimal feed adequacy obtained by pregnant cows. Recipient cattle 4 heads pregnant at BPTU-HPT Padang occur birth of female twins and females of IB and TE 1 recipient results, twin birth but die after birth 1 recipient and single birth of TE 2 recipient results. From the recipient of the 6 pregnant calves, the successful birth of twins is a 1-tailed recipient.

One difficulty in obtaining viable twin births in cows is because the fertilized ovum does not readily transigrate from the ovary past the uterine cornu hence implantation generally occurs in the cornu alongside the ovary that released the ovum [8]. The

primary condition in choosing cows for twin production should, therefore, be the natural occurrence of double ovulations which indicates that the cow is capable of carrying twins successfully [9]. If ovulation can occur in both ovaries gestation of twins is also more likely to occur [10]. Factors influencing the occurrence of twin births in cows include age, parity, season at mating, feed and ability to produce milk [11].

#### **CONCLUSIONS**

Of the sixteen cows treated with AI and ET six successful pregnancies were achieved and one healthy twin birth. Although this represents a higher twinning rate than normal the low conception rate meant that this treatment was not successful in increasing number of offspring.

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