Effect of superovulation on maternal serum progesterone concentration, uterine and fetal weights at weeks 7 and 15 of pregnancy in Javanese Thin-Tail ewes

W. Manalu[a, *], M. Y. Sumaryadi[b], Sudjatmogo[a] and A. S. Satyaningtijas[a]

[a] Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, Jalan Taman Kencana I No 3 Bogor 16151 Indonesia
[b] Laboratory of Physiology and Reproduction, Faculty of Animal Science, Jenderal Soedirman University, P.O. Box 100 Purwokerto 53123 Indonesia
Accepted 23 March 1998.
Available online 3 December 1998.

Abstract

Twenty-one pregnant ewe lambs (nine superovulated and 12 non-superovulated) were used to study the effects of superovulation (injecting 700 IU PMSG at the end of diestrus) on maternal serum progesterone concentrations, uterine and fetal weights at weeks 7 and 15 of pregnancy. In the ewes sacrificed at week 7 of pregnancy, superovulation increased the mean number of corpora lutea ($P<0.01$), fetuses ($P<0.01$), maternal mean serum progesterone concentration ($P<0.01$), mean uterine weight ($P<0.05$), total fetal weight ($P<0.01$), and average fetal weight ($P<0.01$) by 133%, 69%, 354%, 66%, 150% and 40%, respectively, when compared to non-superovulated ewes. In the ewes sacrificed at week 15 of pregnancy, superovulation increased the number of corpora lutea ($P<0.01$), fetuses ($P<0.05$), maternal serum progesterone concentration ($P<0.01$), uterine weight ($P<0.05$), total fetal weight ($P>0.05$), and average fetal weight ($P<0.05$) by 207%, 20%, 84%, 37%, 29% and 24%, respectively, compared to those non-superovulated ewes. It was concluded that the increased number of corpora lutea and, therefore, their hormonal secretions by superovulation could increase uterine and fetal growth and development.

Author Keywords: Superovulation; Progesterone; Uterine weight; Fetal weight; Pregnancy; Sheep

Article Outline

1. Introduction

Birth weight and postnatal life of many domestic animals are profoundly influenced by the prenatal growth and conditions (Dziuk, 1992). During the embryonal phase of pregnancy, growth and development of the embryo are influenced by the uterine growth and development (Denker, 1994), embryo–maternal interactions before implantation (Gandolfi et al., 1992) and nutrients and growth factor exchanges between the embryo and the uterus (Ashworth, 1992).

Growth and development of the uterine tissues with the overall biochemical changes before implantation are initiated by estradiol secreted during preovulation, which are then continued by progesterone (Mulholland et al., 1994) and probably by other hormones and growth factors secreted by the corpus luteum during the luteal phase of the estrous cycle. Growth and development of the fetus during the placental (or fetal) phase of pregnancy are affected by the growth and development of the placenta (Robinson et al., 1995), nutrient availabilities in the maternal, placental and the fetal circulations (Harding and Johnston, 1995; Hay, 1995), and the endocrine status of the mother and the fetus (Fowden, 1995).

The endocrine status of pregnant animals prior to implantation, which for most parts is dictated by the corpus luteum and is represented by progesterone, plays a key role in initiating a cascade of uterine growth and secretions (Knight et al., 1977; Mulholland et al., 1994).

This present experiment was designed to study the effects of superovulation on maternal serum progesterone concentrations, uterine, and fetal weight during the embryonal and placental phases of pregnancy.

2. Materials and methods

2.1. Experimental design and protocol

Forty Javanese Thin-Tail ewes, of similar body weight (14–16 kg) and age (1–1.5 years) at the beginning of experiment, were maintained in an individual pen for 2-month adaptation to the experimental conditions prior to treatment.

The experimental ewes were injected twice with 7.5 mg $PGF_{2\alpha}$ (i.m.) at an 11-day interval. Half of the experimental (20) ewes were injected with 700 IU PMSG (Folligon, Intervet, North Holland), at the same time of the last prostaglandin injection (around the end of diestrus), to stimulate superovulation, and the others (20) with saline. Two days after the last prostaglandin injections, on the onset of estrous cycle, the experimental ewes were mated individually. The experimental ewes were considered superovulated at the ovulation time (around the end of

estrus). In the Javanese thin-tail ewes, normal ovulation rate ranges from 1 to 4 (Sumaryadi and Manalu, 1995). Ewes having corpora lutea of four or more were considered superovulated.

At the beginning of the experiment, 20 ewes were assigned to be sacrificed at the end of embryonal phase (week 7), and the rest (20 ewes) at the middle of placental phase (week 15) of pregnancy. At the respective stages of pregnancy the experimental ewes were sacrificed by slaughtering. Numbers of corpora lutea and fetuses were counted, total and individual fetal weights were determined, and the uterus was excised for determination of weight. The presence of the fetus at slaughtering was used to determine pregnancy. Ewes having a mean serum progesterone concentration of lower than 3 ng/ml during the period of observation were considered to be non-pregnant (Manalu and Sumaryadi, 1998a).

2.2. Blood sampling and processing

Blood samples (10 ml) were drawn with plain vacutainer or sterile syringes from the jugular vein (0900–1000 h) on respective weeks of pregnancy (0, 2, 4, 7, 10, and 15). The weeks of blood sampling were determined with prior knowledge that maternal serum progesterone in Javanese thin-tail ewes did not change significantly from week 1 to week 7 of pregnancy, and increased linearly from week 8 to week 17 of pregnancy (Manalu and Sumaryadi, 1998a and Manalu and Sumaryadi, 1998b). The sampling time was conducted consistently prior to feeding. The blood samples were allowed to clot in a cool box and transported to the laboratory for further separation of serum by centrifugation. The serum samples were then kept frozen for further progesterone analyses.

2.3. Progesterone analyses

Serum progesterone concentrations were measured in duplicate by the solid-phase technique radioimmunoassay (Diagnostic Products, Los Angeles, CA), with slight modification for ovine progesterone concentration ranges (Manalu et al., 1996; Manalu and Sumaryadi, 1998a and Manalu and Sumaryadi, 1998b). The radioactivity of progesterone-bound tubes was counted with an automatic gamma counter. The concentrations of standard progesterone used to construct a standard curve ranged from 0.1 to 20 ng/ml. A sample volume of 100 μl serum was used in the assay for samples with progesterone concentrations ranging from 0.1 to 20 ng/ml. For samples with progesterone concentrations lower than 0.1 ng/ml, sample volume was increased to 200 μl to bring the progesterone concentrations to the range of concentration used in the standard curve. All samples' progesterone concentrations were within the range of concentrations of standard progesterone used to construct the standard curve. Inter- and intra-assay variation coefficients were 7.5% and 2.0%, respectively. The concentrations of progesterone were parallel in the sample volumes of 50, 100, and 200 μl.

2.4. Statistical analyses

Since the number of observations was limited, and the distribution of fetal number within the same treatment in each age of pregnancy was uneven, the differences between the means of parameters measured for non-superovulated and superovulated ewes were analyzed by using

Student's *t*-test (Snedecor and Cochran, 1982). Observations on week 7 and week 15 of pregnancy were also analyzed separately.

3. Results

Due to the low reproductive efficiency in ewe lambs, of 20 ewes assigned to be sacrificed at week 7 of pregnancy, only 13 ewes were pregnant (nine were non-superovulated ewes [7, 1, and 1, carrying single, twin, and triplet fetuses, respectively] and four were superovulated ewes [1, 1, and 2 ewes carrying a single, twin and triplet fetuses, respectively]). Of 20 ewes assigned to be sacrificed at week 15 of pregnancy, only eight were pregnant (three were non-superovulated ewes [1, 2, ewes carrying single and twin fetuses, respectively], and five were superovulated ewes [3, 1, and 1 ewes carrying a single, triplet and quadruplet fetuses, respectively]). From the progesterone analyses, the other ewes were regarded as non-pregnant, as progesterone concentrations were similar to those in non-pregnant ewes (2.2 ng/ml) (Manalu and Sumaryadi, 1998a), and were excluded from data analyses. Therefore, the numbers of non-superovulated and superovulated ewes used in the data analyses were 12 and 9, respectively.

Superovulation increased the number of corpora lutea by 133% (3.0 vs. 7.0) and 207% (2.7 vs. 8.2) ($P<0.01$), and fetuses by 69% (1.3 vs. 2.3) ($P<0.01$) and 20% (1.7 vs. 2.0) ($P<0.05$) in the ewes sacrificed at weeks 7 and 15 of pregnancy, respectively (Table 1 and Table 2). These data indicated that the experimental ewes injected with 700 IU PMSG were actually superovulated (CL number >4). Superovulation dramatically increased ($P<0.01$) average maternal serum progesterone concentrations by 354% (20.77 vs. 458 ng/ml), and by 84% (12.75 vs. 6.92 ng/ml), in the ewes sacrificed at weeks 7 and 15 of pregnancy, respectively. Average weekly serum progesterone concentrations in the ewes sacrificed at weeks 7 and 15 of pregnancy are presented in Fig. 1. These data indicated that superovulated ewes had consistently higher progesterone concentrations during the measurement period, compared to those in non-superovulated ewes. The superovulated ewes sacrificed at week 7 of pregnancy had a significantly ($P<0.01$) greater increase in progesterone concentrations than those sacrificed at week 15 of pregnancy at a comparable stage of pregnancy.

Table 1. Average number of corpora lutea and fetuses, maternal serum progesterone, uterine weight, and total and average fetal weight in non- and superovulated ewes sacrificed at week 7 of pregnancy (Mean±SE)

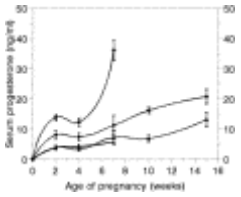
	Treatment	
	Non-superovulation	Superovulation
CL number	3.0 ± 0.4^{a}	7.0 ± 0.6^{b}
Fetal number	1.3 ± 0.2^{a}	2.3 ± 0.5^{b}
Progesterone (ng/ml)	4.6 ± 0.4^{a}	20.8 ± 1.9^{b}
Uterine weight (g)	132.3 ± 32.1^{a}	219.4 ± 39.2^{b}
Fetal weight		
Total/ewe (g)	11.3 ± 1.6^{a}	28.2 ± 6.8^{b}
Average (g)	8.7 ± 0.4^{a}	12.2 ± 1.3^{b}

[a, b]Different superscripts in the same row ($P< 0.05$ or $P< 0.01$).

Table 2. Average number of corpora lutea and fetuses, maternal serum progesterone, uterine weight, and total and average fetal weight in non- and superovulated ewes sacrificed at week 15 pregnancy (Mean±SE)

	Treatment	
	Non-superovulation	Superovulation
CL number	2.7 ± 0.7^{a}	8.2 ± 1.1^{b}
Fetal number	1.7 ± 0.3^{a}	2.0 ± 0.6^{a}
Progesterone (ng/ml)	6.9 ± 1.4^{a}	12.8 ± 2.0^{b}
Uterine weight (g)	482.8 ± 63.4^{a}	663.1 ± 96.0^{b}
Fetal weight		
Total/ewe (g)	885.2 ± 169.3^{a}	1137.8 ± 230.0^{a}
Average (g)	525.5 ± 32.1^{a}	652.0 ± 64.9^{b}

[a, b]Different superscripts in the same row ($P< 0.05$ or $P< 0.01$).



Full-size image (6K)

Fig. 1. Average serum progesterone concentration in the non-superovulated (n=9) (♦) and superovulated (n=4) (+) ewes sacrificed at week 7, and non-superovulated (n=3) (▲) and superovulated (n=5) (▪) ewes sacrificed at week 15 of pregnancy (P<0.01).

The superovulated ewes sacrificed at week 7 of pregnancy had a 66% higher uterine weight than those non-superovulated ($P<0.05$). In the ewes sacrificed at week 15 of pregnancy, superovulation increased ($P<0.05$) uterine weight by 37%, compared to those of non-superovulated ewes. In the superovulated ewes sacrificed at week 7 of pregnancy, total and average fetal weight increased by 150% and 40%, respectively, when compared to those of the non-superovulated ewes ($P<0.01$) (Table 1). In the superovulated ewes sacrificed at week 15 of pregnancy, total fetal weight increased by 29%, but the increase was not significant. Average fetal weight significantly increased by 24% ($P<0.05$) in the superovulated, compared to those of the non-superovulated ewes (Table 2).

4. Discussion

The results of this experiment were difficult to compare, since there were no available data on uterine and fetal weight, as well as serum progesterone concentrations during pregnancy in superovulated animals. Most of superovulation experiments were designed to obtain more embryos for embryo transfer, and observations on hormonal profiles were limited to the luteal phase of the estrous cycle or during the early phase of pregnancy (Bindon et al., 1971; Henricks et al., 1973; McClellan et al., 1975; Amstrong et al., 1983; Stubbing et al., 1986; Schiewe et al., 1991). The results of the present study strongly confirm that superovulation dramatically increased endogenous secretion of progesterone even until week 15 of pregnancy.

Serum progesterone concentration of the superovulated ewes sacrificed at week 15 of pregnancy was consistently lower than that of ewes sacrificed at week 7 at the same stage (weeks) of pregnancy, even though there was no significant difference in the mean corpora luteal and fetal number. This was probably due to the observation that ewes sacrificed at week 15 of pregnancy had a relatively greater number of small corpora luteal compared to those sacrificed at week 7 of pregnancy. The size of corpus luteum could vary greatly within the same number of corpora lutea, and the variation could be greater when the number of corpora luteal increase (McClellan et al., 1975). Variations in the population and diameter of steroidogenic cells in the corpus luteum had been reported to influence progesterone synthesis (Farin et al., 1988; Wiltbank and Niswender, 1992). That could probably explain the variations of progesterone concentrations observed in the superovulated ewes.

The dramatic changes in uterine weight in superovulated ewes during the embryonal phase of pregnancy indicated a greater hormonal stimulation to the uterus. Increased serum progesterone concentrations in superovulated ewes could have dramatic effects on the stimulation of uterine growth, since progesterone is found to direct influence gene expression in uterine stromal cells (Mulholland et al., 1994). Some other hormones and growth factors secreted by the corpora lutea (i.e., estradiol, relaxin and growth factors) might increase and have additive effects on uterine growth. Even though the effect of superovulation in different litter sizes could not be tested statistically in this study, individual data indicate that the superovulated ewes carrying a single fetus had an approximately 59% higher uterine weight, than those non-superovulated ewes with the same fetal number. The superovulated ewes carrying a single fetus had similar uterine

weights as those carrying twin fetuses in the non-superovulated ewes. However, to test the effect of superovulation on uterine growth in different litter sizes merits further study, so that the effect of litter size on uterine weight could be eliminated.

Greater uterine growth could increase uterine secretions (Mulholland et al., 1994). Increased uterine growth in superovulated ewes could have improved uterine secretion and synchrony between uterus and embryo and fetus (Ashworth, 1992; Gandolfi et al., 1992; Geisert et al., 1992), leading to greater fetal growth. Regardless of litter size, average fetal weight per ewe was increased by 40% and 24% in the superovulated ewes sacrificed at weeks 7 and 15 of pregnancy, respectively. These data indicate that the positive effect of superovulation is more evident in the first 7 weeks of pregnancy compared to 15 weeks of pregnancy.

The results of the experiment implied that superovulation has a promising use in increasing endogenous secretion of progesterone and improving prenatal growth in sheep. In the prolific Javanese thin-tail sheep, the higher litter size, and the lower the average birth weight (Bradford et al., 1986; Manalu and Sumaryadi, 1998a) ultimately result in lower weaning weight and higher preweaning mortality rates (Obst et al., 1980; Sutama et al., 1988; Sutama, 1992; Tiesnamurti, 1992). Use of superovulation to increase endogenous secretion of progesterone could have some advantages in improving prenatal growth.

5. Conclusion

Superovulation increased endogenous secretion of progesterone, uterine and fetal growth until week 15 of pregnancy. The dispensable number of follicles in the ovary could be used as a source of continuous endogenous progesterone during pregnancy to improve prenatal growth.

Acknowledgements

This experiment was funded by a grant provided by The Office of the State Ministry of Research and Technology and National Research Council of Republic of Indonesia through the RUT III with contract #: 3247/SP-KD/PPIT/IV/95.

References

Amstrong, D.T., Pfitzner, A.P., Warnes, G.M., Ralph, M.M. and Seamark, R.F., 1983. Endocrine responses of goats after induction of superovulation with PMSG and FSH. *J. Reprod. Fertil.* **67**, pp. 395–401

Ashworth, C.J., 1992. Synchrony embryo-uterus. Anim. Reprod. Sci. 28, 259–267

Bindon, B.M., CH'ang, T.S. and Turner, H.N., 1971. Ovarian response to gonadotrophin by Merino ewes selected for fecundity. *Aust. J. Agric. Sci.* **22**, pp. 809–820 **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (11)

Bradford, G.E., Quirke, J.F., Sitorus, P., Inounu, I., Tiesnamurti, B., Bell, F.L., Fletcher, I.C. and Torrell, D.T., 1986. Reproduction in Javanese sheep: Evidence for a gene with large effect of ovulation rate and litter size. *J. Anim. Sci.* **63**, pp. 418–431 View Record in Scopus | Cited By in Scopus (29)

Denker, H.W., 1994. Cell biology of endometrial receptivity and of trophoblast–endometrial interactions. In: Glasser, S.R., Mulholland, J., Psychoyos, A. (Ed.), Endocrinology of Embryo–Endometrium Interactions. Plenum Press, New York, pp. 17–32
Dziuk, P.J., 1992. Embryonic development and fetal growth. *Anim. Reprod. Sci.* **28**, pp. 299–308 Abstract | **Article** | PDF (588 K) | View Record in Scopus | Cited By in Scopus (14)
Farin, C.E., Moeller, C.L., Mayan, M.H., Gamboni, F., Sawyer, H.R. and Niswender, G.D., 1988. Effect of luteinizing hormone and human chorionic gonadotropin on cell populations in the ovine corpus luteum. *Biol. Reprod.* **38**, pp. 413–421 **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (32)
Fowden, A.L., 1995. Endocrine regulation of fetal growth. Progress in perinatal physiology. *Reprod. Fertil. Dev.* **7**, pp. 351–363 **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (140)
Gandolfi, F., Brevini, T.A.L., Modina, S. and Pasoni, L., 1992. Early embryonic signals: Embryo–maternal interactions before implantation. *Anim. Reprod. Sci.* **28**, pp. 269–276 Abstract | **Article** | PDF (467 K) | View Record in Scopus | Cited By in Scopus (14)
Geisert, R.D., Short, E.C. and Zavy, M.T., 1992. Maternal recognition of pregnancy. *Anim. Reprod. Sci.* **28**, pp. 287–298 Abstract | **Article** | PDF (713 K) | View Record in Scopus | Cited By in Scopus (16)
Harding, J.E. and Johnston, B.M., 1995. Nutrition and fetal growth. Progress in perinatal physiology. *Reprod. Fertil. Dev.* **7**, pp. 539–547 **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (106)
Hay Jr., W.W., 1995. Regulation of placental metabolism by glucose supply. Progress in perinatal physiology. *Reprod. Fertil. Dev.* **7**, pp. 365–375 **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (30)
Henricks, D.M., Hill Jr., J.R., Dickey, J.F. and Lamond, D.R., 1973. Plasma hormone levels in beef cows with induced multiple ovulations. *J. Reprod. Fertil.* **35**, pp. 225–233 **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (3)
Knight, J.W., Bazer, F.W., Thatcher, W.W., Franke, D.E. and Wallace, H.D., 1977. Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: Interrelations among hormonal status, placental development, fetal fluids and fetal growth. *J. Anim. Sci.* **44**, pp. 620–637 View Record in Scopus | Cited By in Scopus (96)
Manalu, W., Sumaryadi, M.Y., 1998a. Maternal serum progesterone concentration during gestation and mammary gland growth and development at parturition in Javanese thin-tail ewes carrying a single or multiple fetuses. Small Rumin. Res. 27, 131–136
Manalu, W., Sumaryadi, M.Y., 1998b. Maternal serum progesterone concentration during pregnancy and lamb birth weight at parturition in Javanese thin-tail ewes carrying different fetal sizes. Small Rumin. Res. 30, 163–169
Manalu, W., Sumaryadi, M.Y. and Kusumorini, K., 1996. Effect of fetal number on the concentrations of circulating maternal serum progesterone and estradiol of does during late pregnancy. *Small Rumin. Res.* **23**, pp. 117–124
McClellan, M.C., Dickman, M.A., Abel, J.H. and Niswender, G.D., 1975. LH, progesterone and the morphological development of normal and superovulated corpora lutea in sheep. *Cell Tiss. Res.* **164**, pp. 291–307 View Record in Scopus | Cited By in Scopus (17)

Mulholland, J., Roy, D., Glasser, S.R., 1994. Progesterone directed gene expression in rat uterine stromal cells. In: Glasser, S.R., Mulholland, J., Psychoyos, A. (Ed.), Endocrinology of Embryo–Endometrium Interactions. Plenum Press, New York, pp. 33–39
Obst, J.M., Boyes, T. and Chaniago, T., 1980. Reproductive performance of Indonesian sheep and goats. *Proc. Aust. Soc. Anim. Prod.* **13**, pp. 321–324
Robinson, J., Chidzanja, S., Kind, K., Lok, F., Owens, P. and Owens, J., 1995. Placental control of fetal growth. Progress in perinatal physiology. *Reprod. Fertil. Dev.* **7**, pp. 333–344 **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (45)
Snedecor, G.W., Cochran, W.C., 1982. Statistical Methods. 7th ed., Iowa State University Press, Ames, IA, pp. 234–235
Schiewe, M.C., Fitz, T.A., Brown, J.J., Stuart, L.D. and Wildt, D.E., 1991. Relationship of oestrous synchronization method, circulating hormones, luteinizing hormone and prostaglandin F-2α receptors and luteal progesterone concentration to premature luteal regression in superovulated sheep. *J. Reprod. Fertil.* **93**, pp. 19–30 **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (17)
Stubbing, R.B., Bosu, W.T.K., Barker, C.A.V. and King, G.J., 1986. Serum progesterone concentrations associated with superovulation and premature corpus luteum failure in dairy goats. *Can. J. Vet. Res.* **50**, pp. 369–373
Sumaryadi, M.Y., Manalu, W., 1995. The effects of corpora luteal number on serum progesterone and estradiol of ewes during luteal phase of estrous cycle and pregnancy. Bull. Anim. Sci. Spec. Ed. 231–235
Sutama, I.K., 1992. Reproductive development and performance of small ruminants in Indonesia. In: Ludgar, P., Scolz, S. (Ed.), New Technology for Small Ruminant Production in Indonesia. Winrock International Institute for Agricultural Development, Morritton, AR, pp. 7–14
Sutama, I.K., Edey, T.N. and Fletcher, I.C., 1988. Studies on reproduction of Javanese thin-tail ewes. *Aust. J. Agric. Res.* **39**, pp. 703–711 **Full Text** via CrossRef
Tiesnamurti, B., 1992. Reducing the preweaning mortality rate of Javanese thin-tail sheep. In: Ludgar, P., Scolz, S. (Ed.), New Technology for Small Ruminant Production in Indonesia. Winrock International Institute for Agricultural Development, Morritton, AR, pp. 71–80
Wiltbank, M.C. and Niswender, G.D., 1992. Functional aspects of differentiation and degeneration of the steroidogenic cells of the corpus luteum in domestic ruminants. *Anim. Reprod. Sci.* **28**, pp. 103–110 Abstract | **Article** | PDF (478 K) | View Record in Scopus | Cited By in Scopus (15)