THE RECOVERY RATE OF EMBRYOS USING EIGHT DIFFERENT PROTOCOLS OF SYNCHRONIZATION AND SUPEROVULATION IN SHEEP

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ABSTRAK

CAROLINE, W. dan P. H. SUMMERS. 1999. Pengumpulan embrio dari ternak domba yang diperoleh dari delapan metoda sinkronisasi dan superovulasi yang berbeda. *Jurnal Ilmu Ternak dan Veteriner* 4(1): 13-19.

Pengumpulan jumlah embrio yang banyak, yang diperoleh dan dapat ditransfer merupakan masalah yang tidak mudah dalam teknik superovulasi dari ternak domba. Tujuan dari penelitian ini adalah untuk mendapatkan sejumlah besar embrio yang dapat ditransfer dengan menggunakan delapan metoda sinkronisasi dan superovulasi yang berbeda untuk masing-masing kelompok domba. Dalam penelitian ini digunakan domba betina Merino umur 1-3 tahun dan pejantan umur 2-5 tahun. Pengumpulan embrio dilakukan secara operasi pada hari ke-7, ke-8, ke-9 dan ke-10 dihitung setelah estrus. Jumlah rataan embrio normal (3,90±1,49) yang diperoleh dari domba yang diberi perlakuan dengan oFSH (4-4,5ml) saja dan kombinasi oFSH (4-4,5ml) dengan PMSG (400 IU) yang dikumpulkan pada hari ke-7, lebih tinggi (P<0,01) dibandingkan dengan domba yang hanya diberi perlakuan dengan PMSG (1.200 IU) saja (0,92±0,32). Hasil dari penelitian ini menunjukkan bahwa kombinasi antara oFSH dan PMSG memberikan hasil yang terbaik dalam pengumpulan jumlah embrio yang dapat ditransfer dibandingkan metoda yang lainnya.

Kata kunci : Embrio, domba, superovulasi

ABSTRACT

CAROLINE, W. and P. H. SUMMERS. 1999. The recovery rate of embryos using eight different protocols of synchronization and superovulation in sheep. *Jurnal Ilmu Ternak dan Veteriner* 4(1): 13-19.

A large number of embryo recovery and also transferable is a major problem in superovulation regime of the ewe. The purpose of the study was to recover a large number of transferable embryos by using eight different protocols of synchronization and superovulation for each group of sheep. In this study, the Merino ewes aged between 1-3 years and rams between 2-5 years were used. The embryos were collected surgically on day-7, day-8, day-9 and day-10 after the onset of oestrus. The mean number of normal embryos recovered (3.90±1.49) at day 7 with oFSH alone (4-4.5ml) and the combination of oFSH (4-4.5ml) with PMSG (400 IU) was significantly higher (P<0.01) than in the sheep treated with PMSG (1,200 IU) alone (0.92±0.32). The results of this study showed that a mixture of oFSH and PMSG gives the best result in embryo recovery of the ewe and also transferable compared to other methods.

Key words : Embryo, sheep, superovulation

INTRODUCTION

Many problems still occured in order to recover embryos in a large number and also in good quality, although there are a number of published data for synchronization and superovulation of the ewe were reported previously. For synchronization, an intravaginal progestagen-impregnated sponge (RYAN *et al*., 1992; DIETRICH *et al*., 1993) or CIDR devices for the progesterone-priming phase are normally used (WHEATON *et al*., 1993; EVANS *et al*., 1994).

A range of superovulation regimes using exogenous gonadotrophins including Pregnant mare's serum gonadotrophin (PMSG) (JABBOUR and EVANS 1991; RYAN *et al*., 1992), porcine follicle stimulating hormone (pFSH) (JABBOUR and EVANS, 1991; RYAN *et*

al., 1992) or oFSH (MCKELVEY, 1994) have been used. Gonadotrophin releasing hormone is often used to enhance ovulation after pessary removal and different doses have been used (SCUDAMORE *et al*., 1993; THOMPSON *et al*., 1995). Some workers also give an injection of an analogue of prostaglandin F2α to ensure the regression of the corpora lutea (HAWK *et al*., 1987; SCUDAMORE *et al*., 1993).

Another consideration for collecting a large number of embryos is using optimum fertilization method . In protocols using natural mating, for optimum fertilization rates each ewe was mated to rotations of the rams have been used (BETTERBED and WRIGHT, 1985; THOMPSON *et al*., 1995). Recently more common to use laparoscopic artificial insemination either with fresh (EVANS and ARMSTRONG, 1984) or frozen-thawed semen (EVANS *et al*., 1994).

Those various methods mentioned above lead to a consideration of choosing protocols that could recover a large number of embryos and also transferable as the aim of this experiment by selecting the simplest and the cheapest regime as main priorities.

MATERIALS AND METHODS

Experimental procedures

Sixty-eight ewes were synchronized and superovulated using eight different protocols to obtain a large number of embryos**.** Merino ewes aged 1–3 years and rams aged 2–5 years were used for these experiments. Embryos were recovered surgically at different times (day-7, -8, -9 and -10) after the onset of oestrus. The number of corpora lutea and large follicles (more than 1 cm) were recorded for each ovary. Animals were held in paddocks at the former Department of Biomedical and Tropical Veterinary Sciences (now School of Biomedical and Molecular Sciences), James Cook University, Australia.

Synchronization of oestrus and superovulation of ewes. Several protocols were used for synchronization of oestrus and superovulation of ewes.

Protocol 1

Intravaginal sponges impregnated with 60 mg of medroxyprogesterone acetate (Repromap: Upjohn Pty. Ltd. Rydalmere, NSW, Australia) were inserted to a group of nine ewes for 13 days and on the eleventh day each ewe was given an intramuscular injection (0.5 ml) of the prostaglandin F2α analogue, cloprostenol (Estrumate: Jurox Pty. Ltd., Silverwater, NSW, Australia). The ewes were injected intramuscularly with 1,200 IU (1,000 IU/ml of diluent) of pregnant mare's serum gonadotrophin (PMSG) (Folligon: Intervet Australia Pty. Ltd., Lane Cove, NSW, Australia) 24 hrs before the removal of the vaginal sponges. After removal of the sponges, the ewes were placed with rams fitted with harnesses with coloured crayons (Crayons Stafix Ltd., Palmerston, New Zealand). Three rams mated each ewe. After one ram had mated a ewe, the ewe was moved to a second ram and after mating moved to the third ram. The ewes were checked for oestrus at 07.00–09.00, 12.00–13.00 and 17.00–18.00 hours.

Protocol 2

The same procedures were used as in protocol one for a group of six ewes, except that the ewes were not treated with Estrumate. Each ewe was mated with three different rams.

Protocol 3

A group of fourteen sheep were treated with a controlled internal drug release (CIDR) type G pessary (EAZI-breed CIDR G: Riverina Artificial Breeders, Albury, NSW, Australia) for 13 days instead of intravaginal sponges to control oestrus. Ewes were not treated with Estrumate. Each ewe was mated with three different rams.

Protocol 4

The same protocol as protocol three was applied to a group of four ewes, but the CIDRs was removed on day 12. Each ewe was mated with three different rams.

Protocol 5

The same procedures as protocol four were used for next group of four ewes but 24 hours after CIDR removal, the ewes received 100 μg of synthetic gonadotrophin releasing hormone (Fertagyl: Intervet) and immediately placed with the harnessed rams. Each ewe was placed with one ram for the entire period of oestrus.

Protocol 6

The same procedures as used in protocol five were applied to groups of eight ewes. However PMSG was replaced by ovine follicle stimulating hormone (oFSH (Ovagen: Immuno-Chemical Products Ltd, Auckland, New Zealand) and each ewe was injected intramuscularly with 4–4.5ml of Ovagen 10 days after the insertion of the CIDR. Each ewe was mated with one ram for the entire period of oestrus.

Protocol 7

Similar procedures as protocol six were used on a group of five ewes. However, Ovagen (4 ml/ewe) was given with 400 IU PMSG in 0.8 ml of diluent 10 days after CIDR insertion. Each ewe was mated with one ram for the entire period of oestrus.

Protocol 8

The same procedures as protocol seven were applied to groups of eighteen ewes. However nine days after CIDR insertion, each ewe was given 0.5 ml Estrumate. Each ewe was mated with one ram for the entire period of oestrus. All of these protocols can be seen on Table 1.

Table 1. The summary of eight protocols that were used in this study in order to recover a large number of sheep embryos

Proto-cols	Sponges	CIDR	PGF2α	PMSG	FSH	GnRH
1	+	-	+	+	-	-
2	+	-	-	+	-	-
3	-	+	-	+	-	-
4	-	+	-	+	-	-
5	-	+	-	+	-	+
6	-	+	-	-	+	+
7	-	+	-	+	+	+
8	-	+	+	+	+	+

Embryo Collection

Embryos were collected from ewes on days-7, -8, -9 and -10 after onset of standing oestrus. Animals were fasted for 24 hr before surgery. Pentobarbitone sodium (Nembutal: Boehringer Ingelheim Pty. Ltd., Artarmon, NSW, Australia) was given intravenously at a dose rate of 1.0 ml per 2 kg body weight to anaesthetize the ewes. Ventral midline laparotomy was done to expose the uterus and ovaries and the number of corpora lutea and large follicles (1 cm or greater in diameter) in each ovary recorded.

In some ewes, a hole about 3–5 mm in diameter was made in the posterior part of each uterine horn by blunt dissection with a pair of fine artery forceps and a Foley catheter inserted (8Fr/Ch, 5 ml balloon; Beiersdorf, AG, Hamburg, Germany). In other ewes, an open-end Tom Cat catheter (3.5 Fr, 14 cm long: Sherwood Medical, St. Louis, MO, USA) was inserted into the anterior ampulla of the oviduct and held in place with the surgeon's fingers for flushing the uterine horn. In some ewes, a blunt 23-G needle attached to a 20-ml syringe was inserted near the uterotubal-junction to flush the uterus. Each horn of the uterus was flushed with about 45 ml of warm (37^{o}C) ova maintenance medium [Commonwealth Serum Laboratories (CSL), Melbourne, Victoria, Australia]. The flushings were collected into sterile plastic Petri dishes (60 × 15 mm; Becton Dickinson, New Jersey, USA) and examined for embryos with a stereo-microscope.

Before replacing the uterus and ovaries into the abdominal cavity, the reproductive tract was washed carefully with normal saline to remove any blood and fibrin. A continuous suture was used to close the abdominal wall using 3.5/0 metric chromic catgut (Ethicon: Johnson and Johnson Medical Pty. Ltd., Sydney, NSW, Australia). Single interrupted chromic catgut sutures were placed in the subcutaneous tissue and interrupted sutures placed in the skin using Vetafil medium 0.40 mm (Bengen: Clements Stansen Medical, North Ryde, NSW, Australia). The ewes were given an intramuscular injection of penicillin and streptomycin [active constituents 250 mg/ml procaine penicillin, 250mg/ml dihydrostreptomycin as sulphate, 20 mg/ml procaine hydrochloride (Troy Laboratories Pty. Ltd., Australia)] at the rate of 2ml/50kg of body weight.

Statistical analysis

Data were analysed using SPSS for Windows, Release 6.1.3 (1995). The occurrence of embryos, corpora lutea and large follicles from ewes were taken into account to estimate the effect of oFSH treatment and were analysed using one-way analysis of variance, where the treatment was the independent variable and the embryos that were collected from sheep on day-7, corpora lutea and large follicles were dependent variables.

RESULTS AND DISCUSSION

The number of ewes exhibiting oestrus following the use of the various synchronization and superovulation protocols are shown in Table 2. One ewe in protocol two, three of 14 ewes in protocol three, seven of eight sheep in protocol six and four of 18 ewes in protocol eight did not exhibit oestrus within 60 hours of removal of intravaginal pessaries.

Not all ewes that exhibited oestrus could be used for embryo collection. In protocol one, all ewes had responded to treatment and all were operated on. In protocol two, one ewe did not respond and two ewes responded to the treatment but they were not operated on. In protocol three, eleven ewes responded to the treatment and ten ewes were operated on. One ewe was not flushed because the corpora lutea had regressed and only unfertilized oocytes were recovered from another ewe.

Table 2. The number of ewes exhibiting oestrus within 60 hours of pessary removal in different protocols for synchronization and

superovulation

Protocols	No. of ewes treated	No. of ewes responding (%)
1	9	9 (100%)
2	6	5 (83.3%)
3	14	11 (78.57%)
4	4	4 (100%)
5	4	4 (100%)
6	8	1 (12.5%)
7	5	5 (100%)
8	18	14 (77.78%)

In protocol four, four ewes were operated on, but no embryos were recovered. In protocol five, four ewes responded and all were operated on. Embryos were obtained from only two ewes, only three unfertilized oocytes were recovered from a third ewe and nothing was recovered from the fourth ewe. In protocol six, only one ewe responded to the treatment and was operated on. In protocol seven, all of the ewes had responded and were operated on, however, two of them had premature luteal regression and were not flushed. Embryos were recovered from only three ewes. In protocol eight, of the 14 responding ewes, two were not operated on.

The number and stage of development of embryos recovered are summarized in Table 3. The range in stage of development of embryos collected on day-7 and day-8 was regarded as normal and is similar to experiences reported by many other authors who have collected embryos from superovulated ewes.

Day-10 embryos were collected from ewes in protocols number one and eight, day-9 embryos from protocol number one, day-8 embryos from protocol number eight, and the remainder were day-7 embryos. A comparison of the embryo recovery results comparing oFSH alone, oFSH with PMSG or PMSG alone for superovulation are shown in Table 4.

The mean number of normal embryos recovered at day 7 with oFSH alone and the combination of oFSH with PMSG was significantly higher ($P<0.01$) (3.9±1.49) than in the sheep treated with PMSG alone (0.92±0.32). Of the 101 embryos recovered from ten ewes treated with oFSH alone and a combination of oFSH with PMSG, 47 (46.53%) were normal whereas only 24 (33.8%) normal embryos were found out of 71 embryos recovered from 25 sheep treated with PMSG alone. In this study, the use of 4-4.5 ml Ovagen in combination with 400 IU PMSG gave a much better superovulation response than with PMSG alone as measured by the mean number of embryos recovered per ewe (see Table 4).

Table 3. Number and stage of development of embryos and number of corpora lutea and large follicles from ewes treated by various synchronization and superovulation protocols

Prot.	Number			Embryo stages							
	Ewes	CL	LF	M	EB	B	EXB	HGB	HDB	DEG	UF
1.	9	62	27	3	-	5	2	-	2	8	-
2.	3	16	19	-	-	-	-	-	-	4	2
3.	9	65	56	1	-	-	-	1	1	5	24
4.	4	14	19	-	-	-	1	-	-	-	3
5.	4	38	16	-	4	2	1	3	-	2	3
6.	1	12	-	-	-	-	6	2	-	4	-
7.	3	43	3	3	-	1	2	-	3	20	4
8.	12	187	5	-	1	8	10	10	37	6	3

Prot.=protocols;CL=corpora lutea; LF=large follicles; M=morula; EB=early blastocyst; B=blastocyst;
EXB=expanded blastocyst; HGB=hatching blastocyst; HDB=hatched blastocyst; DEG=degenerated embryo; UF=unfertilized oocyte

Table 4. Comparison of the results of embryo recovery from ewes treated with oFSH alone, a combination of oFSH with PMSG or PMSG alone

Day of embryo recovery	Number			Stages of embryo development							
	Ewes	CL	Embryos	M	EB	B	EXB	HGB	HDB	DEG	UF
oFSH + PMSG											
Day-7	9	132	89	3	0	9	10	4	5	22	36
Day-8	5	63	37	0	1	0	2	6	27	1	0
Day-10	1	35	14	0	0	0	0	0	8	3	3
oFSH											
Day-7	1	12	12	0	0	0	6	2	0	4	0
PMSG											
Day-7	25	168	71	4	4	7	4	4	1	15	32
Day-9	3	20	2	0	0	0	0	0	2	0	0
Day-10	1	7	4	0	0	0	0	0	0	4	0

CL=corpora lutea; M=morula; EB=early blastocyst; B=blastocyst; EXB=expanded blastocyst; HGB=hatching blastocyst; HDB=hatched blastocyst; DEG=degenerated embryo; UF=unfertilized oocyte

The mean number of normal embryos recovered from ewes treated with the combination of oFSH and PMSG was significantly higher than PMSG alone. MCKELVEY (1994) obtained 2.3±0.56 transferable embryos from Suffolk ewes treated with pFSH which is less than the result from the present study (3.9±1.49). Probably this is because of the difference source of FSH used in these two studies. In the present study FSH from ovine pituitary extract was used, whereas MCKELVEY (1994) used FSH from pigs.

Unfertilized oocytes were found in a combination of oFSH with PMSG and PMSG alone (Table 5). The mean number of unfertilised oocytes for day-7 from ewes treated with oFSH alone and a combination of oFSH with PMSG was 3.60±1.83 (N=10) and this was higher than with PMSG alone (1.23±0.49; N=25). Maybe this was due to sperm transport. Sperm transport in ewes is impeded by PGF2α treatment (HAWK and COOPER, 1977; EVANS and ARMSTRONG 1984; HAWK *et al*., 1987). In the present study however, in protocol eight where PGF2α was used, embryo recovery was better than the other protocols that did not use PGF2α (protocols two to seven), with the combination of oFSH and PMSG. This is probably because the dose of PGF2α used was relatively low compared with that used by HAWK *et al.* (1987) (125μg vs 15 mg) and slightly less than EVANS and ARMSTRONG (1984) (150 vs 125 μg). Also the time difference between PGF2α injection and insemination regime or mating period in the present study was longer than that used by EVANS and ARMSTRONG (1984) (4 vs 2 days) increasing the probability that any detrimental effect of PGF2α on spermatozoa had waned.

Superovulation regimes reduce sperm transport in ewes and the effect is more marked with PMSG than FSH (EVANS and ARMSTRONG 1984). Used of laparoscopic artificial insemination techniques may have allowed for a higher fertilization rate. However, logistical reasons decided use of natural mating in the present study. Multiple mating was performed using three rams for the first four protocols to maximize the fertilization rate. Apparently the use of rotated rams did not increase embryo recovery, as seen from protocol five, where only one ram was used to mate each ewe. The embryo recovery was better than the first four protocols and this may be because in protocol five GnRH was used to enhance ovulation rate.

The number of corpora lutea and large follicles based on superovulation treatments and day of embryo recovery are summarized in Table 6. The results from ewes in which embryos were recovered on day-7 are shown in Table 7.

Table 5. Comparison of the use of oFSH alone, a combination of oFSH with PMSG or PMSG alone on the number of unfertilized oocytes, either with degenerated embryos and/or good embryos or unfertilized oocytes only or without unfertilized oocytes

Day of embryo of ewes recovery	Number of ewes	Number of ewes with UF			Range of UF	Number of ewes without UF
		UF only	UF +DEG	UF + DEG + GE		
oFSH + PMSG						
Day-7	9	2	1	1	0-16	6
Day-8	5	0	0	0	-	5
Day-10	0		0	1	-	-
oFSH						
Day-7	1	0	0	0	0	1
PMSG						
Day-7	25	6	2	0	0-11	18
Day-9	3	0	0	0	-	3
Day-10	0		0	0	-	1

UF=unfertilized oocyte; DEG=degenerated embryo; GE=good embryo

Table 6. Comparison of the number of corpora lutea (CL) and large follicles (LF) in ewes treated with oFSH alone, a combination of oFSH with PMSG or PMSG alone to induce superovulation

Day of embryo recovery	Number of		CL range	Total LF	LF range
	Ewes	CL			
oFSH + PMSG					
Day-7	9	132	4-29	6	0-3
Day-8	5	63	7-19	2	0-1
Day-10		1	35	-0	-
oFSH					
Day-7	1	12	-	0	-
PMSG					
Day-7	25	168	1-14	126	0-16
Day-8	3	20	4-11	10	0-10
Day-10	1	7	-	1	-

The number of corpora lutea in ewes treated with oFSH plus PMSG was significantly greater ($P<0.01$) than ewes treated with PMSG alone. The mean ovulation rate (by counting the total CL) in ewes treated with the combination of PMSG/oFSH was slightly higher (14.4±2.25) than found by MCKELVEY (1994) when ewes were treated only with oFSH (12.4±0.56). The mean ovulation rate in the present study from ewes treated with PMSG alone was higher than that found by MCKELVEY (1994) when he used 18 mg pFSH (6.46±0.67 vs 5.8±0.73).

Table 7. The number (mean ± sem) of corpora lutea and large follicles of ewes at day-7 treated either with oFSH alone and oFSH plus PMSG or PMSG alone

Treatment	Number of ewes	Corpora lutea	Large follicles
oFSH and oFSH + PMSG	10	14.40 ±2.25	0.60 ± 0.34
PMSG	25	6.46 ± 0.67	4.85 ± 0.97

There were significantly more large follicles ($P<0.01$) in ewes treated with PMSG alone. The number of large follicles at the time of embryo recovery was greater ($P<0.01$) in the ewes treated with PMSG alone than in ewes treated with the combination of oFSH/PMSG. This result agrees with the study of JABBOUR and EVANS (1991) who found that the number of large follicles (>5 mm) was greater in ewes treated with PMSG alone (5.8±2.9) than with pFSH (1.1±0.6) or PMSG/pFSH (1.6±0.6). However with the combination of PMSG/oFSH the mean number of unruptured follicles was less than that found byJABBOUR and EVANS (1991) (0.60±0.34 vs 1.6±0.6), possibly because they used a larger dose of PMSG (700 vs 400 IU) and pFSH (11 vs 4mg). They injected pFSH in six divided doses on three successive days, whereas in the present study the ewes received a single injection. The presence of a number of large unovulated follicles is also a common occurrence in goats or cows when superovulation is induced by PMSG (ARMSTRONG *et al*., 1982; MOOR *et al*., 1984).

CONCLUSION

From this study can be concluded that the best protocol for the recovery of acceptable numbers of normal embryos was a combination of oFSH and PMSG, treatment than single treatment of FSH and PMSG alone. Further study is needed to define the optimal concentrations for both gonadotrophins, to reduce the number of the unovulated follicles and to improve the quality of embryos recovered.

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