Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(6): 568-571

Received: 24-09-2019
Accepted: 28-10-2019

SB Harkal
M.V.Sc. Scholar, Department of Animal Reproduction, Gynaecology and Obstetrics. Post Graduate Institute of Veterinary and Animal Sciences, Akola Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

CH Pawshe
Professor and Head, Department of Animal Reproduction, Post Graduate Institute of Veterinary and Animal Sciences, Akola Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

MV Ingawale
Assistant Professor, Department of Animal Reproduction, Gynaecology and Obstetrics. Post Graduate Institute of Veterinary and Animal Sciences, Akola Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

SG Deshmukh
Assistant Professor, Department of Animal Reproduction, Gynaecology and Obstetrics. Post Graduate Institute of Veterinary and Animal Sciences, Akola Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

MB Ambalkar
M.V.Sc. Scholar, Department of Animal Reproduction, Gynaecology and Obstetrics. Post Graduate Institute of Veterinary and Animal Sciences, Akola Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

SD Kale
M.V.Sc. Scholar, Department of Animal Reproduction, Gynaecology and Obstetrics. Post Graduate Institute of Veterinary and Animal Sciences, Akola Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

Corresponding Author:
CH Pawshe
Professor and Head, Department of Animal Reproduction, Post Graduate Institute of Veterinary and Animal Sciences, Akola Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

Effect of frequency of follicular aspiration on recovery of oocytes and follicular development

SB Harkal, CH Pawshe, MV Ingawale, SG Deshmukh, MB Ambalkar and SD Kale

Abstract
The objective of present research work is to study follicular population and oocyte recovery in once a week and twice a week aspiration interval. A total 9 (Bos indicus) healthy animals were selected for trans vaginal follicular aspiration (TVFA) in every once weekly (every 7d; n=9) or twice weekly (every 3 or 4 d; n= 9). During each session, all visible follicles were aspirated using ultrasound guided trans vaginal follicular aspiration by using negative vacuum pressure of 90 mm Hg. Prior to the aspiration, follicular population were identified and measured and data recorded in three classes according to size (< 6mm, from 6 mm to 9 mm and > 9 mm). The COCs harvested from each follicle were counted and classified into 3 categories.
A total of 197 follicles were aspirated by during 60 aspiration sessions yielding 114 oocytes. There was no significant difference in average COCs/follicles recovered but recovery rate of oocyte was higher in twice week than once a week aspiration session (48.24% verses 71.08%). We concluded that follicular aspiration appears in twice a week with a higher number of harvestable follicles and more oocytes recovered than when done once a week.

Keywords: Trans vaginal follicular aspiration, oocyte recovery, COCs recovery

1. Introduction
Ultrasound guided transvaginal follicular aspiration combined with in vitro embryo production is emerging as a successful method for obtaining preimplantation stage embryos (Gibbons *et al.* 1994, Loony *et al.* 1994) [1, 2]. The success of association between the techniques of in vitro ultrasonographically guided transvaginal follicle aspiration (TVFA) and in vitro embryo production of bovine embryos depend upon the quantity and quality of recovered oocytes that directly and indirectly affect the efficiency of maturation, fertilization and in vitro culture systems (Lonergan 1999) [3]. Cattle have waves of ovarian follicular growth and regression during estrous cycles (Knopf *et al.* 1989) [4] and the selection of dominant follicles in successive follicular waves determines the variation in number and development status of follicles. Selection of dominant follicles was also suggested that one of the main sources of variation in results (Adams 1994) [5]. The removal of ultrasonographically detectable ovarian follicular population by transvaginal follicular aspiration results an establishment of new follicular waves of follicular development (Garcia and Salaheddine 1998) [6]. and this procedure is used to synchronize stages of follicular growth (Adams 1994, Bols *et al.* 1995) [5, 7]. The stage of development of the follicles at the time of subsequent aspiration, in females undergoing successive TVFA procedures depend on the interval between aspiration (Gibbons *et al.*1994, Boni *et al.*, 1997) [1, 8]. The results of a transvaginal follicular aspiration (TVFA) schedule also depend on the interval between aspiration and the follicular growth pattern of the animals and this may explain that the differences observed in effect of time of scheduling of oocytes collection different in different cows (Gibbons *et al.* 1994; Kruip *et al.*1994; Boni *et al.*1997) [1, 9, 8]. Several different schemes have been tried for ovum pick up (OPU) method. Generally they can be divided into once weekly and twice a weekly according to the interval between punctures. During certain short periods more oocytes can be obtained with twice weekly puncture but for long term OPU, most popular aspiration schedule is once a weekly (Pieterse *et al.* 1991, Loony *et al.* 1994) [10, 2]. The purpose of present study was to determine and standardize the protocol for obtaining oocytes from live animals. In present experiment once a week verses twice a week trans vaginal follicular aspiration schedules were compared to determine the total recovery rate, oocytes recovery per session and oocyte recovery per

animals.

2. Material and Methods

Selection of animals

For the present study total 9 (Bos indicus) cows were selected, 2 Red Kandhari cows from Instructional Livestock Farm Complex, Post Graduate Institute of Veterinary and Animal Sciences, Akola and 7 Sahiwal cows from Department of Animal Husbandry and Dairy Sciences, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, for ultrasound guided transvaginal ovum pick up. Selected animals were screened for brucellosis and tuberculosis. Reproductive tract of all selected cows was examined by per rectal examination and the cyclicity of ovaries examined per rectally. All the animals were dewormed and vaccinated prior to research work. The animals were fed on mixed ration consisting of concentrate, dry and green roughages as per the routine management practices of farm.

To study the effect of frequency of aspiration on oocytes recovery rate, the experimental cows were aspirated weekly (7 days interval) and twice weekly (3 to 4 days interval). Cows were randomly distributed in two experimental groups and had their ovarian follicles aspirated once a week and twice a week.

Ultrasound guided transvaginal follicular aspiration (TVFA)

Follicular aspiration was performed with a real time ultrasonographic device equipped with sectorial intravaginal 5.0 MHz probe (Pie medical- 240 Parus vet, Philipswea, The Netherland) using disposable 0.9mm × 50 mm, 20 gauge needles and a 90 mmHg negative vacuum pressure. Prior to the aspiration, follicular population was identified, measured and the follicles categorized into three classes according to size i.e., small (< 6mm), medium (from 6 mm to 9 mm) and large (> 9 mm) size follicles. Follicular fluid aspirated was collected in 50 ml conical tube (Corning USA) containing Dulbecco's phosphate buffered saline (DPBS) added with 2% fetal calf serum and 25 IU/ml of heparin. After the recovery, the cumulus oocyte complexes recovered were morphologically classified into 3 categories according to oocyte cytoplasm and morphology of cumulus cell layers i.e. A Grade: compact COCs, more than three layers of cumulus cells and oocyte with homogenous cytoplasm, B Grade: compact COCs, with three layers of cumulus cells and oocyte with slightly homogenous cytoplasm. Partially denuded oocytes with complete removal of cumulus cells from less than 1/3 of zona pellucida and C Grade: oocytes with no cumulus cells over most of ZP surface and shrinkage of cytoplasm

After evaluation, C grade oocytes were recorded and eliminated, while the remaining (A and B grade) cumulus intact oocytes were prepared for in vitro maturation and subsequent in vitro fertilization.

Statistical analysis

The effect of TVFA schedule on ovarian follicular populations, different size of follicle, total number of oocyte recovered and different oocytes categories was analyzed by paired t-test in once a week and twice weekly aspiration groups.

3. Results and Discussion

3.1 Effect of OPU frequency on follicular number

Data for the main ovarian follicular population characteristics at the time of trans vaginal follicular aspiration (TVFA) are shown in Table 1. The mean number of follicles per session was similar in once a week and twice a week aspiration session (3.35 ± 0.27 verses 3.19 ± 0.29). There was no significant difference was detected in the different size of the follicle (small, medium and large) during aspiration of follicle in once a week and twice a week aspiration duration. But follicle distribution among various size classes was affected by oocyte aspiration intervals, with shorter interval (3- 4 days) resulting in greater number of small size follicles (< 6 mm) and smaller number of medium and large size follicles.

Table 1: Effect of aspiration frequency on follicular number and size of follicles

Characteristic	Frequency of aspiration	
	Once –weekly (9 animals)	Twice – weekly (9 animals)
Number of sessions	34	26
Total number of follicles	114	83
Mean number of follicles/ session	3.35 ± 0.27	3.19 ± 0.29
Number of follicles/ animal	12.66	9.22
Number of small size follicles	83	56
Mean number of small follicles	2.44 ± 0.26	2.15 ±0.32
Number of medium follicles	23	19
Mean number of medium follicles	0.67 ±0.12	0.69 ± 0.14
Number of large follicles	8	8
Mean number of large follicles	0.23 ± 0.07	3.15 ± 0.29

The present study evaluated the effect of repeated ultrasound guided transvaginal follicular aspiration on oocyte recovery and subsequent follicular development. In present study, numbers of follicles per session (3.35 ± 0.27) were not significantly differing in once a week and in twice a week follicular aspiration (3.19±0.29). However, Chaubal *et al*. (2006) [11]. Reported that number of follicles per session in once a week (7.8±2.4) were higher than the twice a week (6.5±2.4). This difference is due to unequal number of aspiration sessions in once a week and twice a week in present study.

Li *et al*. (2007) [12]. observed that mean number of aspirated follicles per session in once a week (9.1 ± 1.7) were lower than twice a week (12.1 ± 2.6) and reported that the difference may be due to follicular wave starts at 0 day and at 3rd day there is follicular differentiation and subordinate follicles undergo atresia by day 4 of wave emergence many follicles in follicle pool at day 7 after wave emergence will be in advance stage of atresia therefore higher degree of follicles would be already atretic during once weekly protocol. (Singh and

Adams, 1998) [13].

In present study number of follicles per animal was higher in once a week (12.66) than twice a week (9.22). However, Imai *et al*. (2006) [14]. Reported that number of follicles per animal was higher in twice a week (33.4±12.0) than once a week (26.9±7.8). It may be due to performing the aspiration session twice a week has more chances to aspirate many ovarian follicles in total for a week.

The number of aspirated follicles was directly related to the number of follicles available for the procedure which was similar between experimental groups in the present study. The slight difference number of visualized and effectively aspirated follicles was result in inherent difficulty of the procedure. The greater recovery rate observed in the once a week aspiration session contrast with results reported for other breeds (Gibbons *et al.* 1994, Garcia and Salaheddine 1998) [1, 6].

3.2 Effect of aspiration frequency on oocyte recovery

In the yield and quality of oocytes recovered via TVFA at interval of once a week aspiration and twice a week aspiration, no significant differences were detected. Number of aspirated follicles did not differ between once a week and twice a week aspiration (3.35 ± 0.27 versus 3.19 ± 0.29, P > 0.05), but mean number of oocyte recovered in twice a week group was higher (2.26 ± 0.05 versus 1.64 ± 0.28) than once a week aspiration. It results that greater recovery rate in twice a week aspiration group when compared with once a week aspiration group (71.08 % versus 48.24 % respectively).

3.3 Classification of recovered oocytes

Cows undergoing once a week aspiration and twice a week aspiration, there was no significant difference between A grade, B grade and C grade oocyte recovery. More number of C grade oocytes recovered in twice a week aspiration session as compared to once a week (0.52±0.14 versus 1.08±0.26) however there is no difference in viable COCs for in vitro culture.

Table 2: Effect of frequency on oocyte recovery and classification of COCs recovered

Characteristic	Frequency of aspiration	
	Once –weekly (9 animals)	Twice – weekly (9 animals)
Total number of oocytes recovered	55	59
Mean number of recovered oocyte per session	1.64 ± 0.28	2.26 ± 0.05
Mean number oocyte recovered per animal	6.11 ± 1.01	6.55 ± 0.89
Total number of A grade oocyte recovered	19	19
Mean number A grade oocytes recovered	0.55 ± 0.13	0.73 ± 0.21
Total number of B grade oocyte recovered	13	13
Mean number of B grade oocytes recovered	0.38± 0.11	0.52 ± 0.14
Total number of C grade oocyte recovered	23	27
Mean number of C grade oocyte recovered	0.52 ± 0.14	1.08± 0.26
Overall Recovery Rate (%)	48.24	71.08

The total oocyte recovery rate and oocyte per session was higher in twice a week (71.08%; 2.26±0.50) than once a week (48.24 %; 1.64±0.28). In other parameters there is no significant difference observed between once and twice a week aspiration session. In present study oocytes per session was higher in twice a week (2.26 ± 0.50) than once a week (1.64 ± 0.28). Garcia *et al*. (1998) [6]. Reported that oocytes recovered per session were higher in twice a week (7.7 ± 4.5) than once a week (5.4 ± 3.7) which was similar to present study. However, Gibbons *et al*. (1994) [1]. Imai *et al*. (2006) [14]. Observed that there was no significant difference in oocytes per session in once a week and twice a week.

Viana *et al*. (2004) [15]. Chaubal *et al*. (2006) [11]. Reported that oocytes per session were higher in once a week (8.9 ± 0.8; 4.6 ± 1.9) than twice a week (7.0 ± 0.7; 3.9 ± 2.1) respectively. Garcia *et al*. (1998) [6]. Reported that if the follicle aspiration was done then it induces and synchronizes a new follicular wave and when it is done in twice a week it will yields higher no of follicles and oocytes recovery than once a week.

Boni *et al.* (1997) [8] reported that increasing TVFA interval from 2 to 5 days decreased the quality of recovered oocytes. The relationship between collection interval and oocyte quality probably involves the mechanism of follicular dominance.

Goodhand *et al.* (1999) [16] reported that the pretreatment of cows with FSH undergoing TVFA once weekly overcomes the negative effect of dominant follicles establishment resulting in recovery of oocytes with morphologic quality and in vitro development potential similar to oocytes recovered from animal where follicular content aspirated twice weekly.

In the present experiment the number follicle per session and the number of oocyte recovery were lower as compare to the other studies. It may be due to in the present study we used Red Kandhari and Sahiwal (Bos indicus) cattle and it may be due to the inherent potential of follicular development in indigenous cattle (Bos indicus) is lower as compared to the Bos taurus cattle. Viana *et al.* (2000) [17]. Reported that variable responses are also expected in Zebu breed which shows significant differences in ovarian follicular dynamics including number of waves, growth rate and maximum size of dominant follicles when compared to European breeds.

4. Conclusion

In conclusion, the number of follicular population and oocytes recovered via transvaginal follicular aspiration in twice a week interval is higher than once a week aspiration interval.

5. References

1. Gibbons JR, Beal WE, Krisher RL, Faber EG, Pearson RE, Gwazdauskas FC, *et al.* Effect of once -versus twice weekly transvaginal follicular aspiration on bovine oocyte recovery and embryo development. Theriogenology. 1994; 42:405-419.
2. Loony CR, Lindsey BR, Gonseth CL, Johnson DL. Commercial aspect of oocyte retrieval and *in vitro* fertilization for embryo production in problem cows. Theriogenology. 1994; 41:67-72.

3. Lonergan P. Kinetics of embryo development in vitro: an indicator of oocyte quality. Reproductive Domestic Animals. 1999; 6:33-37.
4. Knopf L, Kastelic JP, Schallenberger E, Ginther OJ. Ovarian follicular dynamics in heifers: test of two wave hypothesis by ultrasonically monitoring individual follicles. Domestic Animal Endocrinology. 1989; 6:111-119.
5. Adams GP. Control of ovarian follicular wave dynamics in cattle: implications for synchronization and super-stimulation. Theriogenology. 1994; 41:19-24.
6. Garcia A, Salaheddin M. Effect of repeated ultrasound guided transvaginal follicular aspiration on bovine oocyte recovery and subsequent follicular development. Theriogenology. 1998; 50:575-585.
7. Bols PEJ, Vandenheede JMM, Soom AV, Kruif AD. Transvaginal ovum pick-up (OPU) in the cow: a new disposable needle guidance system. Theriogenology. 1995; 43:677-687.
8. Boni R, Roelofsen MWM, Pieterse MC, Kogut J, Kruip TAM. Follicular dynamics, repeatability and predictability of follicular recruitment in cows undergoing repeated follicular puncture. Theriogenology. 1997; 48:277-289.
9. Kruip T, Boni R, Wurth WA, Roelofsen MWM, Pieterse MC. Potential use of ovum pick-up for embryo production and breeding in cattle. Theriogenology. 1994; 42:675-684.
10. Pieterse MC, Vos PLAM, Kruip TAM, Wurth YA, Beneden THV, Willemse AH, *et al.* Transvaginal ultrasound guided follicular aspiration of bovine oocytes. Theriogenology. 1991; 35:19.
11. Chaubal SA, Molina JA, Ohlrichs CL, Ferre LB, Faber DC, Bols PEJ, *et al.* Comparison of different transvaginal ovum pick up protocols to optimized oocyte retrieval and embryo production over a 10week period in cows. Theriogenology. 2006; 65:631-1648.
12. Li F, Chen X, Pi W, Liu C, Shi Z. Collection of oocytes through transvaginal ovum pick-up for in vitro embryo production in Nanyang yellow cattle. Reproductive Domestic Animal. 2007; 42:666-670.
13. Adams GP, Matteri RL, Kastelic JP, Ko JCH, Ginther OJ. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. Journal of Reproductive Fertility. 1994; 94:177-188.
14. Imai K, Tagawa M, Yoshioka H, Matoba S, Narita M, Inaba Y. *et al.* The efficiency of embryo production by ovum pick-up and *in vitro* fertilization in cattle. 2006; 52:19-29.
15. Viana JHM, Camargo LSA, Ferreira AM, Sa WF, Fernandes CAC, Junior APM. Short intervals between ultrasonographically guided follicle aspiration improve oocytes quality but do not prevent establishment of dominant follicles in the Gir breed (*Bos indicus*) of cattle. Animal Reproduction and Science. 2004; 84:1-12.
16. Goodhand KL, Watt RG, Staines ME, Hutchinson JSM, Broadbent PJ. *In vivo* oocyte recovery and *in vitro* embryo production from bovine donors aspirated at different frequencies or following FSH treatment. Theriogenology. 1999; 51:951-961.
17. Viana JHM, Ferreira AM, Sa WFA, Camargo LSA. Follicular dynamics in Zebu cattle. Psequisa Agropecuaria Brasileira. 2000; 35:2501-2509.