	experimental groups and vitrified by closed pulled straw at different stages	

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LIST OF ABBREVIATIONS

AI	Artificial Insemination
µm	Micrometer
CIDR	Controlled Internal Drug Release
CL	Corpus Luteam
CLs	Corpora Lutea
cm	Centimeter
CPS	Closed Pulled Straw
d	Day
E2	Estradiol
EB	Estrdiol Benzoate
eCG	Equine chorionic gonadotropin
ET	Embryo Transfer
EV	Estradiol Valerate
F	Follicle
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
IVF	In-Vitro Fertilization
IVP	In-Vitro Production
LH	Luteinizing Hormone
MOET	Multiple Ovulation and Embryo Transfer
mOPS	Modified Open Pulled Straw
ng	Nanogram
OPS	Open Pulled Straw
OR	Ovulation Rate
P4	Progesterone
pFSH	Porcine Follicle Stimulating Hormone
PGF	Prostaglandin
PGF2α	Prostaglandin F2α
PMSG	Pregnant Mare Serum Gonadotropin
PVP	Polyvinyl pyrrolidone
RR	Recovery Rate
SMB	Synchro-Mate-B
SO	Superovulation
SOR	Superovulatory Response
T	Treatment
UOF	Un-Ovulated Follicle

SUMMARY AND CONCLUSION

This study was conducted at El-Karada Animal Production Research Station and International Livestock Management Training Center, (ILMTC)-Sakha, Kafrelshiekh governorate, belonging to Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Ministry of Agriculture in co-operation with Animal Production Department, Faculty of Agriculture, Mansoura University during the period from January to October 2013.

The aim of this study was to determine the effect of different synchronization regimens as exogenous progesterone on; (1) ovarian follicular dynamics and superovulatory response, (2) hormonal profile (P4) during superovulation treatment, (3) embryo production, quality and cryopreservation in Friesian cows.

A total of 24 Friesian cows have live body weight (LBW) of 450-550 kg, 3.5-5.5 years of age and 1-3 parities were used as donor cows. Cows were divided into 3 experimental groups (8 animals in each).

Cows in G1 were injected intramuscularly (i.m) with 3 ml $PGF_2\alpha$/cow to bring them on heat (start of estrous cycles). Cows were i.m. injected with 3000 IU of PMSG/cow on day 10 of the estrous cycle, and 48 hours later with 3 ml PGF2α/cow. Cows received this protocol were considered as control group.

Cows in group (G2) were received Synchro-Mate-B (SMB) ear implant and i.m. injected with 5 mg estradiol benzoate (EB) and 100 mg of progesterone/cow on day of implantation. Seven days later (7 days of treatment), all cows were i.m. injected with 3000 IU of PMSG and 3 ml of PGF2α/cow. Two days later (day 9 of treatment) implants were removed. This protocol was initiated without any information about the previous estrous cycle.

Cows in group (G3) were received controlled internal drug release (CIDR) intravaginal and i.m. injected with 4 mg EB. CIDR were removed 7 days later, and cows were i.m. injection of 3000 IU of PMSG and 3 ml of PGF2α on day of CIDR withdrawal. Also, this protocol was initiated without any information about the previous estrous cycle.

All cows artificially inseminated (AI) when excepted estrous. Ultrasonography device was used during treatment period to record the number of follicles and CL and diameter of the follicles at day 0, during, post treatment, AI and flushing days.

Flushing was conducted 7 days after AI to determine the ovulatory response**.** Collected embryos were evaluated morphologically and were classified into different grades (I, excellent; II, good; III, fair and IV, poor) on basis of their morphological symmetry. Measurements of embryos were recorded (Thickness (µm) of zona pellucida, Diameter (µm) of intrazonal and embryo with its coverings.

Blood samples were collected from all cows in each group at day 0, during, post treatment, AI, post AI and flushing days in all experimental groups until assay of progesterone (P4) hormone.

The obtained results could be summarized as the following:

1. Changes in ovarian characteristics at day 0, during and post–PGF2α (G1), SMB (G2) and CIDR (G3) treatment days:

1.1. Follicular number per ovary:

1. Number of follicles was affected significantly ($P<0.05$) by treatment only at post–PGF2α (G1), SMB (G2) and CIDR (G3) treatment. However, follicular number was not affected significantly by treatment at day 0 and during treatment or by ovarian side or their interaction on different treatment days.

2. Post–treatment, follicular number significantly ($P<0.05$) increased on ovaries of cows in G3 than in G1 and G2 by about 75 and 35% on each ovary, respectively.

3. Results also showed that follicular number was almost higher on the right than left ovaries at day 0, during and post–treatment, but the differences were not significant.

1.2. Diameter of all follicles (cm):

1- Diameter of follicles was affected significantly (P<0.05) by treatment only at post–PGF2α (G1), SMB (G2) and CIDR (G3) treatment. However, follicular diameter was not affected significantly by treatment at day 0 and during–treatment or by ovarian side or their interaction on different treatment days.

2- Results showed that follicular diameter showed insignificant differences among the experimental groups at day 0 and during–treatment. Meanwhile, post–treatment follicular diameter significantly (P<0.05) increased on ovaries of cows in G3 than in G1 and G2 by about 56 and 82%, respectively.

1.3. Diameter of largest follicles (>1.0 cm):

1- Diameter of largest follicles was affected significantly (P<0.05) by treatment only at post–PGF2α (G1), SMB (G2) and CIDR (G3) treatment. However, diameter of largest follicles was not affected significantly by treatment at day 0 and during–treatment or by ovarian side or their interaction on different treatment groups.

2- Results showed that diameter of largest follicles showed insignificant differences among the experimental groups at day 0 and during–treatment days. Meanwhile, diameter of largest follicles on post–treatment day was significantly (P<0.05) higher on ovaries of cows in G1than in G2, but that of G3 did not differ significantly from those in G1 and G2.

1.4. Number of corpora lutea (CLs) per ovary:

1- Number of CLs was affected significantly (P<0.001) by treatment at day 0 and on day post–treatment. However, CLs number was not affected significantly by treatment on days during–treatment or by ovarian side or their interaction at day 0, during and post–treatment days.

2- Results showed that number of CLs at day 0 was significantly (P<0.05) greater in G2 and G3 than in G1. Although CLs number was greater in G3 than in G2, this difference was not significant. It is of interest to note that no CLs were observed on the ovaries of cows in G1 at day 0, whenever estrus occurred and all CLs completely regressed as

affected by PGF2α. However, the CLs were observed on ovaries of some cows in G2 and G3 following the later estrous/ovulation based on the reproductive status of each cow at the beginning of the treatment.

3- Number of CLs during–treatment was not affected significantly by treatment, although CLs number was greater in G1 than in G2 and G3, but the difference was not significant.

4- At post–PGF2α (G1), SMB (G2) and CIDR (G3) treatment, number of CLs was significantly ($P<0.05$) greater in G1 than in G2, meanwhile no CLs were detected on the ovaries of cows in G3. Absence of CLs in G3 on day post–treatment may indicate that effect of PGF2α injection on CLs regression higher in G3 than in G1 and G2 and higher in G2 than in G1, the differences were significant ($P<0.05$).

2. Superovulatory response at AI and flushing days:

2.1. Follicular number per ovary:

1- Number of follicles was not affected significantly by treatment, ovarian side or their interaction at AI and flushing.

2- On day of AI, number of follicles/ovary was greater in G3, followed by G1, while G2 showed the lowest number of follicles. However, on day of flushing, follicular number/cow was the greatest on ovaries of cows in G1, moderate in G2 and the lowest in G3. All differences among groups were not significant.

2.2. Diameter of all follicles (cm):

1- Diameter of follicles was affected significantly ($P<0.05$) by treatment only on day of AI. However, follicular diameter was not affected significantly by treatment on day of flushing or by ovarian side or their interaction on day of AI and flushing.

2- Results showed that follicular diameter showed significant ($P<0.05$) differences among the experimental groups at AI, being wider in G3 than in G1, but G2 did not differ significantly from that in G1 and G3. Also, follicular diameter showed the same differences on day of flushing, but at insignificant level.

2.3. Diameter of the largest follicles (>1.0 cm):

1- Diameter of largest follicles was not affected significantly by treatment, ovarian side or their interaction on day of AI and flushing.

2- Results showed that diameter of largest follicles showed insignificant differences among the experimental groups although it was higher in G3, followed by G1 and the lowest in G2 on day of AI and flushing. Largest follicular diameter was almost higher on the right than left ovaries on day of AI and flushing.

2.4. Estrus response:

1- Results revealed that incidence of estrus occurred in 100% of cows in all treatment groups post–PGF2α injection, being significantly ($P<0.05$) later in G2 and G3 (5.25 and 5.75 day), respectively than in G1 (2.25 day). However, when incidence of estrus was calculated after the end of treatment, estrus incidence was significantly ($P<0.05$) the earlier in G1 (2.25 day), moderate in G2 (3.25 day) and the latest in G3 (5.75 day).

2.5. Ovulation rate and percentage of cows produced embryos:

1- Results showed that superovulatory response of cows in term of average follicular number and mean diameter of largest follicle per cow at artificial insemination was not affected significantly by superovulation protocol. On day of flushing, average number of CLs/cow was affected significantly ($P<0.05$) by superovulation protocol, being greater in G3 (7.5/cow) than in G1 and G2 (4.1 and 4.5/cow), respectively. However, number of un-ovulated follicles showed an opposite trend, but the differences were not significant. Such results were reflected in significantly ($P<0.05$) higher ovulation rate in G3 (71.4%) than in G1 and G2 (47.0 and 55.4%, respectively).

2- It is of interest to note that percentage of cows produced embryos was significantly ($P<0.05$) higher for cows in G3 (62.5%) than in G1 and G2 (37.5% in each).

3. Embryo production:

3.1. Embryo yield and recovery rate:

1- Results revealed that number of embryos/cow was the greatest in cows of G3 (4.75), moderate in G1 (1.78) and the lowest in G2 (1.28). Based on the greatest number of CLs and produced embryos in G3, recovery rate of embryos was significantly ($P<0.05$) higher in G3 (63.3%) than in G1 and G2 (43.2 and 28.4%, respectively).

3.2. Embryonic stage of recovered embryos:

1- Results showed that cows in G3 showed significantly ($P<0.05$) higher number of embryos at morula and compact morula stages (1.6 and 2.5/cow) than those in G2 (0.5 and 0.4/cow) and G1 (0.5 and 0.5/cow), respectively. However, the differences in number of embryos at early blastocyst and blastocyst stages among the experimental groups were not significant.

3.3. Quality of recovered embryos:

1- Results showed that cows in G3 produced significantly ($P<0.05$) higher number of transferable embryos (1.0 excellent and 2.25 good embryos/cow) and even the highest number of fair embryos (0.75/cow for each) as compared to other groups. However, the difference in number of poor embryos among the experimental groups was not significant.

4. Morphometry of recovered embryos:

4.1. Effect of superovulation treatment:

1- Results revealed significant ($P<0.001$) effect of superovulation treatment on intrazonal diameter and total diameter of embryos. However, thickness of zona pellucida was not affected significantly by treatment.

2- Thickness of zona pellucida was nearly similar in all experimental groups, ranging between 17.3 and 18.5 µm. However, diameter of intrazonal and total embryo were significantly ($P<0.05$) the highest in embryos of G2, moderate in G1 and the lowest in G3.

4.2. Effect of embryonic stage:

1- Results revealed significant effect of embryonic stage on thickness of zona pellucida, and diameter of intrazonal and total embryo.

2- Thickness of zona pellucida significantly ($P<0.05$) reduced, being the highest in embryos at morula stage and the lowest in those at blastocyst stage. Meanwhile, both diameters of intrazonal and consequently total embryo significantly ($P<0.05$) increased by advancing embryonic stage, being the lowest in embryos at morula stage and the highest at blastocyst stage.

4.3. Effect of interaction between treatment and embryonic stage:

1- Results revealed that the effect of interaction between treatment and embryonic stage was significant ($P<0.05$) effect of embryonic stage on diameter of intrazonal and total embryo and not significant on thickness of zona pellucida.

2- The recorded insignificant effect of treatment and embryonic stage on thickness of zona pellucid reflected in similar trend of reduction by advancing the embryonic stage in all treatment groups, being higher in G2 than in G1 and G3. However, the significant ($P<0.05$) effect of this interaction on diameter of intrazonal and total embryo reflected similar trend of increase in both diameters of intrazonal and total embryo by advancing embryonic stage, being the highest in embryos produced from cows in G2 at morula and compact morula stages, in embryos of cows in G1 at early blastocyst stage and in those of cows in G3 at blastocyst stage.

4.4. Effect of embryo quality:

1- Analysis of variance revealed significant ($P<0.01$) effect of embryo quality on diameter of intrazonal and total embryonic diameter ($P<0.001$). However, thickness of zona pellucida was not affected by embryo quality.

2- Results revealed that inspite the recorded insignificant differences in thickness of zona pellucida amonge embryo grades, there was a tendency of increase in thickness of zona pellucida by improving embryo quality. Such finding may indicate a protection of embryos by zona pellucida to be in good quality.

3- On the other hand, both diameter of intrazonal and consequently total embryonic diameter significantly ($P<0.05$) increased by improving embryo quality, being the highest in excellent embryos, moderate in good embryos and the lowest in fair and poor embryos.

5. Progesterone concentration (ng/ml):

1- Progesterone (P4) concentration was affected significantly by superovulation treatment only during-treatment ($P<0.001$) and post-treatment ($P<0.01$).

2- Results indicated that P4 concentration at day 0 (initiation of superovultion) was higher in G2 and G3 than in G1, but the differences were not significant.

3- Concentration of P4 during-treatment was significantly ($P<0.05$) higher in G3 than in G1 and G2. However, P4 concentration post-treatment was significantly ($P<0.05$) higher in G1 than in G2, but P4 concentration did not differ in G3 compared to G1 and G2.

4- On day of AI, post-AI and flushing, P4 concentration was not affected significantly by superovulation treatment, although there was a tendency of the highest P4 concentration in G3 and the lowest values in G1.

5- In addition there was marked increase in P4 concentration post-AI and on day of flushing, being the highest in G3, followed by G2 and the lowest in G1**.** This trend was associated with number of CLs in the experimental groups on day of flushing.

6- Results revealed increase in P4 concentration by ≥1 ng/ml in responded than in non-responded cows post-treatment, at AI, post-AI and on day of flushing.

6. Cryopreservation of embryos by vitrification (Closed pulled straw, CPS):

1- Results based on embryos at all stages revealed that embryos of cows in G2 showed the highest post-vitrfication survival and normality rates (60 and 83.3%), flowed by G3 (57.5 and 68%) and G1 (50 and 71.4%), respectively.

2- Concerning the post-vitrification survival and normality rates of embryos at blastocyst stages, also embryos of G2 had the superiority in survival rate (66.7%) with higher normality (50%) than those in G3 (33.3%) and lower normality than those in G1 (66.7%). Regarding the embryos at morula stages, embryos of G2 and G3 showed

higher survival rates (about 57% in each) than those in G1 (50%) with the highest normality (100%) in G2 than those in G1 (75%) and G3 (73.7%).

In conclusion, the obtained results, indicated that using controlled internal drug release (CIDR) device as progesterone source and 4 mg estradiol benzoate (EB) at insersion with 3000 IU of pregnant mare serum gonadotropin (PMSG) in superovulation protocol of Friesian cows resulted in high ovulatory response in terms of ovulation rate, and number of transferable embryos of excellent and good grades. However, further studies are required for improving embryos survival and normality during cryopreservation.