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Effect of pregnancy on retrieval of oocytes for IVF in Deccani ewes

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Abstract

A total number of 76 gravid genitalia of the Deccani breed of ewes was procured from local abattoirs. The morphometrics of the ovaries of gravid genitalia were recorded. The numbers of visible follicles on right and left ovaries were 1.81 ± 0.14 and 1.75 ± 0.14 irrespective sizes of follicle. The mean number of oocytes retrieved from ovaries during different periods of gestation was 7.83 ± 0.63 up to 30 days, 8.27 ± 0.70 in 31-60 days, and 9.50 ± 0.70 in 61-90 days and 9.05 ± 0.70 in 91-120 days of gestation, respectively. The mean number of oocytes retrieved in aspiration, dissection and slicing techniques were 9.33 ± 0.76 , 9.41 ± 0.36 and 7.25 ± 0.50 in ovaries collected from gravid genitalia. Significantly, a longer time was required to process the ovary by dissection technique than slicing and aspiration techniques.

Keywords: Deccani ewes, oocyte retrieval, gravid genitalia, *in-vitro* embryo production

Introduction

Sheep are important protein sources in the diets and support the livelihood of many poor farmers in the tropics and sub tropics of India. Oocytes are the main raw materials for *in vitro* embryo production (IVP) experiments. Therefore, the success of any IVP program in sheep production, either *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) largely depends on the continuous supply of quality oocytes in optimum quantity. A number of methods are currently used for Oocyte Recovery (OR) from live or slaughtered sheep. The laparoscopic method of recovering oocytes in sheep is one of the minimally invasive methods used in the biotechnology of animal reproduction. It allows for good quality oocytes that are suitable for *in vitro* maturation and fertilization to be recovered. Improvement of reproductive traits of sheep can be achieved by using several reproductive technologies. Normally a large number of female gametes are being wasted, as only a few of several thousand follicles present in ovaries are matured and ovulated during the total reproductive life span of ewe. Besides this, large numbers of pregnant ewes are also being slaughtered in abattoirs especially during early gestation along with non-pregnant ewes. Hence, the present study is aimed to retrieve the Oocytes from gravid genitalia and to study the effect of stage of pregnancy on retrieval of oocytes from ovaries.

Materials and Methods

A total number of 76 gravid genitalia collected from a municipal slaughterhouse, Hyderabad was utilized. The fetuses were recovered from gravid uterine horn. The length of gestation was calculated based on crown rump length (CRL) of the fetus *viz.* up to 30 days -1.8 cm; 31-60 days-2.2-7.0 cm; 61to 90 days- 7.1-15.6 cm and 91 to 120 days- 15.7-25 cm as per the technique of Joubert (1956) [5]. The morphology and morphometrics of gravid genitalis were studied. The diameter of individual follicle were recorded with vernier Callipers and follicles were classified as Small (< 3mm), medium (3 to 6 mm) and large (> 6 mm) as per previous description of (Kalita *et al.* 2003) [7].

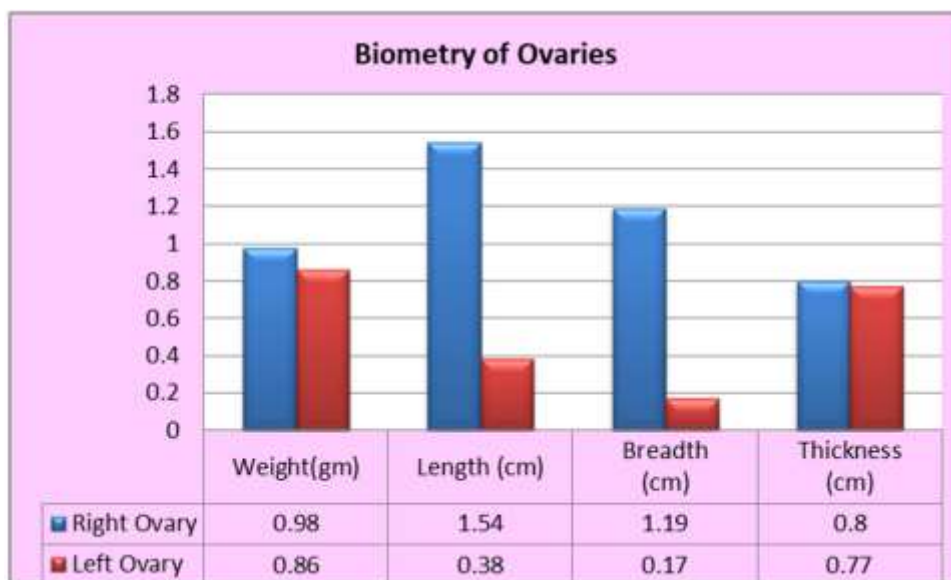


Fig 1: Biometry of Ovaries Collected from gravid genitalia.

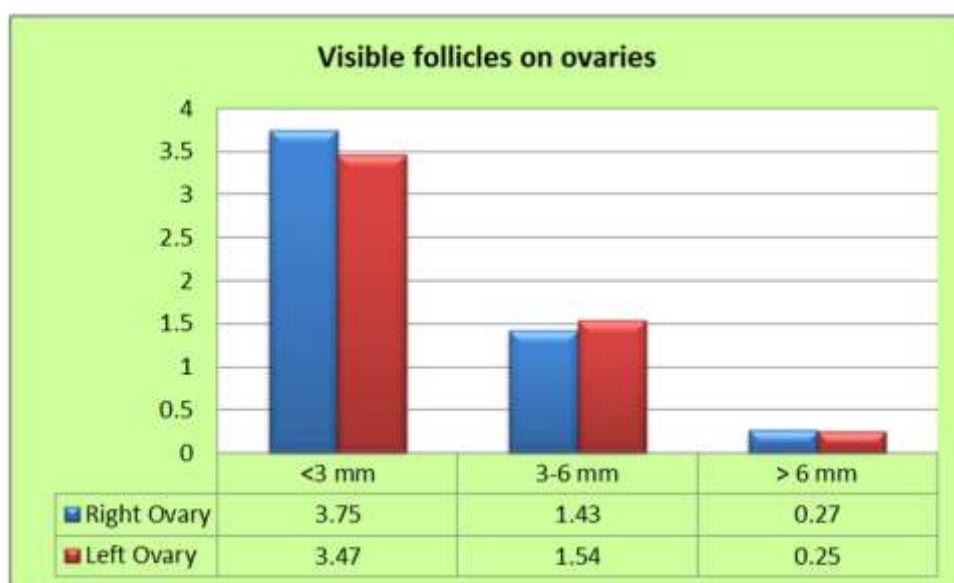


Fig 2: Visible follicles present on ovaries of gravid genitalia.

The oocytes were retrieved from the ovaries by aspiration, (Datta *et al.*, 1993) [2], slicing (Shankarappa and Reddy 1998) [12] and dissection techniques (Datta *et al.*, 1993) [2]. The oocytes were collected by aspiration technique by using 18-gauge needle. The fluid of follicles was aspirated through sterile 18-gauge needle attached to a 5 ml syringe containing a harvested medium (follicular fluid). Aspirated contents were

expelled into a fresh Petri dish containing the medium and ova with cumulus cells were chosen. Similarly, the oocytes were collected by ovarian slicing method, here the collected oocytes were put into a sterile Petri dish contain normal saline and cut into small parts with a blade. The follicular fluid was collected from the Petri dish. Ova that surrounded by cumulus cells was selected from collected media.

Table 1: Oocytes recovered from ovaries of gravid genitalia during different periods of gestation.

S. No.	Technique	Up to 30 days	31-60 days	61-90 days	91-120 days	Average
1.	Aspiration (n=)	8.66±1.08	8.50±1.43	10.50±1.83	9.66±1.87	9.33±0.76 ^b
2.	Dissection (n=)	9.16±0.87	8.83±0.47	10.16±0.65	9.50±0.88	9.41±0.36 ^b
3.	Slicing (n=)	5.66±2.06	7.50±3.9.	7.83±1.47	8.00±1.26	7.25±0.50 ^b
	Overall	7.83±0.63 ^a	8.27±0.70 ^a	9.50±0.70 ^b	9.05±0.69 ^b	

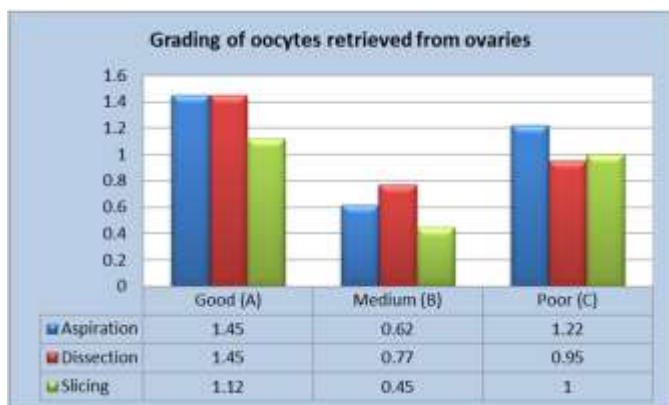


Fig 3: Grading of oocytes retrieved from ovaries of gravid genitalia

The Oocytes collected from slaughtered ewes by aspiration or slicing or puncture were examined under the microscope and graded. Based on the number of cumulus cell layers, the oocytes were classified as A (>3), B (1-2), C (Scattered Cumulus and D (Nude) as described by Kalita *et al.*, (2003) [7]. The unit time taken for Oocyte retrieval by each method was recorded from point of starting the technique to searching out the oocytes under stereozoom microscope

Good = Oocytes with numerous layers of cumulative cells and uniform cytoplasm.

Medium = Oocytes with thin layers of cumulative cells and uniform cytoplasm.

Poor = Oocytes with few or no cumulative cells.

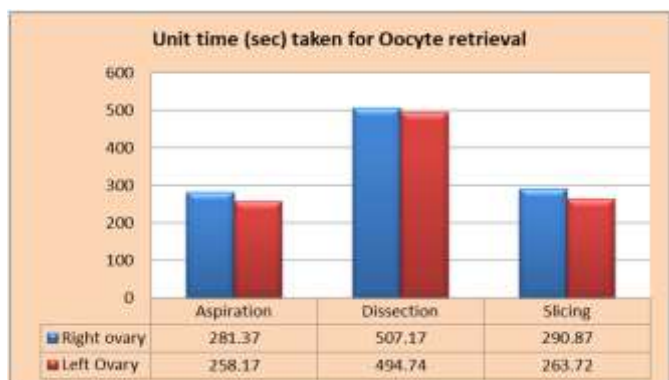


Fig 4: Unit time (sec) taken for Oocyte retrieval from ovaries of gravid genitalia

The values were recorded as means \pm S. E. and subjected to analysis via T-test and analysis of variance.

Results and Discussion

The shape of the ovary of the gravid genitalia was round (12.50%), flat (13.19%), Oval (21.52%), Irregular (28.47%) and almond (24.30%). The weight, length and breadth of right ovary were significantly higher than the left ovary. Similar, studies were conducted by Guha *et al.*, (2000) [4] in goats. (Table 1).

The mean number of small follicles (< 3 mm) size pre-ovary was significantly higher followed by medium (3-6 mm) and large-sized follicles (>6mm) irrespective of right and left ovary (Table 2). Similar findings were also observed by kalia *et al.*, (2000) [6] in local goats of Assam. This might be due to the influence of higher progesterone profiles during the pregnancy which may not support the development of follicle to reach griffin follicle size.

The mean number of oocytes retrieved per ovary by aspiration and dissection techniques were significantly higher than the slicing technique. Significantly, higher number of oocytes was retrieved from the ovaries of gravid genitalia having more than 60 days of gestation than that of groups with gestation period less than 60 days (Table 3). The study indicated that the gravid genitalia procured from abattoirs or from the pregnant ewes by folliculogenesis or ovum pick up methods. Significantly higher number of grade A and D oocytes were retrieved followed by grade B and C irrespective of technique of retrieval (Table 4). Similar findings were noticed by Kalita *et al.*, (2000) [6] Wani *et al.*, (2000) [16], Gogoi *et al.*, 2001 [3], Rao *et al.* 2002 [11], shirazi *et al.*, 2005 [14] nandi *et al.*, (2005) [8] Tibary *et al.* 2005 [15] and Shanmuga sundaram and Devanathan (2006) [13].

Significantly, longer mean time was taken per ovary in dissection technique rather than slicing and aspiration techniques, which may be because of small size of the sheep ovary (table 5)

Similar studies also Conducted by Datta *et al.*, (1993) [2], and Das *et al.* (1996) [1]. It is concluded that the competence of *in vitro* maturation of these oocytes may be studied.

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