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Interaction effect of donor age and body weight on superovulation and embryo production performance in goats

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ABSTRACT

The experiment was conducted to evaluate the interaction effect of age and body weight (BW) on ovarian responses in follicle stimulating hormone (pFSH) superovulated doe. Does (40) were divided into groups 1 (2–4 years old and 15–25 kg BW), 2 (2–4 years old and 26–35 kg BW), 3 (5–7 years old and 15–25 kg BW) and 4 (5–7 years old and 26–35 kg BW). Oestrus was synchronized by inserting a controlled internal drug release (CIDR) and a single injection of prostaglandin F2α. Does were administered 5 mg pFSH/kg BW, twice a day through 6 decreasing dosages. All does of Group 2 showed oestrus signs, while 70, 70 and 80% of does of group 1, 3 and 4 showed oestrus, respectively. Natural mating was carried out when does showed oestrus. Ovarian responses were evaluated on Day 7 after CIDR removal. Group 2 produced higher number of corpus luteum than other groups. Average number of embryo production was higher in group 2 than that in groups 1, 4 and 3. Transferable embryo production was higher in group 2 than in groups 4 and 1. Group 3 did not produce any transferable embryos. Numbers of anovulatory follicles per doe were similar in group 2 and 4, but higher than that in group 1 and 3 and did not show any differences. Does in group 2 gave higher number of embryos than other groups, indicating that donor age and BW interaction is an important consideration for superovulation in goats.

Key words: Age, Body weight, Embryo production, Follicle stimulating hormone, Goat, Superovulation

Superovulation is used for growth and maturation of follicles for fertilization to obtain good number of quality embryos (Baldassarre *et al*. 2003). Using this technology, goat could be a good model animal to transform from animal farming to animal pharming by producing different recombinant pharmaceutical proteins in its milk (Paramio and Izquierdo 2014). Superovulation gave inconsistent performance and could fluctuate from complete failure to total success without any change in standard operating procedure (Baldassarre and Karatzas 2004). Researches were carried out to minimize the inconsistency and find out some affecting factors such as type and dosages of exogenous gonadotrophin, fertilization failure, anovulatory follicles, breed, donor age and reproductive status at the onset of a superovulatory treatment (Gonzalez-Bulnes *et al*. 2004, Rahman *et al*. 2014a,b).

Age and body weight (BW) influence reproductive events of goat such as onset of puberty, kidding, litter size and birth weight. It also had positive correlation with the reproductive traits (Flores *et al*. 2008, Snyman 2010). Effect of donor age on the ovarian response to superovulation are well studied in sheep and cattle than in goats. However, there is limited information and investigation on interaction of age and BW on superovulation performance using follicle stimulating hormone (pFSH) according to per kg BW. Therefore, present research was undertaken to evaluate the interaction effect of age and BW of an embryo donor on the ovarian response to superovulation in goats.

MATERIALS AND METHODS

Crossbred (local × Boer) goats (40) were used to evaluate the effect of donor age and BW on superovulation performance. All animals used in this experiment were in accordance with the guidelines of the University of Malaya. Oestrus was synchronized with a controlled internal drug release (CIDR) device for 14 days and an intramuscular injection of prostaglandin F2α (estrumate) on Day 11. A 2×2 factorial experiment was designed including: (a) 2 age's groups, namely 2–4 years of age and more than 5–7 years of age; (b) 2 BW groups, namely 15–25 kg and 26–35 kg. After oestrus synchronisation, all the donor does were weighed and divided randomly into group 1 (2–4 yr of age and 15–25 kg BW), group 2 (2–4 yr of age and 26–35 kg BW), group 3 (5–7 yr of age and 15–25 kg BW) and group

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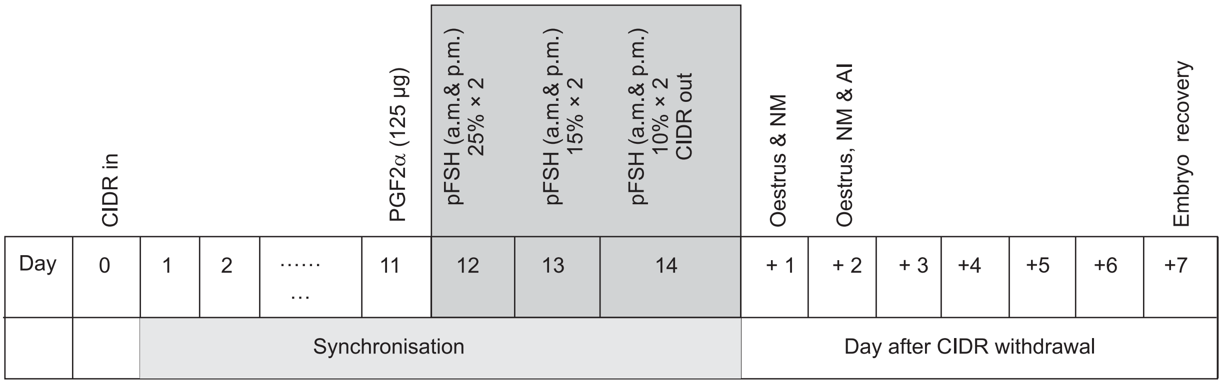

Fig. 1. Schematic representation of the superovulation protocol. NM, natural mating; AI, artificial insemination.

4 (5–7 yr of age and 26–35 kg BW). The superovulatory amount of pFSH (folltropin-V) was calculated according to 5 mg pFSH/kg BW. Total amount of pFSH for each donor was divided into 6 decreasing (25, 25, 15, 15, 10 and 10%) dosages and given twice daily (08:00–09:00 h and 18:00–19:00 h) for 3 successive days. The amount of pFSH per kg BW was 1.25, 1.25, 0.75, 0.75, 0.50 and 0.50 mg. The pFSH treatments were initiated at 2 days before the CIDR removal (Fig. 1). After CIDR removal, oestrus was observed 3 times a day (08:00–09:00 h, 13:00–14:00 h and 19:00–20:00 h) by placing a buck of proven libido. Does showed sign of oestrus within 24 to 30 h after CIDR removal. Subsequently, natural mating was carried out when the does showed oestrus sign. After showing the signs of estrus, the dose was placed in a pen with a male for natural mating. On Day 7 after CIDR removal, ovarian responses were evaluated and embryos were collected during laparotomy session (Rahman *et al*. 2014a). For ovarian response, the number of corpus luteum (CL), number of unovulated follicles, transferrable embryos, number of degenerated embryos and number of unfertilized oocytes were counted for both the ovaries. Ovulation percentage was calculated as:

$$\text{Ovulation percentage} = \frac{\text{No. of CL}}{\text{Total no. of stimulation}} \times 100$$

Recovery rate was calculated as:

$$\text{Recovery rate} = \frac{\text{No. of embryo + unfertilized oocytes}}{\text{Total no. of CL}} \times 100$$

The effects of donor dose and BW on ovarian stimulation and embryo production characteristics were examined by one-way analysis of variance followed by least significant difference (LSD) test using SPSS (version 16.0).

RESULTS AND DISCUSSION

Age and BW along with other factors determine the reproductive events of goat such as onset of puberty, kidding, litter size and birth weight. In farm animals, age and BW of donor dose had positive correlation with the reproductive traits (Flores *et al*. 2008, Snyman 2010). In this experiment, CIDR synchronised oestrus and pFSH superovulated dose from group 2 showed 100% signs of standing oestrus (Table 1). However, dosages of groups 1, 3 and 4 showed 70, 70 and 80% signs of standing oestrus, respectively. The results of oestrus in group 2 were in agreement with the findings of earlier researchers who reported 100% oestrus using goats with various ages (1–4 years) and BW (29–69 kg) and superovulated with 200 mg of pFSH and CIDR (Lehloenya and Greyling 2010, Lehloenya 2013). In addition, goats having 2–6 years of age and 53–83 kg BW, and immunised actively against inhibin showed 100% of oestrus by superovulating with pFSH and Norgestomet ear implants (Holtz *et al*. 2012).

Similarly, result on oestrus of group 2 of this experiment was similar with previous researchers who reported 98% of standing oestrus for dairy does of 2–6 years of age using 180 mg pFSH with FGA intravaginal sponge (Cervantes *et al*. 2007). Moreover, oestrus was observed 94% in Boer crossbred dose of 1.5–5.0 years age and 18–35 kg BW in Malaysia and 88% in Yunnan Black mountain dose of 1.5–

Table 1. Ovarian stimulation (mean±SEM) in goats based on donor age and body weight (BW)

Parameter	Group 1	Group 2	Group 3	Group 4
Number of does	10	10	10	10
Number of does showing oestrus	7	10	7	8
Average number of corpus luteum (CL)/doe	3.30±1.27^{a}	11.70±2.75^{b}	3.30±1.84^{a}	1.40±0.91^{a}
Average number of anovulatory follicle (AF)/doe	3.30±1.67	5.20±2.74	2.90±1.00	5.10±1.78
Average number of ovarian stimulation (CL + AF)/doe	6.60±1.91^{a}	16.90±4.17^{b}	6.20±2.00^{a}	6.50±1.61^{a}
Ovulation percentage	43.33±15.75^{ab}	78.64±11.30^{b}	29.13±13.54^{a}	29.00±14.79^{a}

Group 1, 2–4 year and 15–25 kg; group 2, 2–4 year and 26–35 kg; group 3, 5–7 year and 15–25 kg; group 4, 5–7 year and 26–35 kg. ^{a,b}Mean values with different superscripts within a row were significantly (P<0.05) different. SEM, Standard error of mean.

5.0 years age and 35–50 kg BW in China by using 160 mg pFSH with CIDR (Xiao *et al*. 2013). The lowest result of oestrus was 70% in this experiment which was higher than some other earlier researchers who reported around 50% of oestrus for Boer purebred does with 2–6 years age and 50–80 kg BW using 20 mg pFSH with CIDR and Norgestomet ear implant, respectively (Holtz *et al*. 2012). The oestrus synchronisation and superovulation data proved that donor does with correct age and BW could be enhanced by administering with sufficient and correct amount of pFSH with CIDR.

The pFSH superovulatory response on percentage of dosages producing CL was higher in group 2 than other treatment groups, and they were 50, 90, 40 and 30% for groups 1–4, respectively (Table 2). Numbers of ovulation and ovulation percentages were higher in group 2 than other groups which might be related to optimal specific dosages (5 mg pFSH/kg BW) for specific age (2–4 yr) and BW (26–35 kg BW). Several studies were conducted to evaluate the effect of age on the ovarian response to superovulation. Fertility in the does increased up to about 6 years of age, and after this age the conception rates decreased gradually (Hisham 2007). It is well known that juveniles could be superovulated, and the follicles were sensitively recruited to gonadotrophin administration (Kuhholzer and Brem 1999), but ovarian response to superovulation, embryo recovery and survival rates were not as good as compared to multiparous or adult does (Mahmood *et al*. 1991, Driancourt and Avdi 1993). Torres and Sevellec (1987) reported that highest embryo production in ewe can be obtained at around 6 years of age. On the contrary, Koemann *et al*. (2003) reported a greater number of oocyte recovery from superstimulated 3–7 months old prepubertal does than in adult (2–4 years of age) does. However, the use of older multiparous does as embryo donors in a multiple ovulation and ET programme was favourable (Lehloenya and Greyling 2010).

Reproductive performance of a doe was affected by the changes of her BW (Zarazaga *et al*. 2005), and severe BW loss might cause anoestrus in a cow (Richards *et al*. 1989). Body weight affected the fertility and prolificacy in ewe (Mellado *et al*. 2004, Madani *et al*. 2009). Serin *et al*. (2010) reported that low BW affected the pregnancy rate in synchronised and superovulated doe. The kidding performance of does less than 25 kg BW (mature BW 18 kg) was very poor during the first year and reduced life-time production by 10 to 40% (McGregor 2007). Smith (1985) reported that mature BW of a ewe was positively correlated with breeding specially ovulation. In ewe, increasing BW during mating was positively correlated with the prolificacy (Gaskins *et al*. 2005). Plasma luteinizing hormone levels of growing mithuns (*Bos frontalis*) were positively correlated with BW (Mondal *et al*. 2005). On the contrary, De la Isla-Herrera *et al*. (2011) did not observe any effect of body condition change (positive or negative) on the follicular development or the ovulation rate in ewes under a medium body condition.

In this experiment, the numbers of recovered and transferrable embryos were higher in group 2 than groups 1, 3 and 4. Exogenous pFSH (5 mg/kg BW) could promote ovarian responses in the desired age and BW groups. However, the superovulation effect did not promote by old (5–7 years) and low BW (15–25 kg) as suggested in this present experiment. It was hypothesised that old does had less response to the exogenous pFSH, which might be due to the reduced ovarian sensitivity. Jainudeen *et al*. (2000) reported that age could contribute greatly to the variation in ovarian response to superovulation, because animals of different ages had different physiological needs and responded differently. Serin *et al*. (2010) reported that low BW affected the pregnancy rate in does. On the contrary, Anna *et al*. (2013) reported that age and BW of does had no effect on oocyte recovery of superstimulated does.

Anovulatory follicles were common in superovulatory protocols (Veiga-Lopez *et al*. 2006). In this experiment, the incidence of anovulatory follicles was 30–78%, which was higher in group 4 (78%) followed by group 1 (50%), group 3 (47%) and group 2 (30%). The results indicated that incidence of anovulatory follicles generally increased with the older age and higher BW or younger age and lower BW. However, younger age and higher BW gave the lower anovulatory follicles. Our result was similar with the findings of Abdullah *et al*. (2012) who reported 28–60% anovulatory follicles for superovulated does. However, age

Table 2. Effect of donor does age and body weight (BW) on embryo production characteristics (mean±SEM)

Parameter	Group 1	Group 2	Group 3	Group 4
Number of does with corpus luteum	5	9	4	3
Average number of structures recovered/does	1.10±0.55[a]	5.20±1.62[b]	0.50±0.40[a]	0.80±0.70[a]
Average number of embryos/does	1.10±0.55[a]	5.10±1.64[b]	0.20±0.13[a]	0.80±0.70[a]
Average number of transferable embryos/does	0.60±0.50[a]	4.90±1.70[b]	-	0.70±0.70[a]
Average number of degenerated[d] embryos/does	0.20±0.13	0.20±0.13	0.10±0.10	0.10±0.10
Recovery rate (%)	16.30±7.25[a]	50.50±12.05[b]	7.50±5.34[a]	12.80±8.78[a]
Average number of unfertilised oocytes/does	-	0.10±0.10	0.30±0.30	-

Group 1, 2–4 year and 15–25 kg; group 2, 2–4 year and 26–35 kg; group 3, 5–7 year and 15–25 kg; group 4, 5–7 year and 26–35 kg. [a,b]Mean values with different superscripts within a row were significantly ($P<0.05$) different. SEM, Standard error of mean. All the embryos with regular shape and stage were classified as transferrable embryo. [d]The embryos with retarded growth and irregular shape were classified as degenerated embryo.

and BW were not considered for their experiments. The real explanation for this phenomenon was unknown. However, the causal factor for anovulation might be due the presence of LH in the commercial FSH preparation, which may alter the endocrine and ovarian functions after administration of a high dosage of exogenous pFSH which resulted in smaller size and defective follicular recruitment and selection (Veiga-Lopez *et al*. 2006).

The age and BW had an interaction effect on superovulation and embryo production performance. Two to four years of age and 26–35 kg BW produced a higher number of ovarian stimulation and ovulation percentage, subsequently produced higher number of total and transferrable embryos with the highest recovery rate. Results suggested that 2–4 years old and more than 25 kg BW donor does superovulated with 5 mg pFSH/kg BW was suitable for producing embryos in the Boer crossbred does.

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REFERENCES

Abdullah R B, Shariffah N Y, Rahman M R and Wan Khadijah W E. 2012. Ovarian responses after superovulation with different dosages of oFSH in goats. *Malaysian Journal of Animal Science* **15**: 21–25.

Anna A A, Hisham M N, Wan Khadijah W E and Abdullah R B. 2013. Comparative study on goat oocyte recovery methods and factors affecting the quantity and quality of oocytes. *Asian Journal of Animal and Veterinary Advances* **8**: 437–48.

Baldassarre H and Karatzas C N. 2004. Advanced assisted reproduction technologies (ART) in goats. *Animal Reproduction Science* **82–83**: 255–66.

Baldassarre H, Wang B, Kafidi N, Gauthier M, Neveu N, Lapointe J, Sneek L, Leduc M, Duguay F, Zhou J F, Lazaris A and Karatzas C N. 2003. Production of transgenic goats by pronuclear microinjection of *in vitro* produced zygotes derived from oocytes recovered by laparoscopy. *Theriogenology* **59**: 831–39.

Cervantes M J, Juarez M L, Mejia V O, Berruecos V J M, Vera A H and Valencia J. 2007. Use of fluorogestone acetate after breeding to reduce the effect of premature luteal regression in dairy goats when superovulation is induced with FSH. *Animal Reproduction Science* **97**: 47–54.

De la Isla-Herrera G, Ayala-Burgos A, Ake-Lopez R and Gonzalez-Bulnes A. 2011. Effects of body condition change over oestrus, follicular development and ovulation rate in pelibuey ewes under tropical conditions. *Tropical and Subtropical Agroecosystems* **14**: 337–47.

Driancourt M A and Avdi M. 1993. Effect of the physiological stage of the ewe on the number of follicles ovulating following hCG injection. *Animal Reproduction Science* **32**: 227–36.

Flores R, Looper M L, Rorie R W, Hallford D M and Rosenkrans C F. 2008. Endocrine factors and ovarian follicles are influenced by body condition and somatotropin in postpartum beef cows. *Journal of Animal Science* **86**: 1335–44.

Gaskins C T, Snowder G D, Westman M K and Evans M. 2005. Influence of body weight, age, and weight gain on fertility and prolificacy in four breeds of ewe lambs. *Journal of Animal Science* **83**: 1680–89.

Gonzalez-Bulnes A, Baird D T, Campbell B K, Cocero M J, Garcia-Garcia R M, Inskeep E K, Lopez-Sebastian A, Mcneilly A S, Santiago-Moreno J, Souza C J H and Veiga-Lopez A. 2004. Multiple factors affecting efficiency of multiple ovulation and embryo transfer in sheep and goats. *Reproduction Fertility and Development* **16**: 421–35.

Hisham M N. 2007. 'Effect of oestrus synchronisation and superovulationon progesterone and oestradiol levels in relation to oocyte recovery in goats.' M.Sc. Thesis, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia. p. 292.

Holtz W, Wang X, El-Gayar M and Knight P G. 2012. The effect of exogenous gonadotropins on ovarian function in goats actively immunized against inhibin. *Theriogenology* **77**: 253–59.

Jainudeen H R, Wahid H and Hafez E S E. 2000. Sheep and goats. *Reproduction in Farm Animals*. 7th edn. (Eds) Hafez E S E and Hafez B. Lippincott Williams and Wilkins, Philadelphia, USA.

Koeman J, Keefer C L, Baldassarre H and Downey B R. 2003. Developmental competence of prepubertal and adult goat oocytes cultured in semi-defined media following laparoscopic recovery. *Theriogenology* **60**: 879–89.

Kuhholzer B and Brem G. 1999. *In vivo* development of micro injected embryos from superovulated prepubertal slaughter lambs. *Theriogenology* **51:** 1297–1302.

Lehloenya K C. 2013. Preliminary results evaluating a simplified superovulation protocol in Boer goats. *Small Ruminant Research* **113**: 171–74.

Lehloenya K C and Greyling J P C. 2010. The ovarian response and embryo recovery rate in Boer goat does following different superovulation protocols, during the breeding season. *Small Ruminant Research* **88**: 38–43.

Madani T, Chouia F and Abbas K. 2009. Effect of oestrus synchronisation and body condition on reproduction of anoestrous Ouled Djellal Ewes. *Asian Journal of Animal and Veterinary Advances* **4**: 34–40.

Mahmood S, Koul G L and Biswas J C. 1991. Comparative efficacy of FSH-P and PMSG on superovulation in Pashmina goats. *Theriogenology* **35**: 1191–96.

Mellado M, Valdez R, Lara L M and Garcia J E. 2004. Risk factors involved in conception, abortion, and kidding rates of goats under extensive conditions. *Small Ruminant Research* **55**: 191–98.

Mondal M, Rajkhowa C and Prakash B S. 2005. Secretion patterns of luteinizing hormone in growing mithuns (*Bos frontalis*). *Reproductive Biology* **5**: 227–35.

Paramio M T and Izquierdo D. 2014. Assisted reproduction technologies in goats. *Small Ruminant Research* **121**: 21–26.

Richards M W, Wettemann R P and Schoenemann H M. 1989. Nutritional anestrus in beef cows: body weight change, body condition, luteinizing hormone in serum and ovarian activity. *Journal of Animal Science* **67**: 1520–26.

Rahman M R, Rahman M M, Wan Khadijah W E and Abdullah R B. 2014a. Follicle stimulating hormone (FSH) dosage based on body weight enhances ovulatory responses and subsequent embryo production in goats. *Asian Australasian Journal of Animal Sciences* **27**: 1270–75.

Rahman M R, Rahman M M, Wan Khadijah W E and Abdullah R B. 2014b. Comparison of superovulatory effect of equine chorionic gonadotrophin (eCG) and follicle stimulating hormone (FSH) on embryo production in crossbred (Boer × Katjang) goats. *Pakistan Journal of Zoology* **46**: 819–26.

Serin I, Serin G, Yilmaz M, Kiral F and Ceylan A. 2010. The effects of body weight, body condition score, age, lactation, serum trygliceride, choloesterol and paraoxanase levels on pregnancy rate of Saanen goats in breeding season. *Journal of Animal and Veterinary Advances* **9**: 1848–51.

Smith J F. 1985. Protein, energy and ovulation rate. Genetics on Reproduction in Sheep. pp. 349–59. (Eds.) Land R B, Robinson D W. Butterworths, London.

Snyman M A. 2010. Influence of body weight, age and management system on reproduction of South African Angora goat does. *South African Journal of Animal Science* **40**: 41–53.

Torres S and Sevellec C. 1987. Repeated superovulation and surgical recovery of embryos in the ewe. *Reproduction Nutrition and Development* **27**: 859–63.

Veiga-Lopez A, Gonzalez-Bulnes A, Tresguerres J, Dominguez V, Ariznavarreta C and Cocero M. 2006. Causes, characteristics and consequences of anovulatory follicles in superovulated sheep. *Domestic Animal Endocrinology* **30**: 76–87.

Xiao Z C, Wan Khadijah W E, Abdullah R B and Rahman M M. 2013. Effects of hormonal administration and locality influence on superovulatory responses in goats. *Indian Journal of Animal Sciences* **83**: 927–29.

Zarazaga L A, Guzman J L, Dominguez C, Perez M C and Prieto R. 2005. Effect of plane of nutrition on seasonality of reproduction in Spanish Payoya goats. *Animal Reproduction Science* **87**: 253–67.