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**The Role of German Alumni in  
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Entrepreneurship**

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5<sup>th</sup> International Symposium - cum - ^  
Workshop in Southeast Asia  
23 - 27 August 2004, Phnom Penh, Cambodia  
Proceedings

# **The Role of German Alumni in Rural/Regional Development and Entrepreneurship**

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## FOLLICLE DEVELOPMENT AFTER GONADOTROPHS TREATMENT IN GARUT SHEEP FOR LAPAROSCOPIC OVUM PICK UP

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### Abstract

*Laparoscopy ovum pick up (LOPU) as non invasive method can be used to collect oocytes from live donor with minimum injury. However, to increase number of collected oocytes it is important to stimulate the ovary prior the puncture to increase the number of appearance follicles. An experiment was carried out to observe the effect of gonadotrophin treatment on the follicle development in Garut sheep breed. Animals were divided into three groups according to the treatment and laparoscopic time as follow : Groups A: Laparoscopy was conducted once a week combined with PMSG treatment , Group B: Laparoscopy was conducted once in two weeks combined with PMSG treatment and Groups C as a control group, laparoscopy was conducted once in two weeks on animals without Gonadotrophin treatment. Gonadotrophin treatment on Groups A and B was applied 48h before LOPU by administration 1500 IV Pregnant Mare Serum Gonadotrophin (PMSG- Intervet, Holland). Assessment was performed based on the number of appearance follicles. Result of the experiment indicated that number of follicles were higher in the groups after gonadotrophin treatment than control groups. However, no significant differences in the number of follicle formed in both treatment groups. It is concluded that oocytes collection from live donor in Garut sheep can be done weekly.*

**Key words:** *Laparoscopy, oocyte, follicles, gonadotrophin*

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## **Materials and Methods**

### **Treatment of donor animals**

All animals' donors were Garut sheeps breed with age 2-3 years. Donors were divided into three groups according to the treatment and Laparoscopy time. To make uniform estrus cycle, estrus was synchronized with Prostaglandin 2 alpha (Prosolvin®, Intervet, Boxmeer, Holland) and heat detector (Draminski estrous detector®, Olsztyn, Poland) was used to determine days 0 of cycle. To confirm stage of estrus, adult ram with apron was introduced in folk. Animal with standing heat was determines as heat (day 0).

Group of animals were divided into 3 groups as followed. In Group A, follicles were observed and punctured once a week, In Group B, follicles were observed and punctured once in two weeks and in Group C as control group, follicles were observed and punctured once in two weeks without hormonal stimulation. Hormonal stimulation on Group A and B were treated by 1500 IU Gonadotrophin injection (Folligon®, Intervet, Boxmeer, Holland) 48 hr prior laparoscopy.

### **Laparoscopy technique**

Numbers of follicle were recorded under Laparoscopic observation. The laparoscopy equipment was composed of a 7 mm telescope, light cable, light source, 7 mm trocar for laparoscope, an atraumatic grasping forceps and two 5 mm trocar. Animals were deprived of food and water 18 - 24 hr prior to laparoscopy. Anesthesia was induced with intramuscular (im) administration xylazin (Ilium Xylazil®, Troy Laboratories PTY Limited, Australia) 0.01 - 0.22 ml/20 Kg body weight combined with intramuscular (im) administration Ketamin (Ketavet®, Delvet PTY) 11 - 22 mg/kg body weight.

The anesthetized animals were restrained in a cradle in the position to form 30° angles. Trocar was inserted and the abdominal cavity was filled with CO<sub>2</sub> in order to facilitate visualization of the reproductive tract. The ovaries were exposed by pulling the fimbria in different direction using the grasping forceps. Numbers of follicle were recorded and the follicular content s were aspirated by puncturing follicles with the aspiration needle. At the end of procedure, the surface of both ovaries was flushed with physiological saline in order to clean any blood from the follicle puncture site. The procedure was repeated 3 times in the same animals throughout the experiment.

## Introduction

Repeated collection of oocyte from live donor followed by in vitro embryo production has the potential for producing more offspring from genetically valuable donor than conventional multiple ovulation and embryo transfer (MOET) procedure (Baldassesere et al. 2002). Garut sheep is one of original Indonesian breed sheep which has good characteristic such as non seasonal breeding and prolific. Moreover, Garut ram has a big performance, more demand for "fighting ram championship" in traditional culture particularly in West Java. To increase the reproductive performance capabilities of Garut sheep, assisted reproductive technology such reproductive biotechnology is required. However, the applications of reproductive biotechnology in small ruminant are still hampered by structure of reproductive tract. Therefore, it is required a non invasive method to increase the efficiency in application reproductive biotechnology.

Laparoscopy has provided an efficient and relatively noninvasive method for the collection of oocytes from small ruminants like sheep and goats, in which other techniques may not be feasible (transvaginal ultrasound guided) (Koeman et al., 2003). In addition, recovery period after laparoscopy is faster than normal surgery since laparoscopy requires only small incision. Therefore, the acceptability of the major surgery procedure has been questioned due to the reduce fertility as a results of post operative adhesion and the animal welfare argument (Lymberopoulos et al., 2001).

It is generally accepted that only dominant follicle will be ovulated at the end of estrous cycle, although follicular wave's recruitment occur several times in the period of estrous cycle. It is, therefore, to obtain a high number of good quality oocytes from the ovary; effective hormonal stimulation is required prior aspiration. Treatment of donor by Gonadotrophin is a choice for repeated ovarium stimulation in attempts to maximize availability of follicles and oocytes; and oocytes recovery rates (Armstrong, et al. 1994). Hence, the hormonal treatment approach should be conducted based on the follicular wave recruitments.

The aim of the present study was to observe follicle development after Gonadotrophin stimulation to increase the efficiency to obtain numbers of oocytes for in vitro embryo production.

### Statistical analysis

Differences numbers of observed follicles within treatment and control groups were analyzed by ANOVA. Significant difference between groups were tested with Duncan multiple range test ( $P < 0.01$ ).

### Results

A total of 73 follicles was recorded over period 10 times OPU. These results are shown in Table 1. In group A where one a week repeated OPU combined with PMSG treatment, an average 10.67 appearance follicles were recorded at each OPU.

In group B where once in two weeks repeated OPU combined with PMSG treatment, an average 10.00 appearance follicles were detected at each OPU period. There was no significant difference in the PMSG-stimulated ewes on the number of appearance follicle between a week period OPU (Group A) and two weeks period OPU (group B). However, it is interesting to note, results of this experiment indicated that Laparoscopy ovum pick up can be conducted more than one times in a period of estrous cycle without any detrimental effect on the donor as shown in group A.

Table 1. Number of appearance follicle after Gonadotrophin stimulation

| Treatment program | Total puncture period | Total visible follicles per puncture period |    |     | Total follicles | Average number follicle per puncture |
|-------------------|-----------------------|---|----|-----|-----------------|--------------------------------------|
|                   |                       | I   | II | III |                 |                                      |
| A                 | 3                     | 12  | 9  | 11  | 32              | 10.67                                |
| B                 | 3                     | 7   | 11 | 12  | 30              | 10.00                                |
| C                 | 3                     | 4   | 3  | 4   | 11              | 3.67*                                |

\* Different superscript in the same column are significantly different ( $P < 0.01$ )

In group C where the donor was not treated by PMSG and OPU was conducted once in two weeks, the average number of appearance follicles were lower than both (A and B) treatment groups with an average number 3.67 follicles per puncture period. This result indicated that treatment with Gonadotrophin was required to make an efficient on follicles availability and oocytes collection. However, further study should be conducted on gonadotrophin stimulation regimen for effective treatment and oocytes collection.

In addition, from 3 times puncture periods, no detrimental effect such as adhesion was recorded on the animal's donors. The recovery rate after LOPU was very faster.

### Discussion

Laparoscopy as non invasive method has been widely used in assisted reproductive technology especially in small ruminant; such as, for artificial insemination (Ehling et al. 2002), ovum pick up for in vitro embryo production (Stang et al. 1999) and even for embryo recovery for transgenic animal (Baldassare et al. 2002).

To obtain high number quality oocytes, several gonadotrophin stimulation regimen has been applied in several species such in calf (Armstrong et al. 1994), in prepubertal and adult goat (Koeman et al., 2003), East Frisian Milk sheep ( Stang et al., 1999), Scottish Blackface ewes (Bari et al., 2001), Chios ewes (Lymberopoulos et al. , 2001). This paper reports for the first time of the influence of one shoot gonadotrophin stimulation in Garut sheep on appearance follicular development.

Results of the present study demonstrated that good ovarian response can be achieved by one shoot gonadotrophin regime. On average 10 visible follicles can be recorded on both ovarian after gonadotrophin stimulation in a puncture period. This result demonstrated also that more visible follicles achieved significantly compared in group without Gonadotrophin stimulation.

Although there was no significant difference ( $P > 0.1$ ) in the number follicles appearance in both groups treatment (A and B), it is interesting to note that puncture period in group A is shorter than group B. This result indicated that oocytes collection in Garut sheep can be conducted more than once in one estrous cycle without any detrimental effect. Results of present study could confirm that follicle recruitment in goat and sheep twice in a cycle period (Stick et al. 2003).

Repeated ovum pick up has no negative effect the recovery rate of donor sheep, even when repeated once a week OPU (group A). Furthermore, Stang et al. (1999) reported that no negative effect on the fertility after 20 times repeated OPU. However, number of collected oocytes in the present study is still lower (data not shown), since limited oocytes aspirator and hormonal stimulation regime. In addition, Baldassere et al. (2002) reported that administration hormone 48 hr before LOPU resulted in more follicle showing thick follicular content, which were more difficult to aspirate as well as a larger number of oocytes with expanded cumulus. Therefore it is suggested injection 36 hr prior LOPU appeared to be a more appropriate timing.

In conclusion, weekly repeated laparoscopic ovum pick up combined with PMSG treatment is efficient regimen for the recovery of oocytes in Garut Sheep. Further studies are needed to observe developmental capacity of oocytes derived from LOPU for in vitro embryo production.



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