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Successful Embryo Transfer in Egyptian Buffaloes

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Abstract: Embryo transfer technique has been widely used worldwide, whereas it can increase the number of offspring that can be obtained from supergenetic females. The present work was conducted to compare between mature buffalo cows and heifers in superovulatory response, embryo recovery and calving rates. In 11 buffalo cows and 3 heifers, estrous synchronization was performed using 2 doses of $PGF_{2\alpha}$ 11 days apart. Superovulation regime was done by injection of 400 mg porcine follicle stimulating hormone (FSH-p, Foltropin-V) on 8 fixed doses (50 mg FSH-p/dose). $PGF_{2\alpha}$ was injected at the time of the 6th and 7th FSH doses for induction of estrus. Buffaloes were monitored for estrus using a teasing bull and superovulation response was detected on day 6 post estrus using ultrasonography. In recipient animals, estrous synchronization in buffalo heifers and cows was done by injection of 2 doses $PGF_{2\alpha}$. Embryo recovery was performed on day 6 using non surgical method; and fresh embryos (one or two embryos) were transferred to buffalo heifers and cows recipients (came in oestrus±12hrs to donor oestrus) in ipsilateral horn. Detection of pregnancy was performed on day 40 post embryo transfer using ultrasonography. Results indicated that buffalo heifers came in estrus earlier and possesses higher ($P<0.01$) ovulation rate than buffalo cows (78.3 vs.62.7%). Embryo recovery rate was ($P<0.01$) higher in buffalo cows (52%) than heifers (0%). After embryo transfer of fresh embryos, pregnancy rate and percentage of animals with full term pregnancy were higher ($P<0.01$) in buffalo cows (3/5 (60%) and 60 %, respectively) than heifers (2/4 (50%) and 0% respectively). In conclusion, applications of non- surgical embryo recovery and transfer were successful for 1st time in Egyptian water buffaloes and mature buffalo cows give higher recovery and pregnancy rales more than buffalo heifers.

Key words: Egyptian water buffalo · Heifers and cows · Superovualtion · Ovulation rate · Embryos recovery · Embryo transfer

INTRODUCTION

The technology of embryo transfer as a potential method for buffalo genetic improvement is consequently limited, although the technique has been successfully applied in buffaloes [1], subsequent, in Bulgaria [2], India [3] and Thailand [4]. Superovulation and embryo transfer technology used for genetic improvement are capitalizing on the productive potentials of superior animals at a given age, therefore allowing increased selection intensity among females to obtain the desired donor animals and reduction of the generation intervals. Moreover, embryo quality is very important in embryo transfer programs, because it reflects directly upon pregnancy outcome after

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embryo transfer [5]. The rate of success of multiple ovulation embryo transfer (MOET) is dependent on many factors such as the accuracy of the selection of donor animals. The use of heifer's as a donor in embryo transfer programs presents significant opportunity to accelerate genetic gain in domestic livestock by reducing the generation interval. However, buffaloes were reported to have poor response to superovulation treatments [6]. This is due to, the number of primordial follicles in river buffaloes [7] is lower (30%) than that described in cattle [8]. Follicle stimulating hormone (FSH-p) has been reported to be much lower in buffaloes than in cattle [9-12]. Decreasing of the follicular reserve is involved in age -related reproductive disorders in cattle [13]. The decline in superovulatory response may be due to a reduction in the numbers of follicles capable of responding to gonadotrophin treatment in older cattle [14]. The low responding mature cow had lower LH pulse amplitude, lower estrogen (E_2) and FSH concentrations than heifers [15]. The objectives of this work were: (1) application of embryo transfer in Egyptian water buffalo and; (2) To compare the superovulation response, embryo recovery and calving rate between buffalo's cow and heifer.

MATERIALS AND METHODS

Animal Husbandry: The present work was conducted between January and April 2004 and 2005 on twenty eight female Egyptian buffalos (13 donors and 15 recipients displayed at least one estrous cycle of normal duration (20-25 days). These buffaloes were fed according to the Standard Ration regime for heifers or lactating buffaloes prepared according to the routine management system at Trust Farm, Alexandria.

Synchronization and Estrous Detection: For estrous synchronization, all buffaloes were injected twice with 25 mg Prosolvine i.m. ($PGF_{2\alpha}$ analogue, Intervet and Netherlands) with 11 days apart. Frequent daily observation of estrus after second injection were made to detect external signs of estrus, the response to placing of the hand on the rump and slight vulva massage and confirmed by using teaser buffalo bull [16].

Super Ovulation Region on Donor Buffalo Heifers and Cows: On day 10 of estrous cycle (estrus = D0) super ovulation was performed on 10 buffalo cows (aged 4 to 5 year, with body weight range of 450-600 kg and milk production range of 12-20 Kg/ day) and 3 buffalo heifers (aged 2 years old) and with body weight range of 300-350 kg, using porcine follicle stimulating hormone (Foltropin-V, Bioniche, Canada) in dose of 400 mg/animal, administered in constant doses twice daily for 4 days (50 mg/dose), at the 6^{th} and 7^{th} injection of FSH-P, donors were injected with 25 mg $PGF_2\alpha$ analogue for induction of estrus. Animals were treated with 1500 IU i.m. (Pregnyl, Nile Comp., Egypt) after the end of estrus to assess ovulation. Two buffalo bulls were introduced to the donors at the time of 2^{nd} dose of $PGF_{2\alpha}$ injection for natural insemination and for detection of the onset and duration of estrus.

Recipient Preparation: The recipient buffaloes (10 cows and 13 heifers) were selected based on their health conditions, normal reproductive tract judged by rectal palpation and that showed clear estrus (6 cows and 9 heifers). were selected for estrous synchronization, recipients were injected with 25 mg Prosolven ($PGF_{2\alpha}$) i.m 12 h earlier than donors (at the time of the 5^{th} and 6^{th} FSH-p injection in donor). Monitoring of estrus was performed by using a teaser bull every 4 hours to detect the onset and duration of estrus.

Determination of Superovulation Response: Ovaries were monitored at days 5and 6 after estrus (day 0) by ultrasound scanning machine (Pie Medical, Maastricht, the Netherlands) with7.5 MHz probe for detection the number of corpora lutea and Graafian follicles.

Non-Surgical Embryo Recovery: Epidural anesthesia was induced with 5 ml 2% procaine HCl and 1.5 ml Zylaject (Adwia Co. Egypt) i.m. to calm the animals and avoid strains during flushing. Non-surgical embryo recovery was performed on days 5 and 6 of estrus (day 0) using a two way Folly catheter (Minitüb, Germany). Dulbacco's phosphate buffered saline (DPBS) with 1% bovine serum albumin (BSA) and 100 mg/liter streptomycin sulfas and 100,000 IU/liter penicillin G, 500 ml flushing medium was used as flushing for each uterine horn. The medium, going out of the uterus through the catheter, passes directly through a special plastic embryo filter with pore size of 75 microns.

Embryo Evaluation: The search for embryos is done under a stereomicroscope. Recovered embryos were evaluated morphologically selected [17], the embryo considered excellent or good with no or few extraneous degenerated cells. Calculation of recovery rate (the number of embryos were recovered/ number of C.L were

detected by ultrasound) were carried out. Excellent and good morula or blastocysts were transferred (one or two embryos) to each recipients buffalo.

Embryo Transfer Technique: The fresh excellent and good embryos were transferred non-surgically into synchronized recipients on day 5 or 6 (estrus = day 0) post estrus (recipients that came in estrus at ± 12 hr of donor's estrus were selected (5/6 cows and 4/9 heifers) for embryo transfer). Epidural anesthesia (5 ml of 2% Procain HCl) was injected 10 min before embryo transfer to prevent defecation and to minimize straining and uterine contractions during embryo transfer. The embryos were inserted in 0.25 ml French straw between two air pockets and two columns of TCM-199 with 10% fetal calf serum. The straw was inserted into a special embryo transfer (ET) gun fixed at the end with golden screw round tip, then, it was cleaned by water and sterilized by alcohol to avoid contamination during placing into the vagina. The tip of the ET gun was placed into the external os of the cervix and pushed through it before going into the uterus. The embryo was deposited into the ipsi-lateral uterine horn approximately 5 cm from the uterine bi-forcation and then the gun was slowly withdrawn.

Pregnancy Diagnosis: Pregnancy was diagnosed in recipients after 40 days of embryo transfer by using ultrasound scanning equipment with 7.5 MHZ probe.

Statistical Analysis: Data were statistically analyzed using Chi square analysis according to Snedcor and Cochran [18].

RESULTS

The response of buffalo heifers and cows to superovulation regime is presented in Table 1. Data revealed that in both donor and recipient animals, higher ($P<0.05$) percentage of buffalo heifers (100, 69.7 %, respectively) exhibited estrous symptoms than cows (90.9, 60.0 %, respectively). Time to estrus and the duration of estrus did not significantly differ between the two groups.

The ovarian response of buffalo heifers and cows to superovuation regime as detected by ultrasound is presented in Table 2 and Fig. 1, Ovulation rate was significantly ($P< 0.01$) higher in heifers compared to cows (78.3 and 62.7 %, respectively).

Embryo Recovery Rate: Table 3, Fig. 2 represent the embryo recovery and pregnancy rates of buffalo heifers and cows subjected to superovulation and embryos transfer regimes. 13 excellent and good embryos (morula and blastocyst) with 73% viability were recovered and the average (no. of embryo/donor) was 1.62±0.12, were significantly ($P<0.01$) higher in buffalo cows than heifers, this could in part be due to the inability to perform uterine

Table 1: Estrous manifestation in donors and recipient buffaloes following PGF2α injection

Group	Type	Time to estrus/h	No. buffalo showing estrus after hormonal treatment (%)	Duration of estrus/h
Donor	Superovulated Cow	38.7±1.7	10/11 (90.9)^{b}	18-24
	Superovulated Heifer	36.0±0.0	3/3 (100)^{a}	18-24
Recipient	Cow	48.3±1.8	6/10 (60)^{b}	18-24
	Heifer	46.7±1.3	9/13 (69.7)^{a}	18-24

a,b $P<0.05$ a,c $P<0.01$

Table 2: Ovarian response in superovulation in buffalo heifers or mature cows

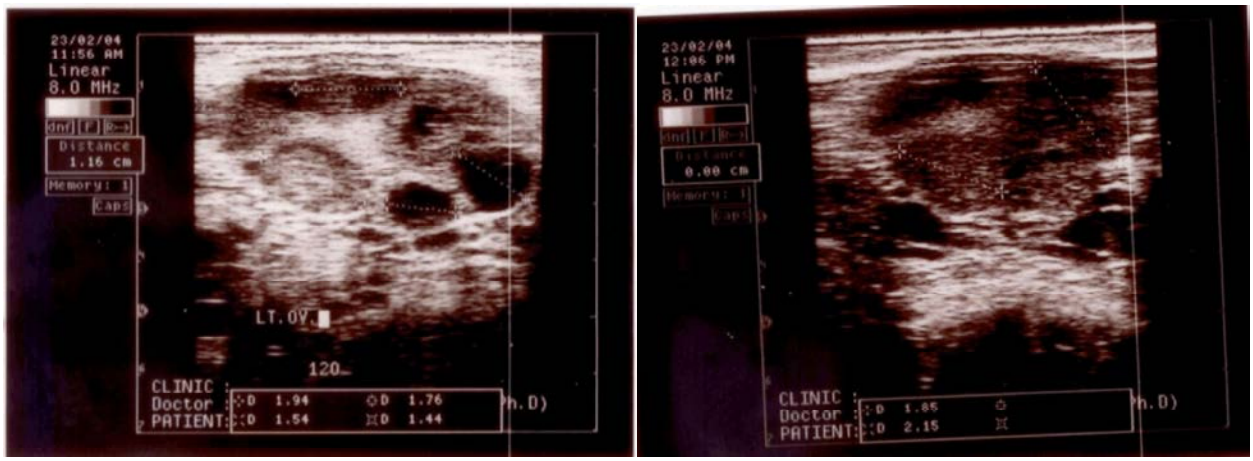
	Donor	
Item	Cow	Heifer
Ovarian response		
No CL	1	-
1 CL	2	1
> 1 CL	8	2
Ovarian response mean±SE using Ultrasonography CL		
Graafian follicles	4.7±1.1	6.0±2.9
	2.8±0.6	1.7± 0.9
Ovulation rate	52/83 (62.7)	18/23 (78.3)**

** $P<0.01$

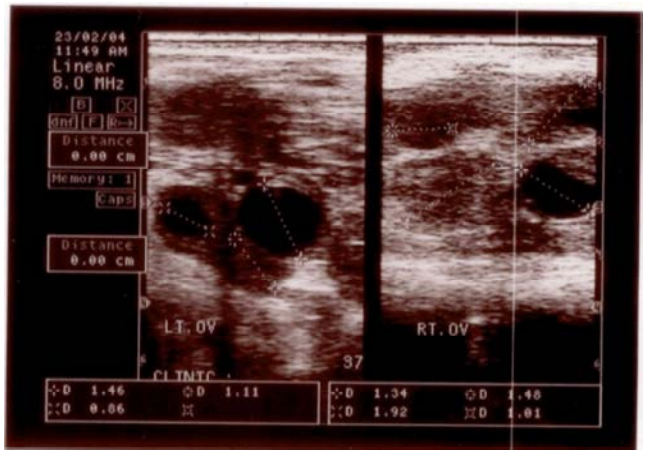
Table 3: Embryo transfer in Egyptian buffalo with emphasis on pregnancy rate and born calves

Item	Mature cows	Heifers
No. donors flushed	8/10 (80%)	0/3 (0%)
No. of embryo recovered (average ±SE)	13 (1.62±0.12)	0
Embryo recovery rate	52%[a]	0[c]
No. recipients responded to $PGF_{2\alpha}$ ± 12 hrs to donors	5/10 (50%)[a]	4/13 (30.77)[c]
Recipients received		
One embryo	3	2
Two embryos	2	2
No. pregnant buffaloes 40 days after embryos transfer	3/5 (60%)[a]	2/4 (50%)[b]
No. of animals with full term pregnancy	3/3 (100%)	0/2 (0%)
No. of calves born	3	0

* P<0.05 ** P<0.01



Ovarian response in Heifer (A):



Ovarian response in Cow (B):

Fig. 1: Ultrasonography scaninning for ovarian response in superovulated buffalo heifers (A) and cows (B)

flushing in heifers as the cervix is very narrow. The number of recipient buffalo cows (5/10) responding to $PGF_{2\alpha}$ within ± 12 hrs prior to donor's oestrus were significantly (P<0.01) higher than heifers (4/13). After embryos transfer pregnancy rate as detected by ultrasound (Fig. 3) on day 40 of pregnancy was higher (P<0.05) in buffalo cows than heifers (60% vs. 50% in cows and heifers, respectively). Moreover, the percentage of buffalo cows with full term pregnancy was higher (P<0.01) than in heifers (100, 0% for cows and heifers, respectively), as the 2 buffalo heifers were aborted after the 3rd month of pregnancy. The 3 buffalo cows have delivered two males and one female calves.

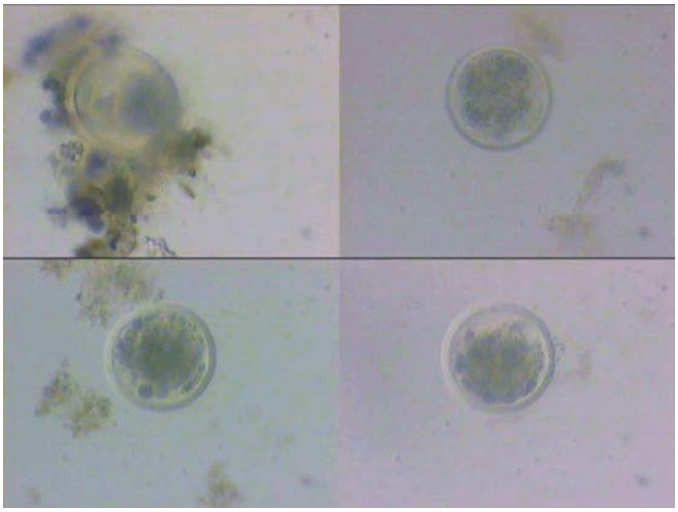


Fig. 2: Non-Surgical Embryos recovered from buffalo cow with viability 73%

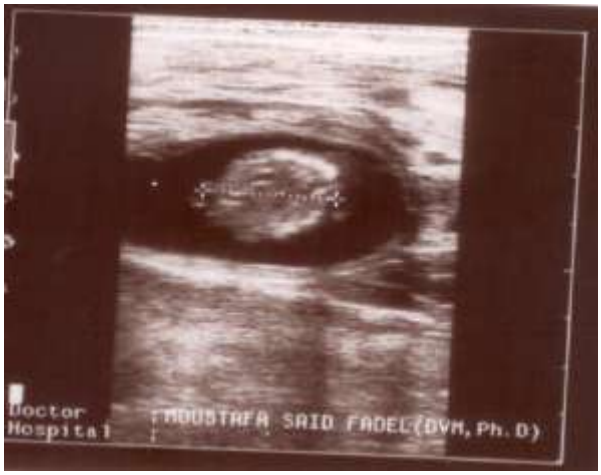


Fig. 3: Pregnancy diagnosis in recipient buffalo after 40 days of embryo transfer using ultrasonography

Fig. 4: 1st Egyptian buffalo calf born through non surgical embryo transfer with 40 kg weight

DISCUSSION

The embryo transfer technique has been widely used worldwide because it increases the number of offspring that can be obtained from females with great genetic value [19, 20]. However, the progress in the field of embryo transfer in water buffalo has been slow and this is primarily due to a poor response to superovulation treatments. Buffalo ovary has a smaller population of recruitable follicles at any given time than the ovary of the cow (89% fewer at birth). In addition, estrus detection is problematic in buffalo compared with cattle [21].

The present work illustrated that higher percentage of both donor and recipients buffalo heifers expressed estrous symptoms earlier than mature cows. Ovulation rate was higher ($P<0.01$) in heifers than cows. These results were comparable to that recorded by

de Silva *et al*. [22] who found that ovulation rate was higher in heifers than cows. The lower superovulatory response of lactating cows in relation to heifers was associated to significantly lower progesterone (P_4) concentrations during diestrus, either before or after the superovualtion (SOV) treatment. These differences in P_4 concentrations at diestrus can be attributed to differences in CL competence (unstimulated cycle) and to CL competence and ovulation rate (superovulation cycle). Other studies recorded that the fewer number of follicles in older cows compared with their daughters was consistent with the tendency for a lesser ovulation rate in older cows; on average, younger cows had eight more ovulations per cow than older cows. Also, the hypothesis that reproductive aging is associated with a reduced follicular and ovulatory response after gonadotropin treatment was supported [23]. The difference observed between heifers and lactating cows in P_4 concentrations, SOV response and embryo yield might be related to the nutritional and metabolic status of donors before or at the SOV treatment [24]. On the other hand Ideta *et al.* [25], found that the longer duration of estrus was associated with higher superovulatory response. This difference could be attributed to species difference. Furthermore, results indicated that the number of flushed donors and the number of embryos recovered per donor were significantly higher in buffalo cows than heifers, this could be in part due to the difficulty of embryo flushing in buffalo heifers, as the cervix is narrow and also the cervical folds makes the flushing more complicated in heifers than in cows. These results completely agree with that previously reported [22], it is a general impression that heifers present fewer problems in terms of response to superovulation (lower non-responding rate), but they produce fewer embryos than high-yielding dairy cows. Also, in the present work the number of transferable embryos is 1.62 embryo/donor. This result was higher than that recorded by Drost [21]. This difference could be due to difference in superovulation regime or breed difference. In cattle, a considerable variation in embryo production is found between donors with a range from 0 to approximately 35 transferable embryos per flushing [26, 27]. The recovered embryos range from four to seven [28, 29]. One reason for this lower yield was that 20-40% of the potential donors superovulated did not respond to the superovulatory treatment, i.e. produced from zero to three transferable embryos only [30, 31]. These figures seem to be more predominant in buffalo heifers than cows. On contrary, other studies have reported that the variation in embryo flushing is predominant in cow than heifers. This difference could be due to the flushing technique or age and breed difference [26, 27]. Furthermore, our data revealed that after transfer of fresh embryos to recipient buffalo cows and heifers, 3 out of 5 cows became pregnant as detected by ultrasonography 40 day later, they proceed to full term pregnancy and giving 3 healthy normal calves, on the other hand, 2 out of 4 heifers conceived and then aborted on the 3rd month of pregnancy. The overall pregnancy rates reported from the MOET schemes range from 50 to 70% [29, 30], depending whether fresh or frozen/thawed embryos were used and whether surgical or non-surgical transfers were applied. In two of the MOET schemes the pregnancy rates improved over time and more strict selection of recipients and better handling of embryos were reported as possible reasons [31, 32]. The low pregnancy rate in recipient heifers could be the uterus may be not developed to support the full term pregnancy or may be due to the weight of heifers which could not provide complete support to the fetus.

In conclusion, Application of non- surgical embryo recovery and transfer were successful for the first time in Egyptian water buffalo and mature cowss give more recovery and pregnancy rates than buffalo heifers.

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