Ovulation Rate, Embryo Recovery and Development in Hormone-Induced Ovulated Goats Treated with $PGF_{2\alpha}$ Analogue

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Abstract: *In embryo transfer, the viability of the embryo collected in vivo or produced in vitro is important for success in the subsequent pregnancy. The present study was conducted to estimate the rate of embryo recovery in hormone-induced ovulated goats and to examine how embryo development would proceed in these animals. A series of FSH injections and a single injection of PMSG under asynchronization of estrus could induce ovulations 2 to 3 fold higher than in the unstimulated control independent of the breeding season. On the other hand, the rate of embryo recovery in FSH and PMSG injection groups differed, whereas the average number of the corpora lutea (CL) in both groups was similar. Profiles of embryo development showed that the embryos at the morula stage were collected from 6 to 8 days post coitus and at the blastocyst stage from 8 to 10 days. These results suggest that in hormonally treated goats healthy embryos could be obtained regardless of the breeding season.*

Key words: *Ovulation, Hormone, Embryo development, Goats*

In embryo transfer, the viability of the embryo collected *in vivo* or produced *in vitro* is important for success in the subsequent pregnancy [1]. When superovulation is performed to collect the embryos [2, 3], one of the major problems with superovulation is the large variability in the ovulatory response, with a relatively high proportion of animals failing to produce any transferable embryos [4]. In addition, since superovulated animals develop more follicles and subsequently more corpora lutea (CL) than unstimulated animals, this results in a drastically changed hormone milieu [5]. The oviduct-uterine environment where fertilization and early embryo development take place is regulated and controlled to a large extent by steroid secretion from the follicles and CL post ovulation [6]. There is therefore a need to keep the physiological environment within the tract as normal as possible, so that embryo development will proceed normally [7], but there has been little study of the hormone-induced ovulated goat on how embryo development would proceed. In the previous study [8], we demonstrated the follicular dynamics and ovulation rate in hormone treated goats and showed that in goats PMSG, pregnant mare serum gonadotrophin could induce ovulation more than in the control (11 vs. 2) and many follicles developed even though in the non-breeding season, although we did not examine the quality (fertilizing ability) of the oocytes recovered.

In the present study, we demonstrated whether healthy embryo could be obtained in hormone-induced ovulated goats after mating and how embryo development would proceed in these animals.

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Materials and Methods

Animals

Mature null- and multiparous females and males of Saanen breed were used. Basically, the estrous cycle was not checked throughout the experiment, but the estrous does were omitted at injection of the hormone as mentioned below. Hay and water were given *ad libitum*.

Hormone treatments and embryo collection

The does were randomly divided into the following treatment groups.

Table 1. Estrus induction and embryo recovery in hormonally stimulated goats

Treatment	No. of does used	No. (%) of does showing signs of estrus (A)	No. (%) of does having CLs in A (B)	No. of CL in B	No. of does with embryos in A	No. of embryos recovered	Rate of embryo recovery*3
FSH	12	7 (58.3)[a]	7 (100)[a]	38 (5.43 ± 2.11)*[a, b]	1	3 (3)*[a]	7.9[a]
PMSG*1	26	19 (73.1)[a]	15 (78.9)[a]	83 (5.53 ± 0.999)[a]	6	37 (6.17 ± 2.00)[a]	44.6[b]
PMSG*2	36	21 (58.3)[a]	19 (90.5)[a]	103 (5.42 ± 1.16)[a]	13	42 (3.23 ± 0.893)[a]	40.8[b]
PG only	4	4 (100)[a]	4 (100)[a]	8 (2 ± 0)[b]	—	—	—

Different superscripts within columns are significantly different (P<0.05). * average/head, mean ± S.E. *1 performed in the non-breeding season. *2 performed in the breeding season. *3 percentage calculated as No. of embryos recovered per No. of CLs counted.

Group 1: a total of 20 AU follicle stimulating hormone (FSH) (Antorin: Denka Inc., Japan) was injected intramuscularly in decreasing doses (5, 5, 4, 3 and 3 AU) over a 3 day period (morning and evening) during the breeding season. One ml (0.263 mg) of cloprostenol, prostaglandin $F_{2\alpha}$ analogue (Estrumate: Sumitomo Inc., Japan) was given as an intramuscularly injection on the day of the 5th injection of FSH.

Group 2: 1000 IU PMSG (Peamex: Sankyo Inc., Japan) as a single intramuscular injection and simultaneously the injection of 1 ml of cloprostenol were given during the breeding and non-breeding seasons.

After injection of the hormone, the estrous behavior was observed daily and on the day of detecting estrus the doe was mated at least twice with different mature males. All of the does mated were slaughtered 2 to 10 days after estrus and the reproductive tracts were collected. The oviducts and uterine horns were immediately flushed to recover embryos by means of a syringe with an 18G needle in prewarmed Dulbeccor's phosphate buffered saline (Nissui Inc., Japan). The ovaries were inspected and the ovulating points or CLs were recorded.

For statistical analysis, the significance of differences was tested by the t-test and the χ^2-test at the level of P<0.05.

Results

Hormone responses and embryo recovery

The numbers of estrous does, CLs and embryos recovered are shown in Table 1. In the FSH treatment group, in the breeding season, the induction of estrus was 58.3% (7 out of 12) and all of the estrous does had 5.4 CLs per head on average, but the number of does in which embryos were recovered, was one out of 7 estrous does. From this doe, three embryos were recovered. In the PMSG treatment groups, 73.1% (19 out of 26) the does injected during the non-breeding season had estrus. In 14 estrous does, the average number of CLs was 5.5. A total of 37 embryos were recovered from 6 does and the rate of embryo recovery calculated as the number of embryos recovered per number of CLs was 44.6%. Among the does injected with PMSG in the breeding season, 58.3% of 36 does treated showed signs of estrus, of which 19 does had a total of 103 CLs and 5.4 on average. The average number of embryos recovered was 3.2 from 13 does. In the controls that were injected only with $PGF_{2\alpha}$, the average number of ovulations was 2.

Profiles of embryo development

Table 2 shows the profiles of the developmental stage of the embryos recovered from 3 to 10 days post coitus in the hormone stimulated does. The embryos at the morula stage were collected from 6 to 8 days post coitus and at the blastocyst stage from 8 to 10 days. Most of the embryos recovered were well developed morphologically (Fig. 1).

Discussion

Ovarian status when gonadotrophin treatment commences appears to be a major determinant of a superovulatory response. This is demonstrated by the differences in response observed when gonadotrophin treatment is started at different stages of the estrous cycle and also by the stronger response obtained when treatment begins in the mid rather than the early or late cycle [9, 21]. In addition, the number of antral follicles present when gonadotrophin treatment begins has been shown to be positively related to the outcome of superovulation [2, 10], but the outcome of FSH superovulatory stimulation in heifers cannot be predicted from a knowledge of prior follicular dynamics [11]. In the goat, the advancement of time of ovulation and of the preovulatory LH surge was most pronounced when PMSG was administered 48 h before rather than at progestagen-sponge removal [12–15]. The injection of

Table 2. Profiles of development of embryos recovered from hormone treated goats

Days post coitus	Stage of development						
	2-cell	4~8-cell	9~16-cell	morula	early blast.	blastocyst	hatched blast.
3	1*	8					
4		1					
6				10			
7			1	19	4		
8		2	1	8	12	7	
9						1	4
10					1	2	

*No. of embryos collected.

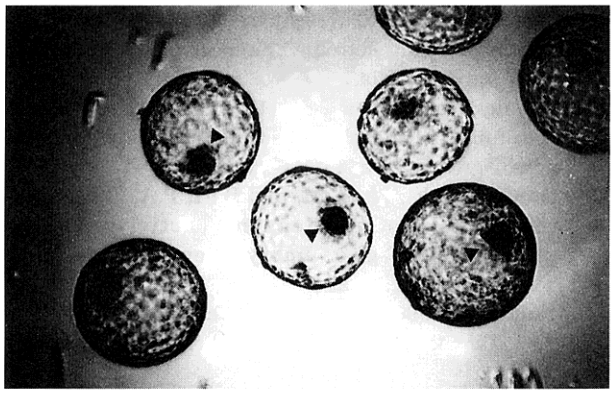

Fig. 1. Goat embryos at stage of hatched blastocyst. They were collected 9 days post coitus. Arrow heads indicate inner cell mass (× 112).

PMSG in conjunction with progestagen-sponge treatment has been shown to partly eliminate the variation in the ovulatory response and ensure that the majority of female goats ovulate whether they are treated in the breeding season or in the anestrous season [16, 17]. In the present study, we showed that a series of FSH injections and a single injection of PMSG under asynchronization of estrus could induce ovulations 2 to 3 fold higher than in the unstimulated control, independent of the season. Nevertheless, the rate of embryo recovery in the FSH and PMSG injection groups differed, although the average number of CLs in the two groups was similar. The reason for this is obscure but is partly related to the different actions of the hormones used [21]. PMSG has been used and associated with large numbers of unovulated follicles, because of its long half-life in blood [4, 20]. On the other hand, FSH, with its shorter half-life, if given frequently, results in equivalent ovulation rates but generally gives a better ovarian response in terms of fewer follicles and a larger number of transferable embryos [9].

Currently available methods of superovulation usually result in higher ovulation rates than the number of viable embryos produced/obtained because of fertilization failure and early embryonic death [1, 2]. There is a need to increase the ovulation rate and at the same time to keep the physiological environment within the tracts as normal as possible, so that embryo development will proceed normally. In the practical MOET (multiple-ovulation embryo transfer) schemes in cattle [1], the overall number of embryos recovered per donor flushed ranges from four to seven, which level is quite satisfactory, considering that the donors were either young heifers or high-yielding dairy cows. In the present study, we found that embryos with normal morphology could be obtained in the PMSG group injected in the non-breeding season better than in the breeding season. Since the estrus in the does used in this study was not synchronized before treatment, the time may result in a condition similar to that of synchronization of estrus; in turn, the animals did not naturally show signs of estrus but still responded to the exogenous hormone.

In Cashmere-Angora does that received treatment for multiple ovulation [18], embryos at the 4-cell, 8- to 16-cell, morula to early blastocyst, expanded blastocyst and hatching blastocyst stages were collected at 48, 96, 120, 144 and 168 h after ovulation, respectively. The results of this study, in which a different breed was used, showed that the time schedule of embryo development is substantially the same as that, although an exact comparison cannot be made.

In vitro maturation, fertilization and culture(IVMFC) and embryo transfer in many species have evolved into a useful research tool [1, 19, 22, 23]. Animal biotechnology programs have promise for achieving, in a single generation, improvements in commercial livestock. Application of such technology in the goat promises more rapid success in gene transfer experimentation than is

possible in cattle and other livestock with longer generational intervals [19]. The goat may serve as a suitable model for the invention of new approaches in biotechnology in ruminants.

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