The relationship between circulating anti-mullarian hormone (AMH) and superovulatory response in buffaloes

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1. Abstract

The current study aimed to investigate the effect of using the superovulatory drug 'follitropin' on the follicular population and number of harvested buffalo embryos. At first, all animals were exposed to AMH analysis, Estrus was synchronized either by progesterone releasing intravaginal device (PRID) for 7 days or by double doses of cloprostenol sodium 11 days interval. A total dose of 40 IU of Follitropin was injected intramuscular for each animal as superovulatory drug (5 IU am and 5 IU pm for 4 consecutive days). AI was done using double straw/time for 3 times with 12 hours interval. Seven days later (day 0=ovulation day), embryos were recovered non-surgically. The results showed that total number of follicles/ animal was significantly increased in the group synchronized by PRID (7.4 ± 0.53) than the group synchronized by cloprostenol (4.8 ± 0.18), Number of the recovered embryos was significantly higher in the group synchronized by PRID (3.2 ± 0.71) than the group synchronized by cloprostenol (2.2 ± 0.37). In conclusion, AMH analysis is a crucial issue in expecting the superovulatory response in buffaloes, injection of follitropin as a superovulatory drug during diestrus phase in buffaloes that previously synchronized by PRID leads to a higher yielded embryo.

Keywords: *Buffaloes, Superovulation, Synchronization, Embryo transfer.*

2. Introduction

Embryo transfer is a valuable reproductive method for improvement of animal breeding and control of reproductive diseases [1].

. Despite embryo transfer is one of the assisted reproductive techniques but its application in buffaloes is still limited, poor superovulatory response and decreased embryo recovery are the most critical issues [2].

Embryo transfer (ET) is a process by which an embryo is collected from a donor female and then transferred into a recipient female where the embryo completes its development. Embryo transfer is profitable for producers of registered purebred animals [3]. Through the use of embryo transfer, a genetically superior female produces more offspring than she could by natural reproduction. The increased number of offspring thus maximizes the donor female's genetic.

Estrus synchronization has become a beneficial tool in the buffalo industry as it facilitates artificial insemination. It is achieved by using PGF 2 α or its analogues through double injection at 11-day intervals or exogenous progesterone given in the form of a progesterone releasing intravaginal device (PRID) [3].

Common superovulatory method is the use of FSH regimen (Follitropin) that is injected in doses of 6,6,4,4,2,2,2 and 2 mg at half-day intervals with prostaglandin F2

alpha given with the sixth or seventh FSH injection [4].

Insemination of the superovulated buffaloes is usually performed at 12, 24 and 36 hours after the onset of standing estrus. Using high quality semen with a high percentage of normal, motile cells is a very critical step in any embryo transfer program. The correct site for semen placement was in the body of the uterus. [5], the embryos were collected non surgically at days 6 or 7 post insemination.

After embryo recovery, the collected embryos were evaluated morphologically depending on some criteria [6]; regularity and compactness of blastomeres, intact zona pellucida, presence of vesicles and overall diameter of embryos.

So, the aim of the current study is to reach a standard protocol of superovulation in buffaloes in terms of studying the role of anti-mullerian hormone, method of synchronization and type of superovulatory drug on the number and quality of the collected embryos.

3. Materials & Methods

The study was conducted at Animal Reproduction Research Institute in combination with Theriogenology Department, faculty of veterinary medicine, Cairo University.

3.1. Animals

Mixed breed buffaloes (Bubalus bubalis) cows (n=22) were housed in barns at reproduction research institute farm, both water and grass were offered ad lib, with nearly 3kg/day of commercial concentrate was offered for each buffalo cow. All animals were reproductively assessed using ultrasound machine (Sonoscape, China) provided with linear transducer of 7.5 MHz. Only reproductively healthy and normally cyclic animals were used.

3.2. Anti-Mullerian Hormone (AMH) Assay

All animals were exposed to blood sampling from coccygeal vein (10 ml).

Blood was collected in EDTA tubes and centrifuged at 3000xg for 15 minutes, plasma samples were stored at -20°c until use.

AMH was analyzed using a bovine AMH Elisa (Mofa Global). Blood samples were taken rather than the stage of estrous cycle as the stage of cycle does not affect the hormone concentration as mentioned by [7]

3.3. Estrus Synchronization

Estrus was synchronized either by progesterone releasing intravaginal device (PRID-Delta, containing 1.55g P4, Ceva) for 7 days, 2cm of $(Estrumate)^{R}$, cloprostenol sodium was injected at the day of PRID removal. Or by double doses of cloprostenol sodium 11 days interval.

3.4. Superovulation

Follitropin (FSH, Kyoritsu Seiyaku Corporation, Japan) was injected either at met estrus phase (two days following ovulation) or at diestrus phase (one week following ovulation) according to the experimental design.

A total dose of 40 IU of Follitropin was injected intra muscular for each animal as following (5 IU am and 5 IU pm for 4 consecutive days). A single dose of 3 cm cloprostenol sodium was injected two days following the beginning of Follitropin injection.

3.5. Heat Detection and Artificial Insemination (AI)

Within 48 hours following the end of Follitropin injection, heat signs were observed and the mature follicles were observed and mature follicles were assayed and counted using ultrasound. AI was done using double straw/time for 3 times with 12 hours interval.

3.6. Embryo Recovery

Seven days later (day 0=ovulation day), embryo were recovered non-surgically, briefly, buffaloes were restrained and confined in a chute, 6 ml of Lidocaine 2% was administrated as posterior epidural anesthesia, rectal ultrasound was done to detect the number of corpora lutea, a supported foley catheter was inserted to the base of the horn, about 500 ml commercial bovine flushing medium was inserted, after mixing with the embryos, the flushing medium containing embryos was recovered on a sterile disposable bovine embryo filter, embryo number and quality were evaluated on the searching dish under dissecting microscope [8].

3.7. Statistical analysis

Data were presented as mean ±SEM and analyzed by one-way ANOVA followed by Tukey's Test. GraphPad prism 5 software was used. The results were considered to be statistically significant at $P \leq 0.05$.

4. Results

4.1. Anti-Mullerian Hormone (AMH) Assay

All buffalo cows were exposed to AMH assay to determine the effectiveness of the ovaries and to study the ovarian response mediated by injection of the superovulatory drug. Ten buffalo cows showed a concentration more than 0.1 ng/ml with mean value of (0.17 ng/ml), while twelve buffalo cows experienced a concentration less than 0.1 ng/ml with mean value of (0.08 ng/ml) (As presented in table **1)**

4.2. Effect of superovulatory drug injection on the follicular population in buffaloes with AMH value of more than 0.1 ng/ml.

Number of large follicles was higher in group 1 than in group 2. Total number of follicles/ animal was significantly increased in group 1 than 2 as presented in table **2.**

4.3: Effect of Follitropin injection on follicular population with AMH concentration less than 0.1 ng/ml.

Number of large, medium sized follicles was significantly higher in group 1 than group 2. Also the total number of follicles was significantly higher in group 1 than group 2. As presented in table **3.**

4.4. Effect of superovulatory drug injection on the number of corpora lutea and the number of the harvested embryos

Number of the recovered embryos was significantly higher in group 1 than group 2 , despite the number of the corpora lutea was higher in group 1 than 2 but the increase was not significant.

5. Discussion

Embryo transfer technology had been used worldwide with increased the number of obtained offsprings from genetically superior females, however the progress in the results of embryo transfer in buffaloes is still limited. The current study investigated at first the role of AMH

analysis on follicular population following synchronization and superovulation in buffaloes, the results showed that, the incidence of large follicles and total number of follicles are significantly higher while AMH concentration is more than 1 ng/ml. In the same way, Redhead et al. [9] concluded that AMH is a good marker that could improve the superovulatory response in buffaloes. In cattle, Baruselli et al. [10] found that measuring the circulating AMH could be useful to identify animals most likely to have improved superovulatory responses to gonadotropin treatment as well as best oocytes donor. Parallel to our study, Baruselli et al. [10] found that the total number of follicles were significantly higher in animals with high plasma AMH than those with low plasma AMH concentration. Our results demonstrated that the number of the recovered embryos was significantly higher in buffaloes synchronized by PRID than those synchronized by double doses of cloprostenol. In the same way, Techakumphu et al. [11] reported that using intra vaginal devices like PRID or CIDR can be efficiently synchronize buffaloes and be ready for superovulation.

Kandil et al. [12] concluded that, application of non surgical embryo collection in buffaloes is applicable and can be led to more recovery of high genetic embryos.

6. Conclusion

AMH analysis is a crucial issue in expecting the superovulatory response in buffaloes, buffaloes with AMH concentration of more than 1 ng/ml are recommended for superovulation, injection of follitropin as a superovulatory drug during diestrus phase in buffaloes that previously synchronized by PRID leads to a higher yielded embryos.

10. References

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Table 1: Anti-Mullerian Hormone in buffaloes (AMH) assay

Mean±SEM	**No. of buffaloes**	**AMH conc.**
0.08ng/ml ± 0.021	12	<0.1 ng/ml
0.17± 0.052	10	>0.1 ng/ml

Table 2: Effect of follitropin injection on follicular population with AMH concentration more than 0.1 ng/ml.

Groups	**No.of buffaloes (AMH=1.7ng/ml)**	**No. of large follicles/Anim. (mean±SEM)**	**No.of medium follicles/Anim. (mean±SEM)**	**No. of small follicles/Anim. (mean±SEM)**	**Total No. of follicles/Anim (mean±SEM)**
Group 1: Synchronized by PRID & Superovulated at Diestrus	5	$(5.6 \pm 0.23)^{a}$	$(1.4 \pm 0.18)^{b}$	$(0.4 \pm 0.08)^{b}$	$(7.4 \pm 0.53)^{ab}$
Group 2: Synchronized by PGF2α & Superovulated at Diestrus	5	$(4.0 \pm 0.26)^{ab}$	$(0.6 \pm 0.13)^{bc}$	$(0.2 \pm 0.05)^{c}$	$(4.8 \pm 0.18)^{c}$

Values with different superscripts were significantly different at P<0.05

Table 3: Effect of follitropin injection on follicular population of buffaloes with AMH concentration less than 0.1 ng/ml

Groups	No.of buffaloes (AMH= 0.08 ng/ml)	No. of large follicles/Anim. (mean±SEM)	No.of medium follicles/Anim. (mean±SEM)	No. of small follicles/Anim. (mean±SEM)	Total No. of follicles/Anim (mean±SEM)
Group A: Synchronized by PRID & Superovulated at Diestrus	6	(0.83 ± 0.28) [a]	(0.50 ± 0.12) [a]	(0.33 ± 0.11) [a]	(1.66 ± 0.33) [a]
Group B: Synchronized by PGF2α & Superovulated at Diestrus	6	(0.33 ± 0.12) [b]	(0.17 ± 0.07) [b]	(0.33 ± 0.11) [a]	(0.83 ± 0.22) [b]

Values with different superscripts were significantly different at P<0.05

Table 4: Effect of follitropin injection on No. of corpora lutea & No. of recovered embryos

Groups	No. of buffaloes	No. of Corpora lutea /Anim. (mean±SEM)	No. of recovered embryos /Anim. (mean±SEM)
Group 1: Synchronized by PRID & Superovulated at Diestrus	5	(4.60 ± 0.81) [a]	(3.2 ± 0.71) [a]
Group 2: Synchronized by PGF2α & Superovulated at Diestrus	5	(3.60 ± 0.53) [ab]	(2.2 ± 0.37) [b]

Values with different superscripts were significantly different at P<0.05

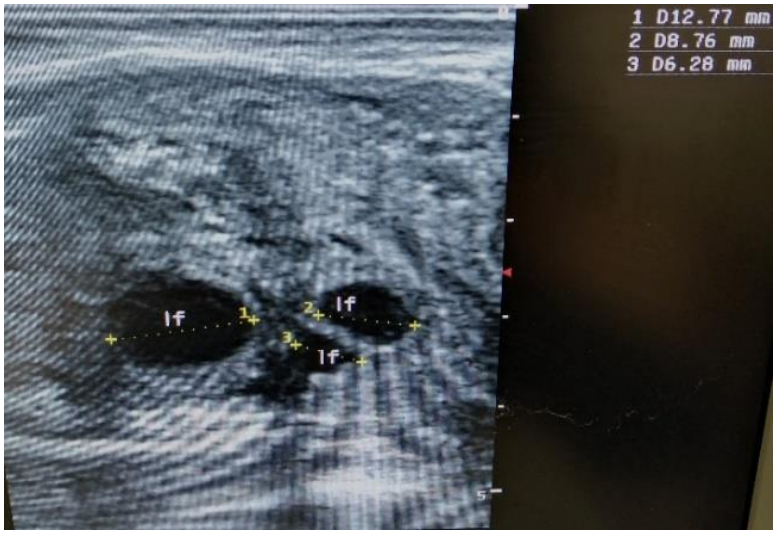
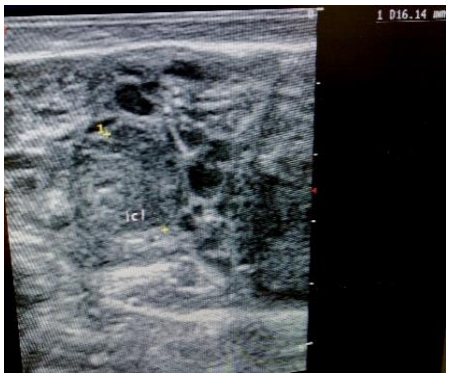
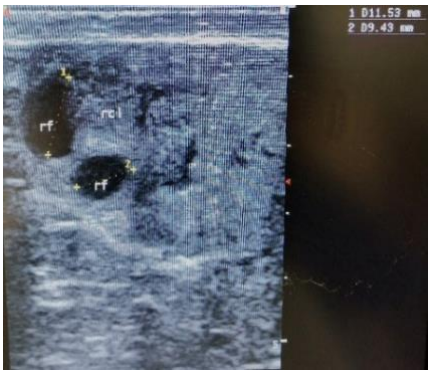


Fig 1: Ultrasonography scanning for ovarian response in superovulated buffalo





Left corpus luteum (A) ***Right corpus luteum (B)***

Fig.2: Ultrasonography scanning show multiple corpus luteum in buffalo A and B