OVULATORY RESPONSE, OF TRANSFERABLE EMBRYOS RECOVERED FROM FRIESIAN COWS SUPEROVULATED BY TWO PMCG DOSES.

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ABSTRACT

The present study was performed to optimize a superovulation protocol of Friesian cows in order to produce a large number of good quality embryos suitable for development in culture *in vitro* and for transfer. Superovulation protocol was done using a single injection of two different doses of pregnant mare serum gonadotropin (PMSG) (Low, 2500 IU vs high, 3000 IU). Cows (n=30) were treated with different PMSG doses at day 10 of normal oestrus cycle , and ovarian response was evaluated by ultrasonography. Results showed that cows treated with low PMSG dose had a greater number of ($P<0.01$) CLs (11.67 *vs.* 9.33) as well as total and transferable embryos (8.3 and 5.3 *vs.* 7.1 and 4.2, $P<0.05$) than cows treated with high dose. The differences in number of unovulated follicles (NOF) and total response (number of CLs and NOF) were not affected by PMSG dose. Recovery rate of total embryos reduced (71.38 *vs.* 76.42%, $P<0.05$) and of transferable embryos increased (63 *vs.* 59.18%) with low compared with high dose. Low dose cows increased number of embryos at morula (2.9 *vs.*2.3, $P<0.05$) and blastocyst (1.4 *vs.* 0.67, $P<0.01$) stages compared with high dose and an opposite trend was recorded for abnormal embryos (0.60 *vs.* 1.1, $P<0.05$). The effect of dose on number of embryos at compact morula stage was not significant. Low dose increased number of excellent (4.3 *vs.*2.1, $P<0.01$) and good (2.8 *vs.* 2.2, $P<0.05$) embryos, and decreased number of fair embryos (1.3 *vs.* 2.9, $P<0.01$) compared with high dose. The highest survival rate of cultured embryos to develop into hatched or complete hatched blastocysts (72.7 and 63.6%) was obtained from embryos at morula stage recovered from cows superovulated by low dose compared with 68.6 and 51.4% for high dose, respectively. In the same respect, excellent embryos gave the best results, being 75 and 59.4%, respectively. For embryo transfer, pregnancy rate was 33% for fresh embryos at morula stage from Friesian cows superovulated by low dose and no pregnant for

vitrified/thawed embryos recovered from superovulated Friesian cows at morula stage.

In conclusion, the obtained results, under the experimental conditions in this study, indicated that the most appropriate hormonal dose for superovulation in Friesian cows was 2500 IU PMSG to reflect the highest ovulatory response, the best survival rate of *in vitro* culture either for stage or grade of recovered embryos and the availability of more pregnancy rate after embryo transfer.

Keywords: Friesian cows, superovulation, PMSG, embryos, survival, embryo transfer.

INTRODUCTION

One of the major advancements in animal reproduction has been the development of embryo transfer techniques. The use of these techniques contributes to the movement of genetic resources with minimal risk of disease transmission and reduced transportation costs in comparison with transport of live animals. Embryo transfer is also essential for application of other reproductive biotechnologies such as in vitro production of embryos, cloning and transgenesis. An efficient and practical technique for bovine embryo cryopreservation is a fundamental issue in the widespread use of embryo transfer.

Cryopreservation of bovine embryos was substantially improved due to factors such as an increases in embryo quality, an adequate selection of the recipient and an apropriate synchronization between donor and recipient **(Gordon, 1996)**. Pregnancy rates achieved using high-quality cryopreserved embryos are only 10% lower than those obtained using fresh embryos **(Niemann, 1991).**

Superovulation is a critical step in embryo transfer success, several gonadotrophine have been utilized for superovulation in different species, but pregnant mare serum gonadotrophin (PMSG) is most often used to induce multiple ovulations from the ovary for increased egg production **(Marte Mucci *et al.*, 1988; Mutiga and Barker, 1982; Walker *et al.*, 1984 and McKiernan and Bavister, 1998)**. Superovulation is a technique used to produce a large number of embryos developmently synchronized. It is employed for protocols such as embryo transfer **(Santiago-Moreno *et al.*, 2001)**, embryonic stem cell production **(Eistetter 1989)**, transgenic animals **(Auerbach *et al.*, 2003)** and to develop animal models of human diseases **(Charreau *et al.*, 1996)**. Superovulation protocols based on gonadotrophic hormones have been standardized in species such as mouse **(Eistetter, 1989)**, pig **(Cuello *et al.*, 2004)**, cattle **(Kanitz *et al.*, 2002)**, sheep **(Santiago-Moreno *et al.*, 2001)** and goat **(Graff *et***

***al.*, 2000)**, and the quality of embryos produced appears to be satisfactory.

With the major advantage of PMSG being that it requires only one injection. However, it has been demonstrated that PMSG results in a highly variable response and reduce both recovery rates and yield of transferable embryos **(Marte Mucci *et al.*, 1988)**. This has been atributed to the long half – life of PMSG in the systemic circulation.

Therefore, the aims of the present study were: 1) detrmine the best dose of PMSG (2500 or 3000 IU) in order to establish the most adequate and consistent treatment to induce superovulation (total response and total recovery) in cows, 2) studing the effect of PMSG dose on pre-implantation embryo development *in vitro* to complete hatching blastocyst according to stage and grade of embryos and 3) comparing transfer of vitrified embryos with fresh ones.

MATERIALS AND METHODS

The current study was carried out at both International Livestock Management Training Center (ILMTC), Sakha and El- Karada Animal Production Research Stations, both belonging to Animal Production Research Institute, Agricultural Research Centre, Ministry of Agriculture during the period from June 2008 to October 2009.

Animals and superovulation protocol:

Thirty Holstein Friesian cows were used in this study. All cows were at 60-120 days postpartum and had 1-4 parities.

Superovulation was induced by i.m. injection of PMSG (Folligon, intervit International BV. Boxmeer-Holand) on day 10 of the normal oestrus cycle. The donor cows were divided into two groups, 15 cows in each. Animals in the 1st group were injected with 2500 IU, while those in the 2nd one were injected with 3000 IU of PMSG. After 48 h of PMSG injection, each cow was received an i.m. injection of PGF2α analogue (2 ml Estrumate, Coopers Animal Health LTD, Berkhamsted-England). Each ml of Estrumate contained 263 µg of Cloporostenol sodium equivalent to 250 µg Cloporostenol. Oestrus was observed for the treated cows within 48 h from Estrumate injection, each cow come in heat was inseminated artificially three times (p.m., a.m. and p.m.). The protocol of oestrous synchronization and superovulation was performed as described by **Ravindranatha and Reddy (1990 & 1999).**

Embryos collection:

Seven days after artificial insemination, embryos were flushed with PBS (Dulbecc's phosphate Buffered Saline, D-PBS, Germany) plus 1% fetal calf serum (FCS). Numbers of corpora lutea (CLs) and unovulated follicles were determined by ultrasonography. Also total embryos (normal or degenerated) and unfertilized ova were recorded, then determined number of transferable embryos.

Total number of recovered embryos and embryo categories were carried out according to the method described by **Reddy (1994)** and **Lonergan (1990)** as the following:

Grade A (Excellent): morphologically normal with all cells in clear spherical shape or apparently normal inner cell mass, absence of vesicles, clear round uniform perivitelline space, and perfectly circular intact zona pellucida shiny and uniform dark/light color.

Grade B (Good): one or two blastomeres extruded from inner cell mass, vesicles present in a few or small amount.

Grade C (Fair): Irregular shape, irregular division in inner cell mass, areas of dark grey color, large vesicles, damaged zona pellucida, cell debris in the perivitelline space.

Evaluation of different stages of embryos: Embryos were scored according to their stage of development and morphological appearance to ***compact morula***: individual blastomeres have coalesced, forming a compact mass. The embryo mass occupies 60-70% of the perivitelline space; ***early blastocyst***: embryos that have formed a fluid-filled cavity or blastocoele and have the general appearance of a signet ring; ***blastocyst***: more compact inner cell mass evident. The blastocoele is highly prominent with the embryo occupying most of the perivitelline space; and ***expanded blastocyst***: overall diameter of the embryo increases 1.2 to 1.5 times with a concurrent, thinning of the zona pellucida to approximately 1/3 of its original thickness.

Embryo freezing: A portion of embryos were loaded individually into 0.25 ml straws (IMV, L'Aigle, France) and cryopreserved using vitrification in liquid nitrogen (LN_2).

Vitrification procedures: Methods as described by **Vicente and Garcia-Ximenez (1996)** were employed for the vitrification of bovine embryos. Holding medium for vitrification was prepared by supplementing PBS with 20% FCS (v/v). Vitrification solution I (VS-

I) [containing 12.5% ethylene glycol (EG) and 12.5% dimethyl sulfoxide (DMSO)] was prepared just prior to use by mixing EG, DMSO and the holding medium in the ratio of 1:1:6. Similarly, vitrification solution II (VS-II) had a composition of 25% EG and 25% DMSO. This was prepared just prior to use by mixing EG, DMSO and the holding medium in the ratio of 1:1:2. Cryoprotective diluent-I (CPD-I) was prepared just prior to use by mixing EG, DMSO and the holding medium in the ratio of 1:1:8. Cryoprotective diluent-II (CPD-II) was prepared by mixing equal volumes of CPD-I and the holding medium. Three to five embryos were initially placed in the holding medium for 10 min followed by VS-I for 4 min. Subsequently, the embryos were transferred into VS-II for 1 min. During equilibration in VS-II the embryos were loaded into 0.25 ml French mini straws as in programmed freezing but using VS-II. Immediately after loading, the straws were immersed vertically into liquid nitrogen. These vitrified embryos were also stored in liquid nitrogen for up to 2 months.

Thawing method of vitrified embryos: For warming, the straws were exposed to air at room temperature (25 °C) for 8–10 seconds and then vertically immersed in water bath at 25 °C until the frozen solutions became liquid. Stoppers were removed, sealed ends were cut and the contents poured into CPD-I for 4 min. Then they were transferred to CPD-II for 8 min. Finally the embryos were washed twice in the holding medium for 8 min each.

Embryo culture: All recovered embryos were washed by DPBS-medium containing 20% fetal calf serum (FCS), then *in vitro* cultured in a 100 µl of tissue culture medium (TCM-199) supplemented with 10% FCS and 1 µg/ml from Gentamycin (Sigma- Aldrich Chemie, Steinheim, Germany) according to **Edashige *et al.* (1999)**. Fresh embryos collected from the cow donors in both treated groups (2500 IU or 3000 IU of PMSG) were cultured in groups according to the stages and grades in 4-well culture dishes under mineral oil (Sigma-Aldrich Chemie, Steinheim, Germany) and incubated at 38°C in a humidified atmosphere of 5% CO_2 in air for up to 72 h. Checks for developmental stage were done at 24 h intervals. Development to hatched blastocyst stage at the end of culture period was considered indicator of success of embryos *in vitro* culture. Also, the vitrified/thawed embryos were cultured in the same culture medium for 4 h and transferred to recipients.

Embryo transfer: Total of 7 Holstein Friesian heifers were used as recipients (body weight 360 kg). Oestrus of all recipients were synchronized by using a single dose of 2 ml Estrumate. Oestrus was detected after 48 h from PGF2α injection. Embryos were non-surgically transferred on 6-8 days after detection of oestrus (one embryo per recipient) into the uterine horn using an embryo transfer syringe (I.M.V., L'Aigle, France).

after two months of cryopreservation, the vitrified/thawed embryos were transferred, while fresh embryos were transferred immediately after flushing. Pregnancy was diagnosed at 40-45 days after embryo transfer by transrectal ultrasonography using a real-time, B-mode linear array ultrasound scanner (model: Scanner Aquila vet version:58B11ENO1-200301, Pie Medical company, Masstricht, Netherlands). The Scanner was provided with transrectal transducer (probe) and has a dual frequency (6.0 and 8.0 MHz).

Statistical analyses: Chi-square test was used to determine differences in survival rates of embryos at different stages and different grades. While, data of total response and embryo recovery traits were analyzed using computer programme of **SAS (1987)** according to **Snedecor and Cochran (1982)**.

RESULTS

Superovulatory response and recovery rate:

Table (1) shows superovulatory response, in term of averages number of ovarian structures (CLs and unovulated follicles/cow), total recovered embryos (normal, degenerated embryos and unfertilized ova/cow) and transferable embryos/cow. Cows treated with 2500 IU PMSG showed significantly greater ($P<0.01$) CLs (11.67 *vs.* 9.33/cow) as well as total and transferable embryos (8.33 and 5.25 *vs.* 7.13 and 4.21/cow respectively, $P<0.05$) than cows treated with 3000 IU PMSG. However, the differences in number of unovulated follicles (UOF) and total response (number of CLs and UOF) between both treatment groups were not significant.

It is worthy noting that the significant ($P<0.05$) increase in the average numbers of total and transferable embryos with low PMSG dose reflected a reduction ($P<0.05$) in recovery rate of total embryos (71.38 *vs.* 76.42%) than cows treated with 3000 IU and nearly similarity in recovery rate of transferable embryos in the both two

treated groups (45.0 *vs.* 45.12%) (table 1). In addition, there was a tendency of increase in number of transferable embryos relative to total embryos for the cows treated with low PMSG dose (63.02 *vs.* 59.18%) as compared to cows treated with high PMSG dose (Table 1).

Table (1): Effect of PMSG dose on ovarian response and embryos recovered from superovulated cows. (mean±SE)

Item	PMSG dose		Sign.
	Low (2500 IU)	High (3000 IU)	
Average number of ovarian structure/cow:			
Corpora lutea (CL)/cow	11.67±0.67	9.33±0.56	**
Unovulated follicles (UOF)/cow	1.33±0.25	1.93±0.25	NS
Total ovulatory response[1]	13.00±0.89	11.27±0.78	NS
Average number of recovered embryos/cow:			
Total embryos[2]	8.33±0.39	7.13±0.43	*
Transferable embryos	5.25±0.23	4.21±0.19	*
Recovery rate (%):			
Total embryos	71.38±1.72	76.42±0.85	*
Transferable embryos	45.00±1.36	45.12± 2.86	NS
Transferable relative to total embryos			
Ratio (%)	63.02	59.18	NS

NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01.
[1]: Total response to superovulation = Number of CL+UOF.
[2]: Number of total embryos (normal and degenerated) and unfertilized ova.

Stage and grade of recovered embryos:

Results in Table (2) showed that cows treated with 2500 IU PMSG significantly increased average number of embryos at morula (2.93 *vs.*2.33/cow, P<0.05) and blastocyst (1.40 *vs.* 0.67/cow, P<0.01) stages compared with 3000 IU PMSG. An opposite trend was recorded for average number of abnormal embryos (0.60 *vs.* 1.13/cow, P<0.05). In the mean time, the average number of embryos/cow at compact morula stage was higher in cows treated with low PMSG dose than in cows treated with high PMSG dose but the difference was not significant (Table 2).

Table (2): Effect of PMSG dose on embryo classification (stage and grade) collected from Friesian cows. (mean±SE)

Embryonic stage	PMSG dose		Sign.
	Low (2500 IU)	High (3000 IU)	
Average number of embryos at different stages/cow:			
Morula	2.93 ± 0.18	2.33 ± 0.19	*
Compact morula	3.40 ± 0.19	3.00 ± 0.17	NS
Blastocyst	1.40 ± 0.13	0.67 ± 0.13	**
Abnormal(1)	0.60 ± 0.13	1.13 ± 0.17	*
Average number of embryos at different grades/cow:			
Excellent	4.27 ± 0.23	2.07 ± 0.21	**
Good	2.80 ± 0.17	2.20 ± 0.17	*
Fair	1.26 ± 0.12	2.86 ± 0.18	**

NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01.
(1): Degenerated embryos and unfertilized ova.

Regarding the effect of PMSG dose on embryo grade, low PMSG dose significantly increased both the average number of excellent (4.27 *vs.* 2.07/cow, P<0.01) and good (2.80 *vs.* 2.20/cow, P<0.05) embryos, and decreased average number of fair embryos (1.26 *vs.* 2.86/cow, P<0.01) as compared to high PMSG dose (Table 2).

***In vitro* culture and development of embryo category:**

After *in vitro* culture of recovered embryos at morula stage showed the best survival rate to develop to hatched blastocysts or to complete hatched blastocysts (72.7 and 63.6 % or 68.6 and 51.4 % for 2500 and 3000 IU respectively as compared to the other stages. In the same trend, compact morula stage showed insignificantly lower ability than morula stage. While, in both PMSG doses blastocyst stage showed the low survival rates of hatched or complete hatched blastocysts, the differences between both PMSG doses were not significant (Table 3).

Table (3): Effect of PMSG doses on culture and development of embryos at different stages to hatched blastocyst stage. (mean±SE)

PMSG dose	Embryonic stage	No. of cultured embryos	Developed embryos			
			Hatched blastocysts		Complete hatching	
			n	%	n	%
2500 IU	Morula	44	32	72.7	28	63.6
	Compact *	51	37	72.6	28	54.9
	Blastocyst	21	14	66.7	12	57.1
3000 IU	Morula	35	24	68.6	18	51.4
	Compact *	45	28	62.2	23	51.1
	Blastocyst	10	6	60.0	4	40.0

The differences between stages are not significant. * Compact morula

***In vitro* culture and development of embryo grade:**

Recovered embryos from superovulated cows by each PMSG dose were graded to excellent, good and fair embryos. I*n vitro* cultured, excellent embryos had the highest survival rate to develop into hatched blastocysts or to complete hatched blastocysts, being insignificantly higher for low (75.0 and 59.4%) than high (64.5 and 51.6%) PMSG dose(Table 4).

Table (4): Effect of PMSG doses on culture and development of embryos at different grades to hatched blastocyst stage. (mean±SE)

PMSG dose	Embryo grade	Number of cultured embryos	Developed embryos			
			Hatched blastocysts		Complete hatching	
			n	%	n	%
2500 IU	Excellent	64	48	75.0	38	59.4[a]
	Good	42	28	66.7	21	50.0[ab]
	Fair	19	9	47.4	6	31.6[b]
3000 IU	Excellent	31	20	64.5	16	51.6[a]
	Good	33	20	60.6	13	39.4[ab]
	Fair	43	18	41.9	12	27.9[b]

a and b : Means denoted with the same column with different superscripts are significantly different at P<0.05).

However, good embryos recovered by low PMSG dose had insignificantly higher survival rate of hatching (66.7 vs. 60.6%) than high PMSG dose the percentage of developing good embryos to complete hatching was higher (50.0%)in cows treated with 2500 IU PMSG than cows treated with 3000 IU PMSG (39.4%) but differences was not significant. While, the fair embryos had insignificantly, the lowest survival rates of hatched or complete hatched blastocysts in high PMSG dose (Table 4).

Embryo transfer:

Three fresh embryos at morula stage collected from Friesian cows superovulated by low PMSG dose (2500 IU) transferred into 3 Friesian recipient heifers, only one heifer became pregnant with pregnancy rate of 33%. Another, two Friesian recipient heifers received two vitrified/thawed embryos at morula stage, but they failed to become pregnant.

DISCUSSION

Primary factors limiting embryo production in cattle are the variability of the ovarian response following induction of superovulation with commercially available gonadotrophin preparation and the competence of the oocytes ovulated. Result obtained in this study clearly indicated at the cows treated with 2500 IU PMSG resulted in significantly ($P<0.05$) higher ovulatory response and greater total and transferable embryos than the cows treated with 3000 IU PMSG. **Greve and Jensen (1979)** found that using 3000 IU PMSG yielded more embryos than 2500 IU PMSG, but the later dose yielded significantly higher number of viable embryos than 3000 IU PMSG. Similar results were reported by several authors **(Holy, 1987; Gonzalez *et al.*, 1995; Mohammed and Ismail, 1999 and Aboul-Ela, 2000).** Also, **Manik *et al.* (1999)** suggested that treatment of buffaloes with 2500 IU PMSG (eCG) for superovulation reduced the number of large follicles 5-7 days after PG treatment. In the present result, the obtained relatively higher number of unovulated follicles and poor embryos with high (3000 IU PMSG) than low (2500 IU PMSG) is considered as one of the disadvantages of the use of PMSG. Such effect may be related to the relatively long half-life of PMSG causing increased production of oestradiol from small unovulated follicles, which is maintained for few days after the female comes into estrus **(Agarwal *et al.*, 1993). Cortell and Viudes (2008)** evaluate the reported recombinant human (rh) FSH superovulation treatments in

rabbit females induced an immune response and have a negative effect on ovulation rate. The immune response shows an important individual variability and may be related with the decreased reproductive response and embryo quality has been related to the interval among treatments in different species **(Swanson *et al.*, 1996; Kanayama and Osada, 2000 and Van Blerkom and Davis, 2001).** In agreement with the obtained results regard to culture results on cows, **Cornejo-Cortes *et al.* (2006)** found that the embryos derived from rats groups treated with high dose of PMSG (PMSG 50 IU / hcG 30 IU) or (PMSG 50 IU / hcG 50 IU) had a lower capacity to survive in culture (13.9-27% *vs.* 59.8-71.4%) than those obtained from rats groups treated with low dose (PMSG 30 IU / hcG 30 IU) or (PMSG 30 IU / hcG 50 IU). Also, **Tain *et al.* (2001)** observed that additional hCG resulted in more complete ovulation and increased quality of oocytes and subsequent embryos, but addition of HCG was unable to reverse the embryo demise caused by high dose of PMSG administered earlier. On the other hand, **Popova *et al* (2002)** found that the rates of development to blastocyst stage *in vitro* did not depend on the superovulation protocol, being 31.4% for PMSG and 23.3% for FSH. In hamster, **McKiernan and Bavister (1998)** found that the morula and blastocysts produced by females super-stimulated with PMSG have fewer cell numbers after culture and post transfer viability than embryos recovered from non-stimulated females. Also, a negative effect of PMSG on embryos development was reported by **(Ertzeid *et al.*, 1993, Paulson *et al.*, 1997).** Regarding the results of embryo transfer in this study, being higher with fresh than vitrified/thawed embryos (33 *vs.* 0%), **Greve and Del Compe (1986)** recorded a percentage of 30-40% embryonic loss following non-surgical embryo transfer in cows. So, the low pregnancy rate might be attributed to some managerial factors in embryo manipulation and the available number of recipients was small.

Generally, the present results come in line with the results of **El-Baz (2003)**, who concluded that 2500 IU PMSG dose could be recommended as an optimal dose for most of the criteria studied in supperovulatory response of Baladi cows. Therefore, the present study indicate that the most appropriate PMSG dose for superovulation is 2500 IU, which has impact on number of CL as well as number of total and transferable embryos having a more even development, a better surviving capacity *in vitro* culture on the basis of stage and grade of embryos. Further studies are needed to evaluate the effect of different doses of PMSG (<2500 or >3000 IU) in comparing FSH

doses on superovulatory response and *in vitro* culture of cow embryos to increase the available number of recipients to obtain pregnancy rate more accuracy after transfer.

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REFRENCES

Aboul-Ela, M.B.E. (2000). Superovulation in the buffaloes: constraints and manipulation. Review Article, Buffaloes Journal, 1: 1-20.

Agarwal, U.; M.C. Shankar; P.C. Yadav; S. Sanwal and V.P. Varshney (1993). Superovulation, embryo recovery and endocrine response in crossbred cattle treated with PMSG and FSH-P. Indian J. Dairy Sci., 46: 450.

Auerbach, A. B.; Norinsky R. and Ho W, (2003). Strain dependent differences in the efficiency of transgenic mouse production. Transgenenic Research 12, 59 -69.

Charreau, B.; Tesson, L.; Soulillou, J. P.; Pourcel, C. and Anegon, I. (1996). Transgenic in rats : technical aspects and models. Transgenic Research. 5, 223 - 234.

Cortell, C. and M.P. Viudes (2008). Immune response to repeated rhFSH superovulation treatment in rabbit does. 9th World Rabbit Congress, June 10-13, Verona, Italy.

Cuello, C.; F. Berthelot and F. Martinat-Botte (2004). Transfer of vitrified blastocysts from one or two superovulated large white hyper prolific donors to Meishan recipients: reproductive parameters at day 30 of pregnancy. Theriogenology, 61: 843-850.

Edashige, K.; A. Asano; T.Z. An and M. Kasai (1999). Restoration of resistance to osmotic swelling of vitrified mouse embryos by short-term culture. Cryobiology, 38: 273-280.

Eistetter H.R. (1989). Pluripotent embryonal stem cell lines can be established from disaggregated mous morulae. Development Growth & Differentiation, 31: 275-2782.

El – Baz, M. M. (2003). Physiological studies in farm animals. PhD. Fac, of Agri. Mansoura University.

Ertzeid, G.; R. Storeng and T. Lyberg (1993). Treatment with gonadotrophins impaired implantation and fetal development in mice. J. Assist. Reprod. Genet., 10:286-291.

Gonzalez, A; H. Wang; T.D. Carruthers; B.D. Murphy and R.J. Mapletoft (1995). Superovulation in the cow with pregnant mare serum gonadotropin: effect of dose and anti pregnant mare serum gonadotropin serum. Canadian Vet. J., 35:158.

Gordon, I. (1996). Embryo transfer and associated techniques in cattle. In: Gordon I, editor. *Controlled Reproduction in Cattle and Buffaloes.*

Cambridge: CAB International, University Press, UK., 245-371.

Cornejo-Cortes, M.A.; C. Sanchez-Torres; J.C. Vazquez-Chagoyan; H.M. Suarez-Gomez; G. Garrido-Farina and M.A. Meraz-Rios (2006). Rat embryo quality and production efficiency are dependent on gonadotrophin dose in superovulatory treatments. Ltd. Laboratory Animals. 40:87-95.

Graff, K.J.; M. Meintjes and Y. Han (2000). Comparing follicle stimulating hormone from two commercial sources for oocyte production from out of season dairy goats. Journal of Dairy Science,. 83: 484-487.

Greve, T. and Del Compe (1986). Embryo loss following embryo transfer in cattle and swine. In: Sreenan, J.M. and Diskin, M.G. (Eds) Embryonic Mortality in Farm animals. Martins Nijhoff for Eec, pp. 179-194.

Greve, T. and H. Jensen (1979). Current status of embryo transplantation in Denmark. Theriogenology, 11:99.

Holy, L. (1987). Results of the use of non-surgical embryo transfer in the breeding of heifers. Vet. Med., 32:634.

Kanayama, K. and H. Osada (2000). The yield of abnormal unfertilized eggs observed after repeated gonadotrophin-induced ovulation. J. Int. Med. Red., 28:24 -27.

Kanitz, W.; F. Becker and F. Schneider (2002). Superovulation in cattle: practical aspects of gonadotrophin treatment and insemination. Reproduction Nutrition Development, 42:587-599.

Lonergan, P. (1990). Studies in the *in vitro* maturation, fertilization and culture of bovine follicular oocytes. Ph. D. Thesis, National University of Ireland, Dublin.

Manik, R. S.; Singla, S. K.; Palta, P. and Madan, M. L. (1999). Changes in follicular populations following treatment of

buffaloes with PMSG (eCG) and Neutra-PMSG for superovulation. Anim. Reprod. Sci : 56. 31-38.

Marte Mucci, G.; F. Toteda; A. Manchisi; G. Lacalandra and M. Gambacorta (1988) Risposta al trattamento di superovulazione con PMSG in pecore di razza Gentile di Puglia e Altamurana. Fecondita successive a trapianto di embrioni, Zoo Nutr Anim.14:165-172.

McKiernan, S. H. and D. B. Bavister (1998). Gonadotrophin stimulation of donor females decrease post-implantation viability of cultured one-cell hamster embryos. Human Reprod. Vol. 13 no.3 : 724 – 729.

Mohammed, K.M.E. and S.T. Ismail (1999). Application of embryo transfer in Friesian cows under Egyptian conditions. Egyptian J. Agri. Res., 77:1415.

Mutiga, E.R. and A.A. Baker (1982). Superovulation response, ova recovery and fertility in Merino ewes superovulated either during the luteal phase of their estrus cycle or after intravaginal progestagen treatment. Theriogenology, 17:537-544.

Niemann, H. (1991). Cryopreservation of ova and embryos from livestock: current status and research needs. Theriogenology, 35:109-24.

Paulson, R.J.; M.V. Sauer and R.A. Lobo (1997). Potential enhancement of endometrial receptively in cycles using controlled ovarian hyperstimulation with antiprogestins: a hypothesis. Fertil. Steril., 67:321-325.

Popova, E.; A. Krivokharchenko; D. Ganten and M. Bader (2002). Comparison between PMSG and FSH induced superovulation for the generation of transgenic rats. Molecular Reproduction and Development, 63:177-182.

Ravindranatha, B.M. and S.M. Reddy (1990). Superovulatory response in improved Holstein Friesian cows under field conditions. Indian, J. Anim. Reprod., 20:88-90.

Ravindranatha, B.M. and S.M. Reddy (1999). Effect of single and double dose of PGF2α on oestrous synchronization in purebred Holstein Friesian cows. Indian, J. Anim. Reprod. Sci., 69:1040-1041.

Reddy, S.M. (1994). *In vitro* fertilization and embryo transfer in cattle. Ph. D. Thesis. Bidhan Chandra Krishi Viswavidyalaya. Mohanpur, West Bengal.

Santiago-Moreno, J.; A. Gonzalez-Bulnes and A. Gomez-Brunet (2001). Procedure for successful interspecific embryo transfer

from mouflon (Ovis gmelini musimon) to Spanish merino sheep (Ovis aries). Journal of Zoo and Wildlife Medicine, 32:336-341.

SAS (1987). SAS User' s Guid. Statistical Analysis System. Institute, Inc. Cary., NC.

Snedecor, G.W. and W.G. Cochran (1982). Statistical Methods. 7th Ed. Iowa Univ. Press, Ames. Iowa, USA.

Swanson, W.F.; T.L. Roth; K. Graham; D.W. Horohov and R.A. Godke (1996). Kinetics of the humoral immune response to multiple treatments with exogenous gonadotrophins and relation to ovarian responsiveness in domestic cats. Am J Vet Res., 57, 302 – 307.

Tain, C. F,; Goh, V. H. H, and Ng, S. C. (2001). Dose response effects of equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG) on early embryonic development and viable pregnancy rate in rats. Reproduction 122, 283 – 287.

Van Blerkom, J. and P. Davis (2001). Differential effects of repeated ovarian stimulation on cytoplasmatic and spindle organization metaphase II mouse oocytes matured *in vivo* and *in vitro*. Hum. Reprod., 16:757-764.

Vicente, J.S. and F. Garcia-Ximenez (1996). Direct transfer of vitrified rabbit embryos. Theriogenology, 45:811–815.

Walker, S.K.; D.H. Smith; D.L. Little; G.M. Warnes; P. Quinn and R.F. Seamark (1984). Artificial insemination and transfer of embryos by laparoscopy. In: DR Lindsay, DT Pearce (eds), Reproduction in sheep. Canberra. Austral. Academy of Science. 306 – 309.

الملخص العربى

الاستجابة التبويضية للأجنة القابلة للنقل المستردة بالتبويض المتعدد لأبقار الفريزيان بمستويين من هرمون مصل الفرس الحامل الجونادوتروفين

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**قسم الإنتاج الحيواني-كلية الزراعة -جامعة المنصورة

* أجريت هذه الدراسة لتحديد أمثل بروتوكول للتبويض المتعدد فى أبقار الفريزيان من أجل انتاج أكبر عدد من الأجنة الجيدة والمناسبة للنقل والزراعة المعملية. تم احداث تعدد التبويض بالحقن مرة واحدة بهرمون الجونادوتروبين لأنثى الفرس الحامل بمستويين ٢٥٠٠، ٣٠٠٠ وحدة دولية. تم معاملة ٣٠ بقرة بالجرعات المختلفة من الهرمون فى اليوم العاشر من الشياع الطبيعى، وتم تقييم الاستجابة التبوبيضية باستخدام جهاز الموجات فوق الصوتية.

* أظهرت النتائج أن الأبقار التى حقنت بجرعات منخفضة كانت أفضل عند مستوى ١% بالنسبة للأجسام الصفراء(١١,٦٧% مقابل ٩,٣٣%) وأيضا كانت أفضل عند مستوى ٥% فى الأجنة الكلية والأجنة القابلة للنقل عن الأبقار التى حقنت بجرعات عالية حيث كانت (٨,٣% و ٥,٣% مقابل ٧,١% و ٤,٢%) . بينما الحويصلات التى لم يحدث لها تبويض والاستجابة الكلية (عدد الأجسام الصفراء وعدد الحويصلات التى لم يحدث لها تبويض) لم تتأثر بجرعة هرمون الجونادوتروبين للفرس الحامل، انخفض معدل استرداد الأجنة الكلية معنويا عند مستوى ٥% (٧١,٤ % مقابل ٧٦,٤%) وزادت الأجنة القابلة للنقل زيادة غير معنوية (٦٣% مقابل ٥٩,٢%) عند استخدام الجرعات المنخفضة مقارنة بالجرعات العالية، أيضا زادت عدد الأجنة فى أطوار المربولا معنويا عند مستوى ٥%(٢,٩% مقابل ٢,٣%) والبلاستوسست عند مستوى ١% (١,٤% مقابل ٠,٦٧%) عند استخدام الجرعات المنخفضة مقارنة بالجرعات العالية بينما كان الاتجاه عكسى فى الأجنة المشوهة حيث كانت أقل معنويا عند مستوى ٥% فى الجرعات المنخفضة (٠,٦ مقابل ١,١).

لوحظ أن الحقن بجرعات مختلفة من الهرمون لم يكن لها تأثير معنوى على عدد الأجنة فى مرحلة الكومباكت مريولا. زادت عدد الأجنة الممتازة والجيدة معنويا عند مستوى ١% و٥% باستعمال الجرعات المنخفضة مقارنة بالجرعات العالية حيث كانت (٤,٣ مقابل ٢,١) و (٢,٨ مقابل ٢,٢) وقلت عدد الأجنة المتوسطة الجودة معنويا عند مستوى ١% (١,٣ مقابل ٢,٩).

كما أظهرت النتائج أن أحسن معدل حيوية للأجنة المزروعة معمليا لتتطور الى Hatched or complet hatched blastocysts (٧٢,٧% و٦٣,٦%) من الأجنة فى مرحلة المريولا للأبقارالتى حدث لها تعدد تبويض بجرعات منخفضة مقارنة بلأبقار التى حقنت بجرعات عالية حيث كانت (٦٨,٦% و٥١,٤%) على التوالى و أعطت الأجنة الممتازة أحسن نتائج حيث كانت ٧٥% و ٥٩,٤% على التوالى. بالنسبة لنقل الأجنة كان معدل الحمل ٣٣ % للأجنة الطازجة ولايوجد حمل عند نقل الأجنة المجمدة عند مرحلة المريولا للأبقار التى حقنت بالجرعات المنخفضة من الهرمون.

الخلاصة

النتائج المتحصل عليها تحت الظروف التجريبية فى هذه الدراسة أشارت إلي أن أفضل جرعة هرمونية من الجونادوتروبين لأنثى الفرس الحامل لإحداث تعدد تبويض فى أبقار الفريزيان كان ٢٥٠٠ وحدة دولية حيث أعطت أعلى استجابة تبويضية، أحسن معدل حيوية من حيث طور الجنين وجودته سواء فى الأجنة التى زرعت معمليا أو فى معدل الحمل من خلال نقل الأجنة.