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SUPEROVULATORY RESPONSE AND EMBRYO RECOVERY IN BUFFALO HEIFERS AND COWS TREATED WITH FSH (SUPER-OV) AND LH

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SUMMARY

Superovulation and nonsurgical embryo collection were tried on 5 heifers and 16 buffalo cows. These animals were at mid luteal phase before treatment. Purified porcine FSH (Super-Ov) was administered intramuscular, morning and evening for 3 consecutive days. $PGF_{2\alpha}$ (Lutalyse) 25mg was injected with the fifth Super-Ov injection. Chorionic gonadotropin (2000 USP unit) was injected in heifers and 10 of the cows during oestrus. The other six buffalo cows were considered as control without LH supplement. The onset and duration of oestrus were detected using buffalo bulls which frequently mated the females. Corpora lutea (CL) and unovulated follicles (UF) were counted rectally before non-surgical embryo collection. Oestrus started earlier and lasted longer in heifers than in cows and the differences were significant. The numbers of CL were significantly lower in heifers while the numbers of UF were higher. The ovulation rates in heifers and cows were 36.6 ± 12.8 % and 65.2 ± 8.4% respectively.

In control buffalo cows, significant increase in the number of UF has been concomitant with significant reduction in the ovulation rate as compared to LH injected-cows ($P<0.05$). Uterine flushings from 10 and 6 animals revealed in the recovery of 12 and 6 embryos with a recovery rate of 27.9% and 23.8% in buffalo cows with and without LH respectively. The used protocol appeared satisfactory in buffalo cows rather than buffalo heifers and addition of LH improved ovarian response and embryo recovery .

INTRODUCTION

Recent application of biotechnology in animal reproduction is a key in the generation of an adequate food supply to cope with the dramatic increase of world human population especially in developing countries.

In cattle, superovulation and embryo transfer have been improved year by year using genetically superior animals. In many developed countries, animal production incorporating such biotechniques has become a big business (Bahr et al., 1998).

Although superovulation and embryo transfer techniques had been fairly well refined in cattle, this process still lags far behind in buffalo. A limited number of successful attempts have been reported in the last decade with regard to superovulation and embryo transfer in buffalo (Drost et al., 1983,1985; Karaivanov 1986; Ismail 1991; Misra 1993 ;Taneja et al., 1995 and Misra et al., 1999).

In buffalo, the response of the ovaries for superovulation using FSH or PMSG is quite variable and much lower than in cattle. A low rate of response up to 55.5% was recorded in buffalo by Vlakhov et al.(1986) versus 90.3% reported by Megahed (1991) in Friesian breed after FSH treatment.

In cattle, Bouters et al. (1978) found an average of 9.4 corpora lutea per individual cow after superovulation with a range from 0-42. Ismail (1991) reported a lower value of 5.5 ± 1.28 corpora lutea in non-lactating buffalo cows superovulated with FSH. In swamp buffalo, Songsasen et al.(1999)reported similar results.

Armstrong et al. (1983) cited that the superovulatory response after FSH administration was higher than after PMSG treatment. Moreover, although the number of corpora lutea was often similar in FSH- and PMSG- treated buffaloes, the recovery of embryos after flushing often favored FSH (Alvarez et al., 1990 and Taneja et al., 1990).

Zicarelli et al. (1997) observed a high rate of ovarian cysts (50%) or unovulatory follicles following superovulatory treatment with 1000 IU porcine gonadotropin in buffaloes. Misra (1993) cited in his review that the most effective superovulation treatment in buffalo involved multiple doses of porcine FSH. The effect of hCG given at oestrus on the ovulation rate and embryo production together with 3000 IU of PyMSG (FSH) at the mid luteal stage of the cycle is reported by Ismail et al. (1993). They found that the embryo recovery percentage was higher after hCG treatment. The results published by Kafi and McGowan (1997) in cattle and Manik et al. (1999) in buffalo indicated that variability in the superovulatory response is still one of the major limiting factors in the extensive usage of embryo transfer technology in buffalo.

The aim of the present work is to use a purified porcine FSH (Super-Ov) supplemented with a defined amount of chorionic gonadotropin (LH) to induce superovulation and to recover embryos in buffalo heifers and cows.

MATERIALS AND METHODS

From a local large governmental buffalo farm (El-Hawatka, Assiut) 5 heifers (2 years old) and 16 cows (3-5 years old) were selected for superovulation and nonsurgical embryo recovery. These animals were healthy, non pregnant, non lactating and cycling. After thorough gynecological examination, all animals were assigned to be at mid luteal phase before treatment paid the attention on which side the CL was present.

The feeding and management for all animals were the same during the period of study and according to the regular breeding system of the farm. Two or three buffaloes were selected at each trial (total 10 trials during Spring) and were subjected for the hormone treatment as follow:-

A dose of 1.6/ml Super-Ov contained 12.5 NIH purified porcine FSH (Mfd. in Canada by W.A.Montreal Inc) was injected i.m. morning and evening for 3 consecutive days.

At the morning of the 3ed day 5-ml lutalyse contained 25 mg $PGF_{2\alpha}$ /was injected at the time of the 5th Super-Ov injection according to the manufactures instructions of the Drug Company.

In addition to this and aiming to improve rate of ovulation and embryo recovery, 2 ml chorionic gonadotropin (Steris Laboratories INC) contained 2000 USP units was injected i.m. at the morning of the 5th day, during oestrus, in all buffalo heifers and in 10 of the buffalo cows. The rest six buffalo cows were considered as control group without LH supplement.

The superovulated buffaloes in each trial were kept untied in open yard and 2-3 fertile buffalo bulls were paraded for natural mating when required and at least 2 or more matings were performed for each female during oestrus.

Moreover, the onset and duration of heat were recorded by 2 herdsmen who are expert in oestrus detection and closely observed the animals 3-4 times for 1-2 hours per day and at least one time during the night, just after the last Super-ov injection. With regard to the known symptoms of oestrus, some buffaloes showed in addition, a lateral side to side head movements beside raising of the tail root and exhibiting some muscle contractions around the vulva and perineum on touching or manipulating the external genitalia.

At the 11th day from the beginning of hormone treatment, rectal palpation was performed to count the numbers of developed corpora lutea and unovulated follicles when present. Clinically, the CL felt firm and protruded from the ovarian surface while the UF felt as a raised tense blister.

Moreover, non-surgical uterine flushing was don for each buffalo using phosphate buffer saline (PBS) media to collect and identify the numbers of day 7 embryos in the lab (Megahed, 1991).

The statistical analysis of the obtained results were carried out after PC-Stat computer program.

RESULTS

It is evident from table 1 that the onset of oestrus began earlier in buffalo heifers than cows with or without LH supplements and the difference was significant (P<0.05). The duration of oestrus was significantly longer in buffalo heifers than other cows (P<0.05). The number of CL was significantly higher in cows with LH (P<0.01) than heifers while the number of unovulated follicles was significantly higher in heifers (P<0.05) than in cows with LH.

In buffalo cows without LH, the number of CL was lower but not at a significant level from the other cows but the number of UF was significantly higher (P<0.05). Moreover, the ovulation rates showed significant differences between groups (p<0.05) and the highest value was recorded for cows with LH additives .The ovulation rates were calculated by dividing the number of CL by the number CL plus the number of UF.

Significant correlation was present between onset of heat and each of numbers of unovulated follicles (r = 0.9177) and duration of heat (r = 0.6844) within all individuals.

The obtained results revealed no significant difference in the response of the right and left ovaries to superovulation treatment although the total number of developed CL was higher in the right ovaries (32 versus 22 CL).In addition, ovaries carried a cyclic CL before treatment responded higher than the other static ovaries after hormone injection (30 versus 24 CL). It might be of interest to mention that all of the selected buffaloes were responded to the performed superovulated scheme of hormonal treatment with or without LH but in variable degrees. A range from 2-6 corpora lutea and 1-7 unovulated follicles were developed after treatment.

As far as embryo collection is concerned, uterine flushing failed in two heifers due to narrow cervix. The rest of flushing revealed a total of 12 and 6 compact transferable morulae with a recovery rate of 27.9% and 23.8% from cows with and without LH respectively. The embryo recovery rate was calculated by dividing the number of recovered embryos by the number of developed CL in both ovaries.

Table1: Oestrus behaviour, ovarian response and embryo recovery in superovulated buffalo heifers an cows.

Buffaloes	N	Onset of oestrus after last Super-ov injection (hr)	Duration of oestrus (hr)	Ovarian response Total		Ovulation Rate (%)	Recovered day 7 embryos	Embryo recovery Rate (%)
				CL	U.F.			
Heifers Treated With LH	5•	16.0 ± 1.4* (14-16)	39.2 ± 8.1* (30-52)	2.7 ± 0.6* (2-3)	4.4 ± 1.6* (3-7)	36.6 ± 12.8* (22-50)	0.0	0.0
Cows Treated With LH	10	24.7 ± 9.9* (12-40)	30.2 ± 3.7* 24-36	4.2 ± 1.0** (3-6)	2.3 ± 0.7* (1-3)	65.2 ± 8.4* (50.75)	1.3 ± 0.7 (0-2)	27.9 ± 20.5 (0-66)
Control Cows	6	22.3 ± 5.2* (15-28)	31.2 ± 5.5* 23-36)	3.8 ± 1.2 (2-5)	3.5 ± 1.1* (2-5)	52.3 ± 8.9* (40.66.7)	1.0 ± 0.6 (0-2)	23.8 ± 13.6 (0-40)

Mean ± St. Dev. ( ) = Range hr= hour

CL = Corpus Luteum U.F. = Unovulated Follicle

• = Two heifers failed to be flushed

* = Significant $P < 0.05$

** = Significant $P < 0.01$

DISCUSSION

The significant differences between buffalo heifers and cows with or without LH supplement especially with regard to numbers of developed corpora lutea and unovulated follicles as well as the onset and duration of heat might reflect the high sensitivity of heifers to the dose of Super-Ov recommended by the company. In this respect, Riha and Frelich (1997) administered lower dose of pFSH in beef heifers (400.I.U.) than in cows (560 I.U.) to induce superovulation. Contrary to these findings, Desaulniers et al. (1995) reported poor response to superovulation in mature cows of high genetic potential (7.8 ± 2.8 ovulated follicles) when compared to heifers less than 2 years old (15.2 ± 2 ovulated follicles).

The positive significant correlation between duration of heat and number of unovulated follicles in buffaloes reflects the activity of such developed follicle to produce estrogen. Moreover, the significant correlation between onset of heat and numbers of UF and duration of heat denoted that earl appearance of oestrus with prolonged duratio was usually accompanied with larger number (

UF in both ovaries. However, the prolonged duration of heat in the studied buffalo heifers (30 to 52 hours) is less than 48-120 hours reported by Singh and Madan (1998) who used PMSG for superovulation. Thus, to recommend a fixed time for insemination in superovulated buffalo heifers appeared unpractical and such females should be kept freely with buffalo bulls till the end of oestrus to get successful fertilization rate and lower dose of Super-Ov may improve the results.

The careful selection of buffaloes to be at mid luteal phase before treatment appeared responsible for the positive response of all individuals for superovulation. A range from 2-5 developed corpora lutea and 1-7 unovulated follicles were recorded. Zicarelli et al. (1997) cited that superovulation usually considered positive when the buffalo yielding more than one CL after treatment. Karaivanov (1986) reported 78.9% and 89.5% response rate for lactating buffalo cows superovulated with FSH and PMSG respectively. Ismail et al. (1992) reported a response rate of 75-87.5% in non lacting buffaloes treated with different doses of FSH.

As far as the role of LH in improving the rate of response of the studied buffalo cows, Donaldson and Ward (1986) and Yamamoto et al. (1993)concluded that the luteinising content of the FSH has to be controlled and limited to improve the proportion of transferable embryos since high LH decreased rate of fertilization and increased rate of embryo degeneration. Sugie et al. (1980) recommended 1500-3000 IU LH to assure ovulation in cow superovulated with FSH. In the present study, the administration of LH during oestrus significantly favor the ovulation rate in buffalo cows with certain improvements in the numbers of CL and recovered embryos.

Uoc et al. (1997) cited that the supplementation of hCG treatment improved the level of ovarian stimulation in swamp buffaloes superovlated with eCG. Within the scope of this topic, Ismail et al. (1993) reported about triple fold increase in the embryo recovery percentage in buffalo cows superovulated with PMSG after the addition of hCG. It seems possible that the amount of LH used in this work (2000 USP units) might be suitable for the higher response of buffalo cows for superovulation treatment.

In the present trial, the number of developed CL averaged 2.4 ± 0.55 and 4.2 ± 1.03 in buffalo heifers and cows respectively. These values are within the scope of 4.3 ± 0.87, 5.13 ± 1.49 and 2.8 ± 0.7 as published by Karaivanov (1986), Ismail et al. (1991) and Taneja et al. (1995) respectively. However, the ovarian response of buffalo to superovulation with FSH although variable was much lower than the response obtained in cattle by Donaldson (1985), Guilbault et al. (1991), Reinhard (1992) and Riha and Frelich (1997) especially with regard to the number of ovulation and CL formation. This might be attributed to the inherent small sized ovaries in buffaloes with a consequent low follicular oocyte population and endocrine diversity before puberty. Osman (1984) cited that in buffaloes the ovaries are much smaller in size than cattle and an average weight of 4.25 ± 0.29 g was recorded during oestrus in slaughtered buffalo cows. According to the evidence of Erickson (1966) in cattle, the number of germ cells in the fetal ovaries reached 15000 by the time of birth in heifer calf. This number is expected to be dramatically reduced in buffaloes with a consequent lower capacity for producing follicles after puberty. Within the scope of this respect Le Van Ty (1989) found that the overall population of antral follocles in buffaloes was only 20% of that of cows (47.5 ±23.8 vs 233.0 ± 95.8) .

The ovulation rates reported for buffalo heifers and cows are comparable to those recorded by Sing and Madan (1998) in buffalo heifers (63%)and Ismail(1991) in two groups of buffalo cows (66.6% and 42.11 %).

The prolongation of oestrus in certain buffalo heifers might reflect the transfer of some unovulated follicles to cyst. Zicarelli et al. (1997) observed a high rate of ovarian cysts in superovulated buffaloes.

The obtained recovery rate for buffalo embryos after uterine flushing (27.9%) is nearly similar to 30.77 % reported by Ismail et al. (1991). Higher and Lower values (31% and 23.08%) were reported by the same authors as well as Drost et al. (1988) in buffalo. In cattle, however, Riha and Frelich (1997) recorded variable recovery rates up to 100% which is much higher than those reported in buffaloes.

The fertilization rate in the present work reached 100% since unfertilized ova could not be recovered .The obtained fertilization rate is nearly equal to 97.6% as reported by Drost et al. (1988) in the same species.

As a major conclusion from the present investigation it seems possible that administration of LH with Super-Ov might be responsibie for the relatively high ovarian response to superovulation in buffalo cows with maximum rate of fertilization.

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