NOTE *Theriogenology*

Transvaginal Follicle Aspiration in Thai Swamp Buffalo Heifers Using Different Vacuum Pressures after FSH Pretreatment (*Bubalus bubalis*)

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ABSTRACT. The objective of the experiment was to study oocyte recovery by transvaginal, ultrasound-guided, follicle aspiration, from Thai swamp buffalo using different vacuum pressures. Six adult buffalo heifers, aged 2.5–3.0 yrs were treated with a total dose of 280 mg FSH, given twice a day in a divided doses over a three day period (60/60 mg, 50/50 mg, 30/30 mg) at d7 after progesterone implant. Three vacuum pressures were used; 100 (n=12), 80 (n=12) and 60 mmHg (n=12) and all of the pressures were performed in each anima l. The animals were treated repeatedly and collection took place using 2 sets of each pressure every 2 months, giving a total of 36 collections from each animal. The oocyte recovery rates from each pressure were 81.2% (69/85) 79.1% (53/67) and 90.3% (93/103) for 100, 80 and 60 mmHg respectively. The number of oocytes collected per donor were 5.33 ± 3.27, 4.42 ± 2.71 and 7.75 ± 4.31 respectively. The quality of the oocytes did not improved with the lower vacuum pressure. In conclusion, the application of FSH pret reatment improves the yield of oocytes from Thai, swamp buffalo heifers after gonadotropin treatment when using the vacuum pressures betw een 60–100 mmHg.

KEY WORDS: OPU, swamp buffalo, vacuum pressures.

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In swamp buffalo, the reproductive technology has not been studied as extensively as those of cattle and river buffalo. The limiting factors are poor *in vivo* embryo production [15] and a very low recovery rate of immature oocytes from slaughterhouse ovaries [1, 14] which make it difficult to apply the technology in this species. Transvaginal, ultrasound guided, oocyte retrieval (Ovum Pick Up, OPU) is a non invasive procedure for collecting oocytes from live animals. This technique, together with *in vitro* fertilization, could be the way to produce a large number of superior animals [5, 9]. Our earlier report [12] showed that it was possible to collect oocytes from swamp buffalo with a recovery rate of 63%, however, a large number of denuded oocytes and free zona, without cytoplasm, were found more than 50% of the recovered eggs. In cattle, it was shown that the vacuum pressure of OPU could play a role on both the recovery rate and the oocyte quality [2, 3], although no studies in swamp buffalo were performed. The objective of this experiment was to study oocyte recovery in swamp buffalo after FSH pretreatment and using different vacuum pressures.

Follicle aspiration by OPU was performed in 6 Thai swamp buffalo heifers (2.5–3.0 yrs old). The program of superstimulation by gonadotropin was a modification of our previous work in prepubertal buffaloes [16]. The animals were stimulated with a total dose of 280 mg Follicle Stimulating Hormone (FSH, Folltropin®, Vetaphaarm, Australia) after a progesterone implant (Crestar®, Intervet, The Netherlands). The treatment was started on d7 after the implant. A total of 6 injections was given twice a day (60/60 mg, 50/50 mg, 30/30 mg), intramuscularly, over a period of three days. An intramuscular injection of 100 micrograms of Gonadotropin Releasing Hormone (GnRH, Cystolerin®, Sanofi, France) was administered 24 hr after the last dose of FSH. The ovarian responses, in terms of the number of follicles and the different categories of follicles [16]; 2,<4 mmØ, 4-<6 mmØ, 6-<8 mmØ, 8-<10 mmØ and >10 mmØ, were noted during the investigation by the introduction of a transvaginal probe, before oocyte collection.

The oocytes were collected by an OPU technique, in a standing position, in a chute. The animals were kept without water and food for 24 hr. A sedative, Xylazine HCl (Rompun®, Korea; 5 mg/100 kg, IM) with an epidural injection of 1 m*l*, 2% Xylocaine HCl, given to prevent movement of rear part of the animal and to reduce pain during ovarian puncture. A convex, transvaginal, human probe transducer (5 MHz, Aloka SSD-550V, Japan) was introduced into the vagina after cleaning the perineal area with water and alcohol. The needle was attached to the transducer giving a length of 34.5 cm and a 17-g single lumen. The needle was withdrawn at intervals during aspiration and rinsed with a solution of lactated ringer supplement, containing 2% heparin. The oocytes from all >2 mmØ were aspirated by using a closed system of aspiration (Cook, Australia) and controlled by observation through a monitor. One hundred thousand IU penicillin-streptomycin was given IM, to prevent infection. Each buffalo was aspirated at weekly intervals for 3 consecutive weeks, according to their vacuum pressure group which was random arranged. The three vacuum pressures 100, 80 and 60 mmHg were compared. Collection with 3 different vacuum pressures were repeated for a second time after 2 months of convalescence, so in total six collections were made from each buffalo. All animals were raised in the university experimental station and fed

with concentrate and roughage according to our standard feeding program.

All aspirants were strained through a 0.45 μm filter which precluded the oocytes from passing. The oocytes were searched immediately and placed in TCM 199 2.5 mM Hepes (Gibco, U.S.A.), for classification, according to the degree of their investment with cumulus cells and the quality of the cytoplasm, into 5 groups as follows; cumulus-oocyte-complex (COC, >4 layers), single layered cumulus oocytes (S, 2–4 layers), denuded cumulus oocytes (D), expanded cumulus oocytes (EXP), degenerated oocytes (DEG) and free zona without cytoplasm (FZ). The means and ± SD of follicles and oocytes were compared between the different vacuum pressure groups, by analysis of variance (ANOVA). The different types of oocytes were calculated in percentage terms.

After 36 treatments, the buffaloes responded with an average number of follicles per animal of 7.42 ± 3.65 (n=267) being 7.42 ± 4.21(n=89) in the 100 mmHg group, 5.83 ± 2.08 (n=70) in the 80 mmHg group and 9.0 ± 3.86 (n=108) in the 60 mmHg group (Table 1). Ninety five point five percent (255/267) of the follicles, 7.08 ± 2.81 per animal were aspirated and were 7.08 ± 4.18(n=85), 5.58 ± 2.19 (n=67), 8.58 ± 4.33 (n=103) in the respective groups. There were no differences in the number of follicles recovered between the first and second rounds of stimulation. The responses of right and left ovaries also showed no differences. There were 27% of follicles 2-<4 mmØ, 54.7% were 4-<6 mmØ, 1.9% were 8-<10 mmØ while only 0.3% were in >10 mmØ.

The average number of recovered oocytes per animal was 5.97 ± 3.95 (n=215) with a recovery rate of 84.3% (215/255) and a variation of 0–100% (Table 1). The average number of oocytes per animal in the 100, 80 and 60 mmHg groups was 5.33 ± 3.27 (n=69), 4.42 ± 2.71 (n=53) and 7.75 ± 4.31 (n=93) respectively. The three vacuum pressures used showed no differences as to the type of oocytes recovered. More than 50% of recovered oocytes were in the denuded, degenerated and free zona group (Fig. 1).

The results showed that OPU can be performed successfully and repeatedly on swamp buffalo heifers after pretreatment with FSH. The gonadotropin treatment was necessary for OPU in swamp buffalo because their ovaries was small [8] and possess few follicles, averaging only 1–2 follicles of 2–3 mmØ in size (unpublished data). In this study, hormonal treatment increased size of the follicles to an average of 7.0 and more than 80% of them were 2 to >6mmØ and were suitable for collection. It is possible that the supplementation of FSH by GnRH as used in this program helped to increase the number of medium-sized follicles and accords with our previous observations in prepubertal swamp buffalo calves and heifers [12, 16]. It is suggested that the size of the follicles influenced the oocyte recovery rate [11, 13]. They increased by 13% for 3–5 mm follicles to 35, 31 and 66% for 5–10, 10–15 and 15 mmØ follicles respectively. However the large-sized follicles were not required for OPU because they can easily be ruptured at the time of ovarian manipulation and contained a more viscous follicular fluid. The matured oocytes with expanded cumulus and only granulosa cell masses were commonly found in our observation.

When comparing the three vacuum pressures, it seemed that a decrease in aspiration pressure slightly increased the recovery rate but not the oocyte quality. This is the first time this has been reported in swamp buffalo. It is noted that more bovine oocytes were recovered from slaughterhouse ovaries at the high vacuum pressure, 110 to 130 mmHg [2, 3] and this is contrary to our result. In cattle, OPU had been performed in various studies, reviewed by Bols *et al.* [2]. Different vacuum pressures, from 20–40 mmHg to 100–400 mmHg, were used in OPU and contrary to buffalo, a low pressure, 40 mmHg was suggested [4, 10]. The vacuum pressure for OPU in buffalo may different from that of cattle because the swamp buffalo ovary is smaller [8], compared to those of cattle and riverine buffalo [14]. In addition, optimal aspiration conditions for the ovaries of slaughtered animals are not necessarily applicable in live ones [6]. However, according to Fig. 1, it suggests that the

Table 1. The mean number of follicles, aspirated follicles and oocytes recovered by OPU in swamp buffalo heifers, using three different vacuum pressures, 100, 80 and 60 mmHg

Vacuum pressure (mmHg)	No. of buffaloes	No. of aspiration sessions	No. of produced follicles	No. of aspirated follicles (n)	No. of oocytes (n)	% recovery rate (n)
100 mmHg	6	12	7.42 ± 4.21 (n=89)	7.08 ± 4.18 (n=85)	5.33 ± 3.27 (n=69)	81.2[a)] (69/85)
80 mmHg	6	12	5.83 ± 2.08 (n=70)	5.58 ± 2.19 (n=67)	4.42 ± 2.71 (n=53)	79.10[a)] (53/67)
60 mmHg	6	12	9.0 ± 3.86 (n=108)	8.58 ± 4.33 (n=103)	7.75 ± 4.31 (n=93)	90.1[b)] (93/103)
$\overline{X}$+SD total		36	7.42 ± 3.65 (267)	7.08 ± 2.81 (255) [95.5%]	5.97 ± 3.95 (215)	84.3 (215/255)

Value presented in $\overline{X}$ ± SD, n=number a)b); P<0.05.
[ ] aspiration rate.

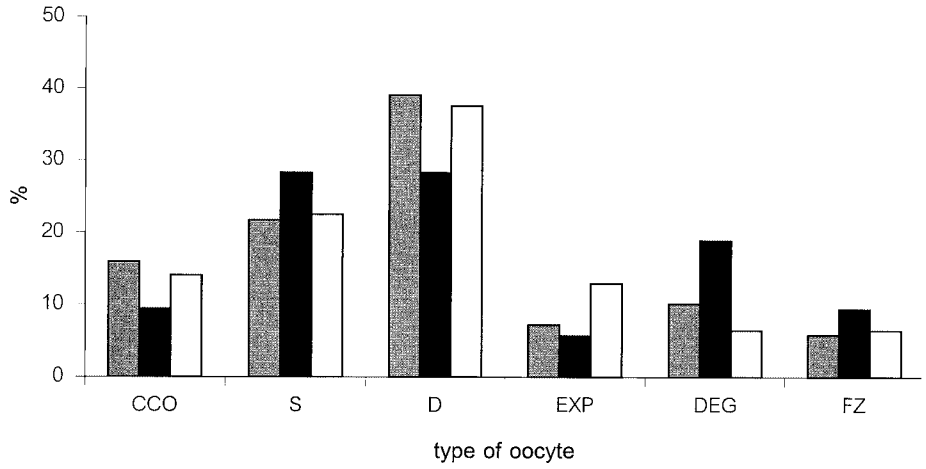

Fig. 1. The type of oocytes recovered by OPU using 100 (grey bars), 80 (black bars) and 60 (white bars) mmHg vacuum pressures.

ovarian response of the 60 mmHg group was the best, due to the high percentage of medium-sized follicles. Thus there is the possibility that the highest recovery rate for oocytes in the 60 mmHg group may be strongly affected by the FSH pretreatment, making it difficult to conclude that oocyte recovery rate is improved by the lower vacuum pressure. The proportion of good oocytes surrounded with a cumulus mass decreased progressively when the vacuum pressure increased [2, 3]. In the study the number of good oocytes with a cumulus mass did not improve as a result of the vacuum pressures used. About 50% of oocytes are of poor quality; denuded, degenerated and free zona. Boni and his colleagues [4] reported that poor quality oocytes was twice as numerous in buffalo than in cattle. The quality of the oocytes in our study was distributed in a similar way to previous observations on swamp buffalo [7, 12] and river buffalo [4]. Furthermore, it is not only vacuum pressure that affects the oocyte quality but it is also related to the needle diameter, the needle tip bevel, the length of needle or combination of all these factors [2]. In conclusion, the application of FSH pretreatment can improve the yield of oocytes in Thai swamp buffalo. Optimizing the techniques for ovum pick up in swamp buffalo required further studies in order to improve the oocyte quality which vital used in in vitro embryo production. In the study the developmental competence of recovered oocytes for maturation and fertilization was not undertaken, future research on this aspect is under investigation.

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