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RECOVERY OF UNFERTILIZED OVA FROM SLAUGHTERED CATTLE

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The syringe and oocyte recovery unit designed for the human ovary was modified for the bovine ovary. From a local slaughterhouse, ten Holsteins and 7 Herefords were selected for experimental purposes. The recovery of 70 ova required aspirating 228 follicles for an overall recovery rate of 30.7%. There was no difference in the results obtained by using the syringe aspiration only or by using the modified ova recovery unit. The recovery results of the Holstein oocytes compared with the Hereford and of the small follicles compared with the large were approximately the same.

INTRODUCTION

Recent advances in the field of in vitro culture of ova (EDWARDS, '65; SREENAN, '68; WHITTINGHAM, '68; THIBAUT, '69; TERVIT, '72), in vitro fertilization (AUSTIN, '51; CHANG, '51; YANAGIMACHI, '69; BRACKETT, '70; SEITZ et al., '70), embryo transfer (BENNETT & ROWSON, '61; ADAMS, '68; ROWSON et al., '69; ROWSON, '71; SUGIE et al., '72^{14,15}); TERVIT & ROWSON, '72; BEDIRIAN & BAKER, '73) and freezing ova (WHITTINGHAM et al., '72; WILMUT, '72) in experimental animals have opened opportunities to extend such investigations to farm animals. These experimental approaches were not possible a few years ago. Application of these techniques to farm animals depends to a large extent on an adequate supply of ova. At present, cattle ova are usually recovered surgically or non-surgically from donors who have been superovulated by hormones; however, these methods involve a great deal of time and labor, and produce inadequate numbers of ova for experimental purposes, particularly in those experiments requiring a large number of ova. Also, the age of recovered ova is too advanced for studies on in vitro fertilization and maturation. Ova recovered from slaughtered cows could become an important source for basic studies on ova transfer in cattle.

The present study was undertaken to examine the possibility of ova recovery from slaughtered cattle.

MATERIALS AND METHODS

Ten Holsteins and 7 Herefords were selected at random from a slaughterhouse.

Immediately after slaughter the abdomen was opened, the reproductive organs were removed and the ovaries were put in a plastic bag containing ice. After 20 to 30 minutes transportation to the laboratory, the ovaries were measured and the diameter of each follicle was recorded. Follicles of two different sizes were detected; small follicles (ranging in diameter from 0.30 to 0.50 cm) and large follicles (ranging from 0.51 to 1.50 cm). The total number of follicles observed was 128 from the Holsteins and 100 from the Herefords.

Two methods were used for the recovery of ova: one approach involved the use of a 6 ml disposable plastic syringe fitted with an 18 gauge needle for follicular fluid aspiration and the other utilized the ova recovery unit for the human ovary (MORGENSTERN & SOUPART, '72) modified to suit the bovine ovary. The assembled ova recovery unit is shown in figure 1. It consists of three components: a 5 ml ova collection vial (silicone coated glass vial) with a 20 gauge double-end needle, a plastic extension tube with a 20 gauge needle and an aspiration syringe (10 ml plastic syringe).

Follicle fluid aspiration from the ovary was carried out with the tip of the needle in the follicle and by withdrawing the syringe plunger to create a gentle vacuum. The follicular contents were aspirated into the syringe or into a collection vial and aspiration was terminated as soon as a collapse of the follicle became apparent and/or a drop of blood was detected at the end of the needle.

Immediately after aspiration, the follicular fluid was mixed in a Petri dish with 2 ml of warm (37°C) TCM 199 for examination of the ova under a dissecting microscope. Each individual ovum was transferred to a microscopic slide with a drop of medium prior to covering it with a cover glass for phase contrast microscopic examination. Some of the ova were fixed with ethanol and acetic acid (3:1) for 24 hours and stained with acetic orcein.

RESULTS

The recovery rate of the ova from slaughtered cattle ovaries is shown in the table. All 34 ovaries from both sides of the 17 cows were found to have follicles present (0.3 cm or larger in diameter) ranging from 2 to 21 follicles per ovary. Ova were re-

TABLE *Recovery of ova from follicle*

SIZE OF FOLLICLE	NO. OF FOLLICLE EXAMINED	NO. OF OVA RECOVERED	%
Small*1	190	57	30.0
Large*2	38	13	34.2
Total	228	70	30.7

Notes: *1 Small follicle: 0.30~0.50 cm in diameter

*2 Large follicle: 0.51~1.50 cm in diameter

covered from 24 out of 34 ovaries. The recovery of 70 ova required the aspiration of 228 follicles for an overall recovery rate of 30.7 %.

There was no difference in the results obtained by using the syringe aspiration only or by using the ova recovery unit. The recovery rates of Holsteins and Herefords were approximately the same. Also, no difference in the recovery rate was noted between small and large size follicles.

The largest number of ova recovered were surrounded by cumulus cell layers (figs. 2 & 3). However, 9 out of 70 ova were naked and without any surrounding cells (fig. 4). One out of the 9 naked ova had an abnormal nucleus. Two very small naked ova were found in the small follicles.

DISCUSSION

This report describes a technique for the recovery of ova from the bovine ovary obtained from slaughtered cows. This procedure has the advantage of obtaining large numbers of ova for ova transfer experiments in cattle and for further studies, such as in vitro fertilization, in vitro culture, as well as biochemical and physiological studies on ovum maturation and development.

In humans, STEPTOE & EDWARDS ('70) developed a laparoscopic aspiration apparatus and obtained similar oocyte recovery rates of 31.8~32.4 %. MORGENSTERN & SOUPART ('72) reported that a recovery rate of 30.6 % was obtained in humans using a combination of surgery and an oocyte recovery unit. In this study, the recovery rate was very close to the result on humans, however, the materials were from a random selection from the slaughterhouse, so that some abnormal and degenerating follicles may have been included. Histological sections were made from some of the unrecovered follicles and it was found that the granulosa layers were completely degenerated (fig. 5).

A simple aspiration method is described here for the recovery of ova which can be used for a surgical approach to the in situ ovary exposed in the course of vaginal and abdominal surgery. For ova recovery from large follicles in the course of surgery, especially after pregnant mare's serum gonadotrophin treatment, a heparinized syringe or collection tube should be used in order to avoid coagulation of blood.

The results from this study indicate that through further refinement of the aspiration technique, it may be possible to use slaughtered and surgical material as a routine source of viable ova for ova transfer in cattle in the future.

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EXPLANATION OF PLATE

PLATE

Fig. 1 The assembled ova recovery unit (right) and bovine ovary with follicle (left)

The ova recovery unit consists of a 5 ml ova collection vial with a 20 gauge double-end needle, a plastic extension tube with 20 gauge needle and an aspiration syringe. (scale : cm)

Figs. 2 & 3 The recovered ovum was surrounded by cumulus cell layers (fig. 2) and later placed between a cover glass (fig. 3).
× 250

Fig. 4 A naked ovum without surrounding cells × 250

Fig. 5 A histological section from some of the unrecovered follicles shows complete degeneration of the granulosa layer. × 300

