The Indian Journal of Animal Reproduction; 29(2):138-143; December 2008 **Research Article**

Effect of Season on Oocyte Recovery, Quality and Morphometry in Goat

G. K. SANGHA, H. BHATIA AND K.S. KHERA

Department of Zoology, College of Basic Sciences and Humanities
Punjab Agricultural University, Ludhiana-141004 (Punjab)

ABSTRACT

Seasonal effects have the potential to influence the follicular dynamics. The prerequisite for the contemporary embryo transfer technology is the availability of good quality oocytes. The present study was undertaken to observe effects of season on oocyte retrieval, quality and morphometry in goat. Maximum numbers of small (<3mm) as well as large (>3mm) follicles were obtained during rainy season. The percentage recovery of oocytes was highest during rainy season though the proportion of good quality oocytes was more during winter. The size of the oocyte showed a direct relation with the follicular size. The diameter of small as well as large oocytes was reduced during summer. The present study reports a seasonal relationship of ovarian morphology as implicated by the quality of oocytes in slaughtered goats.

Key words: Goat, oocyte, Recovery, Quality, Morphometry, Season and Heat stress

INTRODUCTION

Temperature of the reproductive tract is influenced by exogenous estradiol concentrations and distinct thermal changes of the tract are associated with the transition in plasma estradiol approaching estrous and ovulation (Thatcher 1973). Follicular growth patterns are influenced by changes in the environmental temperature (Hahn 1999). Heat stress affects ovulatory oocyte (Baumgartner and Chrisman 1981) as well as functions of follicular granulosa and theca cells. Follicular dynamics of ovary and corpora lutea change under heat stress (Wolfenson *et al.* 1995).

There is a decrease in the number of small (3-5 mm) and medium (6-9 mm) follicles after exposure to heat stress in cows (Wolfenson *et al* 1995, Wilson *et al.* 1998, Osman and Shehata 2005). The numbers of cumulus oocyte complexes (COCs) recovered in different seasons were significantly lower during summer in cattle and buffalo (Hussain and Shahim 2005). Rocha *et al.* (1998) reported that the quality and developmental capabilities of bovine oocytes after in vitro fertilization decrease during hot season. Decline in follicular growth and dominance during the preovulatory period due to heat stress has also been observed in cattle (Wilson *et al.* 1998). Heat stress impairs oocyte quality and embryo development and increases embryo mortality in cattle (Wolfenson *et al.* 2000, Hamam *et al.* 2001). Seasonal effects on follicular dynamics have the potential to influence on all the follicular cells and significant differences were observed between winter and summer in the capacity for in vitro development of bovine oocytes (Zeron *et al.* 2001). Significant changes in the cytoskeleton of oocytes after heat shock are associated with the reduced development under hyperthermia and low pregnancy rates during hot seasons in cattle (Tseng *et al.* 2004) and pigs (Ju and Tseng 2004). Seasonal effects on follicular dynamics and oocyte morphology need to be explained in goats, as this will relate to their differential developmental capacity in vitro.

The aim of the present study was to determine the influence of seasons, acute heat stress and follicular size on the oocyte retrieval, quality and morphometry in goat. The observations regarding the season best suited for oocyte retrieval will prove to be beneficial for in vitro fertilization programme as selection of oocyte allows a prior assessment of successful and subsequent fertilization.

MATERIALS AND METHODS

Goat ovaries were collected throughout the year from the local abattoir and transported to the laboratory in phosphate buffered saline (PBS) pH 7.4 supplemented with 50-µg/ml gentamycin at 37°C within 1-2 hours of the slaughter. Only adult ovaries were used for the oocyte recovery as described by Sangha and Sharma (2006). The ovaries were excised from the genitalia and washed twice with saline (37°C) and dried on blotting papers.

The number of visible follicles was counted on the surface of ovaries and measured using vernier caliper. Based on their sizes, follicles were divided into two groups: (a) small follicles, <3mm in diameter (b) large follicles, >3mm in diameter. The data were pooled in five seasons - winter (December-February), spring (March-April), summer (May-June), rainy (July-September) and autumn (October-November). At least 38 – 45 animals were collected during each season for evaluation.

The oocytes were aspirated from normal small and large follicles along with follicular fluid using 18-gauge needle in different seasons. Cumulus oocyte complexes thus recovered were washed three times in PBS and observed under an inverted microscope. The oocytes were divided into four grades on the basis of their morphology and the number of cumulus layers as (a) good quality oocytes with 3-4 layers of compact cumulus cells (b) fair quality oocytes with at least 2 layers of cumulus cells (c) poor quality oocytes with partial cumulus cell layers and (d) bad quality oocytes comprised denuded oocytes without cumulus.

Oocytes were subjected to measurements of diameter with ocular and stage micrometers under an inverted microscope at a magnification of 20X and percentage recovery was assessed in different seasons. All results were subjected to statistical analysis by ANOVA. Level of significance was checked at 5%.

RESULTS AND DISCUSSION

Maximum numbers of small and large follicles per ovary were present in ovaries during the rainy season (July-September) (Table 1). The number of follicles decreased significantly (P>0.05) during summers (May-June). The decrease in the number of small and large follicles may be due to decreased estradiol concentration (Wilson *et al.* 1998). Wolfenson *et al.*(2000) observed that in bovines when heat stress was applied, estradiol concentrations in the peripheral blood are decreased. Follicular growth and development were lowered in heat stressed cows (Wilson *et al.* 1998). A reduction in the number of follicles per ovary has also been reported in bovines during summer (Zeron *et al.* 2001, Osman and Shehata 2005).

The percentage recovery of large oocytes was maximum during rainy season and least during summers (Table 1). The numbers of COCs recovered in different seasons were also significantly lower during summer in cattle and buffalo (Hussain and Shahim 2005). In our studies, we have obtained a recovery rate of 39.85% in small and 68.69% in large oocytes (Table1). Goswami *et al.* (1992) and Sarkhel *et al.* (1997) have also observed oocyte recovery to be 75.22 and 50.29%, respectively in goat. However Sangha and Sharma (2006) observed a low recovery rate of 34.3% in goats. Naik *et al.* (1999) obtained a recovery rate of 52.31% in buffaloes. A non-significant (P>0.05) effect of seasons has been observed on the oocyte recovery rate (Table 1). Hamam *et al.* (2001) also observed non-significant effect of seasons on oocyte recovery rate in bovines. The average recovery rate was found to be 90.2% from 624 aspirated follicles without marked variations among the seasons in buffalo (Osman and Shehata 2005).

The average number of small as well large oocytes obtained per ovary was significantly higher during monsoon as compared to summer season (P<0.05) (Table 1). Agrawal (1992) has reported that the oocytes recovered per ovary were 13.4 in monsoon season and 8.2 in winter season in goat. Zeron *et al.* (2001) have reported the average number of oocytes per ovary in bovines to be 7.5 in winter and 5.0 in summer. Average number of culturable oocytes in cattle and buffalo were higher in winter than in summer season (Hamam *et al.* 2001, Hussain and Shahim 2005). Oocytes obtained per ovary in goat have been reported to be 2.08 (Chakravarty *et al.* 1994), 3.68 (Bonde 1995), 2.54 (Sarkhel *et al.* 1997) and 3.41 (Malakar and Majumdar 2002). Average number of good quality COCs recovered in cattle and buffalo were 3.9±0.21 and 1.9±0.14 per ovary, respectively (Hussain and Shahim 2005).

Table 1: Effect of seasons on the recovery, quality and morphometry of goat oocytes

Category	Winter (Dec-Feb)		Spring (Mar-Apr)		Summer (May-Jun)		Rainy (Jul-Sep)		Autumn (Oct-Nov)	
	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large
No. of follicles per ovary	17.83[b]	4.04[ab]	13.14[b]	3.02[a]	7.26	2.01[a]	22.84[b]	5.23[ab]	14.22[b]	3.17[a]
No. of oocytes recovered per ovary	7.00[b] (39.26)	3.00[ab] (74.26)	5.5 (41.68)	2.00[ab] (66.22)	3.50 (48.21)	1.00[a] (49.75)	8.00[b] (35.03)	4.00[ab] (76.48)	6.00[b] (42.19)	2.00[ab] (63.09
Good quality oocytes per ovary	4.00[b] (57.07)	1.36[ab] (45.17)	1.38 (25.00)	0.85[ab] (42.49)	0.92 (26.26)	0.24[a] (24.14)	3.16[b] (39.44)	1.51[ab] (37.71)	2.33[b] (38.84)	0.70[ab] (35.19
Fair quality oocytes per ovary	1.30[b] (18.51)	1.98[b] (36.07)	1.98[b] (36.07)	0.47[a] (23.32)	0.55 (15.70)	0.30 (30.28)	2.42[b] (30.28)	1.18[ab] (29.51)	1.09[b] (18.18)	0.33[a] (16.67
Poor quality oocytes per ovary	1.25[b] (17.81)	0.5[ab] (18.43)	1.13 (20.49)	1.03[b] (18.65)	0.73 (20.93)	0.23[a] (22.54)	1.80[b] (22.54)	0.79[ab] (19.67)	1.88[b] (31.4)	0.56[ab] (27.78)
Bad quality oocytes per ovary	0.46 (6.59)	0.47 (15.51)	1.01 (18.44)	0.85[b] (15.54)	1.30 (18.65)	0.23[a] (23.04)	0.62[b] (7.75)	0.52[b] (13.12)	0.69[b] (11.57)	0.56[b] (27.78)
Oocyte diameter Mean±SE (μm)	120.12[b] ± 4.63	162.76[ab] ± 2.88	116.76 ± 2.89	150.34[a] ± 3.17	104.67 ± 3.17	142.3[a] ± 5.66	112.27 ± 3.01	145.6[a] ± 4.71	118.3 ± 2.17	159.6[a] ± 1.36

( ) Figures in parentheses represent percentage figures

[a] Values differ significantly from the corresponding small category at 5% level

[b] Values differ significantly from the corresponding value of summer season at 5% level.

Higher number of good quality small as well as large oocytes was recovered during winter season as compared to summer season. The proportion of poor and bad quality oocytes increased during summer (Table 1). This may be non-exhibition of estrous in goats during summer season. Agrawal (1992) observed that the percentage of good quality oocytes was higher in winter compared to monsoon in goat. A higher percentage of good quality oocytes were obtained in all

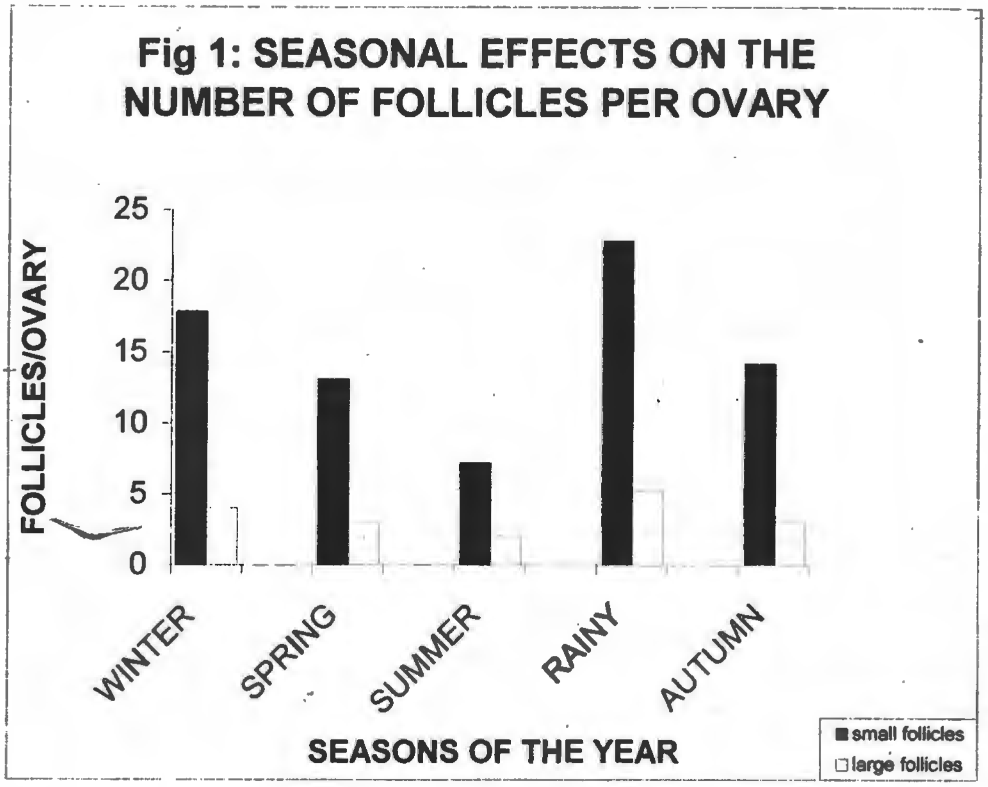

Fig 1: SEASONAL EFFECTS ON THE NUMBER OF FOLLICLES PER OVARY

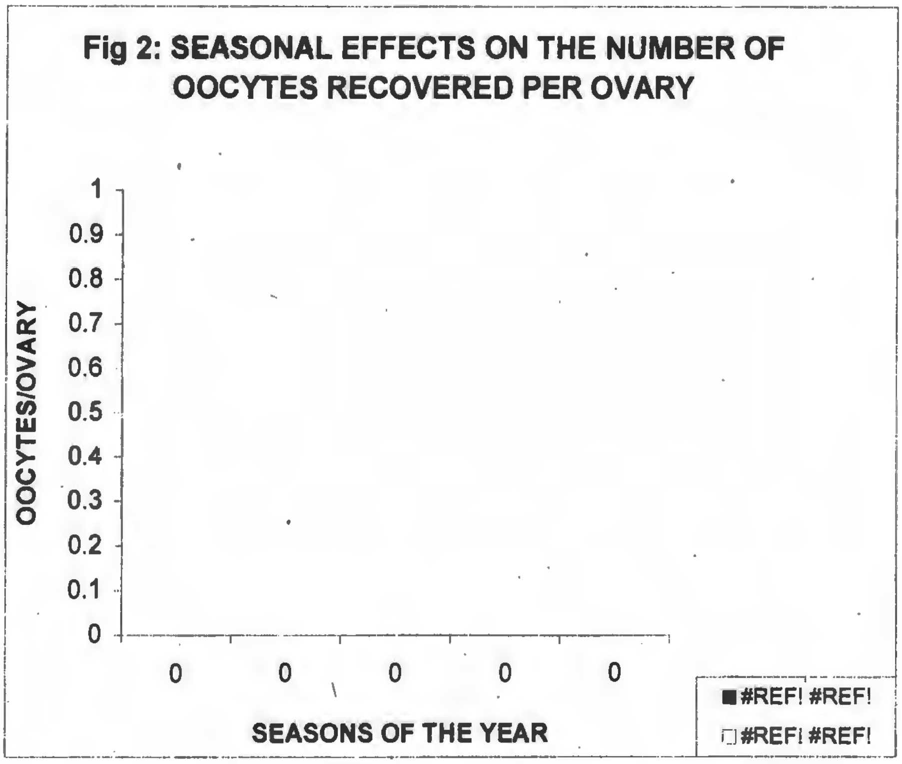

Fig 2: SEASONAL EFFECTS ON THE NUMBER OF OOCYTES RECOVERED PER OVARY

seasons except summer, where the percentage of poor quality oocytes was increased significantly in buffalo (Hamam *et al.* 2001). Summer heat stress impairs oocyte quality, and embryo development and increases embryo mortality in cattle (Wolfenson *et al.* 2000), although Zeron *et al.* (2001) and Osman and Shehata (2005) have reported that the percentage recovery rates in bovines were not affected by season. Abdoon (2001) observed that the growth and development of ovarian follicles as well as total yields of category 1 oocytes were greater during breeding season in camels. Average number of culturable oocytes in cattle and buffalo were higher in winter than in summer season (Hussain and Shahim 2005).

The average diameters of small and large oocytes during winter, spring, summer, rainy and autumn seasons have been depicted in Table1. Oocyte diameter increased significantly ($P<0.05$) with increase in the size of the follicle (Table1). A significant reduction ($P<0.05$) in the size of the oocyte was observed during summers as compared to winter season. A positive correlation between the oocyte diameters and follicular size has also been reported by other workers in goats (Sarkhel *et al.* 1997, Sarkhel *et al.* 1999, Sangha and Sharma 2006) and buffalo ovarian follicles (Naik *et al.* 2002). Izumi *et al.* (2003) have reported that when the follicles in bovine ovaries grow more than 4 mm in diameter, the oocytes reach full size and cease to grow. An increase in diameter of oocytes in cow as compared to calf has also been observed (Yeh *et al.* 2004). Follicular growth patterns are influenced by changes in body temperature (Zeron *et al.* 2001). Heat stress affects the follicular dynamics and reduces the diameter and the volume of the dominant follicles (Hahn 1999). Kalita *et al.* (2002) in goats observed that the diameter of oocytes varies with the quality of the oocyte.

In conclusion, it can be stated that summer season of the year adversely affects the recovery, quality and morphometry of caprine oocytes, which in turn, may influence the ability of the oocytes to develop to the blastocyst stage.

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