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A simplified superovulation protocol using split-single administration of Folltropin®-V in hyaluronan: application to purebred sheep

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ABSTRACT: Superovulation is an important step in assisted reproductive technology. Due to its short half-life, follicle stimulating hormone is usually given twice daily to ewes for three to five days, which is both time- and labour-intensive. However, dissolving follicle stimulating hormone in degradable polymers to delay absorbtion has been effective in ruminants. Experiment 1 was performed to compare a split-single follicle stimulating hormone dissolved in hyaluronan (S group; 150 mg follicle stimulating hormone on the first day and 30 mg 48 h later; n = 21) and six decreasing doses of follicle stimulating hormone (M group; 50, 50, 30, 30, 10 and 10 mg; n = 22) at 12-h intervals. Ovarian responses and numbers of recovered ova/embryos did not differ significantly between groups. However, there tended to be more Grade 1 and 2 embryos in S vs M groups (mean ± SEM, 5.1 ± 4.9 vs 2.9 ± 2.9, respectively; P = 0.08). Experiment 2 tested the effectiveness of a simplified split-single follicle stimulating hormone in purebred sheep on a commercial farm. The numbers of recovered good-grade embryos (day 2) were 4.8 ± 5.0 and 4.0 ± 2.5 per donors in Corriedale and Bond sheep breeds, respectively. We conclude that this modified technique for ewe superovulation improved animal welfare, reduced animal handling and labour and yielded results similar to or better than conventional twice-daily follicle stimulating hormone treatments.

Keywords: ovine; follicle stimulating hormone; FSH

The sheep industry in Thailand is modest but expanding. Purebred sheep, wool and/or meat types, have been increasingly imported for crossbreeding or to maintain purebred flocks. However, costs of transportation and adaption are major obstacles. Reproductive biotechnologies, e.g., laparoscopic artificial insemination and/or embryo transfer (ET), may be alternatives. Recently, laparoscopic artificial insemination/ET was very successful in purebred goats raised in tropical conditions (Anakkul et al. 2013).

Follicle stimulating hormone (FSH) has a short half-life and multiple injections are needed to achieve a superovulatory response. However, that requires considerable time and labour and may adversely affect animal welfare. To overcome these limitations, alternative delivery of FSH has been proposed. For example, a single superovulation injection with FSH in degradable polymers to sustain hormone release has been studied in ruminants (Lopez-Sebastian et al. 1993; Yamamoto et al. 1994; Satoh et al. 1996; Sugano and Shinogi 1999; D'Alessandro et al. 2001; Kimura et al. 2007; Tribulo et al. 2012). In cattle, administration of FSH dissolved in various substances, including polyvinylpyrrolidone, aluminum hydroxide gel

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or hyaluronan (HA), increased ovarian responses compared to multiple FSH injections (Bo et al. 1994; Yamamoto et al. 1995; Kimura et al. 2007; Tribulo et al. 2012; Chasombat et al. 2013). HA is a biological polymer that is abundantly expressed in many tissues. Recently, it was also reported that single or split-single injections of FSH dissolved in HA increased the number of transferrable embryos in cattle. Ovarian characteristics and the number of collected embryos were not significantly different between split-single *i.m.* injection of Folltropin®-V dissolved in HA (HA-FSH) and traditional twice-daily *i.m.* injections (Tribulo et al. 2011; Tribulo et al. 2012).

Our goal was to develop superovulation protocols for ewes that require fewer injections, less labour and animal handling, better animal welfare and with no reductions in ovarian response. To the best of our knowledge, the effects of split-single HA-FSH injection on superovulatory response in ewes has yet to be examined. Therefore, the overall objectives of this study were to: (1) assess the efficacy of a simplified superovulation protocol (a split-single dose of HA-FSH) for embryo production compared to the traditional FSH programme (several FSH treatments); and (2) to determine pregnancy rates after embryo transfer to two superovulation programmes.

MATERIAL AND METHODS

Chemicals. All chemicals in this study were purchased from Sigma-Aldrich Chemical Company (St Louis, USA), unless otherwise stated.

Experimental design. Experiment 1 was designed to assess ovarian responses, hormonal changes, and embryos produced in two superovulation procedures (split-single HA-FSH vs multiple FSH injections) in mixed breed ewes. Ewes were randomly selected to receive one of the following two treatments: split-single HA-FSH injection (S) or multiple FSH injections (M). Next, 22–24 h after showing oestrus signs, donor ewes were inseminated by laparoscopic artificial insemination. Embryos were collected by flushing uterine tubes two days after laparoscopic artificial insemination and embryos were transferred to a recipient's uterine tube. Pregnancy was confirmed using transabdominal ultrasonography (HS-2000, Honda Electronics Co, Ltd, Aichi, Japan) 60 days after ET (n = 21 and 19 in S and M groups, respectively). Experiment 2 aimed to validate this simplified superovulation procedure in purebred sheep raised on a commercial scale.

Animals. Both experiments were approved by the Institutional Animal Care and Use Committee (IACUC), Chulalongkorn University (Approval No. 13310050). In Experiment 1, 43 apparently healthy crossbred ewes with a good body condition score (BCS) ranging between 3 and 3.5 (1–5 scale) were randomly allocated to undergo one of two superovulation protocols. Recipients (healthy crossbred ewes; n = 44) were oestrus-synchronised before receiving embryos. All sheep were housed under natural environmental conditions. In Experiment 2, the purebred ewes, including Corriedale and Bond breeds, were kept under typical ranching conditions on a commercial sheep farm located in western Thailand.

Donor and recipient synchronisation protocols (Figure 1). All donors were superovulated as described (Shin et al. 2008; Mayorga et al. 2011) with minor modifications (summarised in Figure 1). Briefly, oestrus was synchronised using a CIDR-G device (Interag, Hamilton, New Zealand) for 10 days. In the split-single HA-FSH protocol, 180 mg of FSH (Folltropin®-V, Bioniche Animal Health, Belleville, Canada) were dissolved in 10 mg/ml of 750 kDa hyaluronan (MAP-5®, Bioniche Animal Health, Vetrepharm, Canada) and mixed well to achieve a final concentration of 60 mg/ml HA-FSH. Then, 150 and 30 mg were administered subcutaneously on the mornings of days 8 and 10 in CIDR-G-implanted ewes. In the multiple FSH protocol for superovulation, FSH was divided into six doses (50, 50, 30, 30, 10 and 10 mg) administered twice daily (12-h intervals) in the control group. The first dose of FSH was started on day 8 after insertion of a CIDR-G. In all ewes, two 125-μg doses of cloprostenol (Estrumate, Schering-Plough Animal Health, USA) were given im at CIDR-G removal and repeated 12 h later. After CIDR-G removal, oestrus behaviour was determined every 6 h using apronised rams until standing oestrus was detected. Once ewes were found to be in oestrus, 200 IU of hCG (Chorulon, Intervet Schering-Plough Animal Health, Boxmeer, The Netherlands) was given to induce ovulation. All recipients were oestrus-synchronised using insertion of a CIDR-G for 10 days and 300 IU eCG (Folligon®, Intervet Schering-Plough Animal Health, Summit, USA) given im at implant removal. Oestrus was detected

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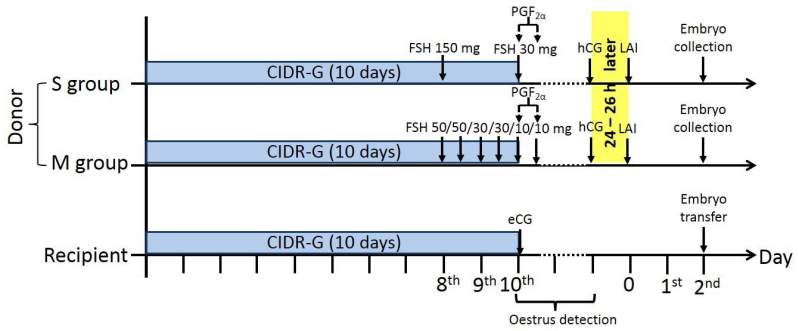

Figure 1. Scheme of donor superovulation protocols in split-single (S) and multiple (M) follicle stimulating hormone (FSH) injection groups and the recipient synchronisation program

$PGF_{2\alpha}$ = prostaglandin F2α, hCG = human chorionic gonadotropin, eCG = equine chorionic gonadotropin, LAI = laparoscopic artificial insemination

after progesterone removal every 6 h using apronised rams. Timing of oestrus was recorded.

Laparoscopic artificial insemination. Donor ewes were inseminated using laparoscopic artificial insemination, as described above, 24–26 h after detection of oestrus signs (Panyaboriban et al. 2015). Briefly, ewes were anaesthetised with an intraveneous injection of 1.1 mg/kg ketamine HCl, 0.025 mg/kg xylazine HCl and 0.04 mg/kg phenylbutazone. Then, 100×10^6 freeze-thawed sperm were inseminated into the middle of the uterine horn, using a direct-view 5-mm laparoscope (Scholly, Denzlingen, Germany).

Embryo flushing and evaluation. At two days after insemination, embryos were collected from the uterine tubes of donor ewes under general anaesthesia. The flushing media was Dulbecco's phosphate-buffered saline (DPBS, Gibco, USA) supplemented with 2% (v/v) foetal bovine serum (FBS, JR Scientific, Inc., Woodland, USA). In brief, the reproductive tract was accessed through a mid-ventral incision. The number of recovered embryos or ova was recorded in relation to numbers of corpora lutea (CL). The flushing medium (10 ml) was introduced ~1 cm above the uterotubal junction (UTJ) to the fimbria. Flushing fluid was collected via a polyethylene tube with an outer diameter of 1.57 mm and inner diameter of 1.14 mm (Intramedic, Becton, Dickinson and Company, Franklin Lakes, USA). Embryos were identified under a stereomicroscope (Nikon SMZ645, Japan) with 10 × magnification. Recovered embryos were evaluated and graded as described (Wright 1998).

Blood sampling, plasma progesterone and estradiol 17-β assays. To compare the hormonal profiles of the two programmes, a total of 144 blood samples were collected from 12 randomly selected ewes per group, every two days after the first injection of FSH. Samples were collected in heparinised tubes by veinipuncture of the jugular vein. Plasma was immediately separated by centrifugation at 1500 × *g* for 15 min and stored at –20 °C until analysed. Plasma concentrations of progesterone and oestradiol 17-β were determined using a radioimmunoassay, as described (Kamonpatana et al. 1976).

Validation of progesterone assay. The reliability of this progesterone assay was tested using three pools of standard progesterone – low, medium and high – added to blank plasma pools. The coefficients of variation (CV) of these three pools were 10.26% (0.1 ng/ml), 9.95% (0.5 ng/ml) and 8.60% (1.0 ng/ml), respectively, and interassay CVs were 13.1, 14.8 and 10.3%, respectively. The sensitivity of the assay was 0.03 ng/ml.

Validation of oestradiol 17-β. The reliability of this estradiol 17-β assay was tested using three pools of standard oestradiol 17-β – low, medium and high – added to blank plasma pools. Intra- and interassay CVs were 9.48 and 12.05%, respectively. The sensitivity of the assay was 0.36–100 pg/ml. Mean ± SD percentage recovery in plasma was 89.44 ± 3.05. The specificity of the oestradiol 17-β antibody (#8932 180381: Dr. R.I. Cox Csira Prospect, NSW, Australia), defined as cross-reactivity in the described RIA system, was as follows: oestradiol 17-β 100%; oestradiol 17-α 0.48%; oestrone 8.40%; other steroids < 1.68%.

Embryo transfer protocol. Embryos were transferred to recipients. Only good quality (Grades 1 or 2) embryos were transferred to recipients when they were in oestrus ± one day compared to donors. Recipients were chosen randomly and two *in vivo* embryos retrieved from either an M or S superovulation protocol were transferred. Embryos were transferred into one uterine tube ipsilateral to the corpus luteum.

Pregnancy detection. At 60 days after transfer, pregnancy diagnosis was made with transcutaneous real time B-mode ultrasonography using a

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transcutaneous probe (3.5 MHz probe, HS-2000, Honda Electronics Co., Ltd., Aichi, Japan). Ewes were determined to be pregnant as described (Ganaie et al. 2009).

Statistical analyses. The normal distribution of data was verified using a Univariate procedure (SAS version 9.0; SAS Institute, Inc., Cary, USA). Quantitative data (numbers of follicles, CL, ova and embryos, were analysed using one-way ANOVA followed by the Turkey-Kramer test or, in the case of unequal variation among treatments, NPAR-ANOVA with the Wilcoxon signed-rank test. The statistical significance of rates of recovery and pregnancy were determined using the Chi-square test. Plasma hormone concentrations (progesterone and oestradiol 17-β) were subjected to repeated measures analyses of variance (PROC GLM). For all analyses, $P < 0.05$ was considered to denote a significant difference between groups.

RESULTS

Experiment 1: Simplified HA-FSH superovulation protocol for *in vivo* embryo production

All ewe donors in both groups (S and M) showed signs of oestrous after completion of the superovulation program (43/43). On average, the onset of oestrus after CIDR-G removal in the S group (25.7 ± 3.2 h; range 22.7 to 33.1) was earlier ($P < 0.05$) than in the M group (28.3 ± 4.2 h; range 22.8 to 35.7 h).

Mean plasma concentrations of progesterone and oestradiol 17-β hormone according to day of superovulation protocol (day 0: day of first dose of FSH injection) and group are shown in Figure 2. On day 8, the group S ewe donors with split-single injection of HA-FSH had higher plasma progesterone concentrations compared to the M group (7.4 ± 2.8 vs 4.5 ± 2.4 ng/ml, $P < 0.05$). In both groups, plasma oestradiol 17-β concentrations increased gradually after the first FSH injection. However, there were no significant differences between groups in progesterone concentrations on days 2, 4, 6 and 10. Differences between groups in average plasma oestradiol 17-β levels were also not significant. However, the peak of oestradiol 17-β concentrations in the M group

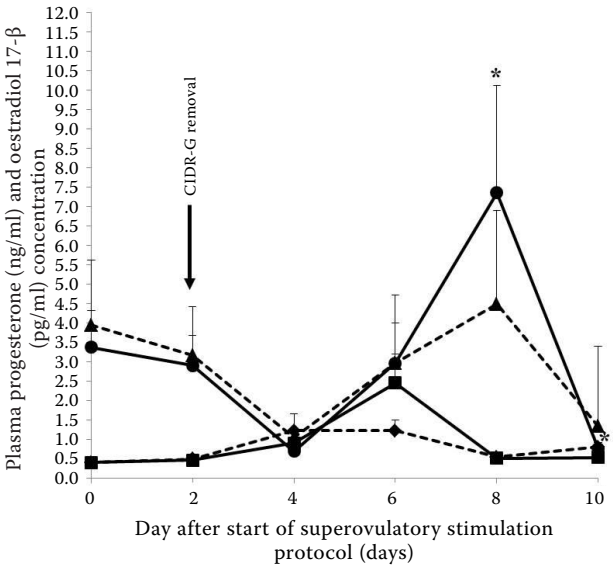

Figure 2. Plasma concentrations of progesterone (P4) and oestradiol (E2) 17-β in superovulated donor ewes in split-single (S) or multiple (M) injection groups during superovulation

*Difference between groups significant at $P < 0.05$

–▲– = P4 (M group), –●– = P4 (S group), –◆– = E2 (M group), –■– = E2 (S group)

(0.8 ± 0.4 pg/ml) was higher than that of the S group (0.5 ± 0.2 pg/ml) at day 10 ($P < 0.05$).

For ovarian responses, there was no difference in the average number of ovulations (i.e., mean number of CL) and average number of recovered ova/embryos ($P > 0.05$) between two superovulation protocols (Table 1). However, the S group tended to have more good quality embryos (5.2 vs 2.9 embryos/ewe, $P = 0.08$) when compared to the M group. The mean number of unovulated follicles ranged from

Table 1. Mean ± SEM responses in superovulated ewe donors given split-single (S) or multiple (M) follicle stimulating hormone injections

Treatment group	S	M	*P*-value
Treated ewes	21	22	–
Oestrous signs	21 (100%)	22 (100%)	–
Positive response	21 (100%)	22 (100%)	–
No. ovulations	10.2 ± 3.4	10.8 ± 4.7	0.67
No. ova/embryos per ewe	9.9 ± 3.6	10.5 ± 5.2	0.66
No. fertilised embryos per ewe	5.2 ± 5.0	3.9 ± 3.5	0.32
Grades 1 and 2 embryos	5.1 ± 4.9	2.9 ± 2.9	0.08
Fertilised per ewe (%)	52.0 ± 42.4	40.9 ± 31.5	0.33

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1.7 to 2.1 with no difference between treatments (S or M groups; $P = 0.386$). Recovered ova/embryos did not differ between S and M groups (96.1 vs 93.1%, respectively). In addition, four of 22 (18.2%) ewes in the M group did not produce embryos compared to three of 21 (14.3%) in the S group.

Regarding the recipient group, 90.9% (40/44) of the ewes exhibited oestrus within 48 h after oestrus synchronisation. Pregnancy rate with embryos from the S group was higher than with those from the M group (90.5% (19/21) vs 78.9% (15/19); $P < 0.05$, respectively).

Experiment 2: Application of a simplified superovulation procedure on commercial farms

The objective of this experiment was to determine whether a simplified superovulation protocol for embryo production was effective and practical on the scale of a commercial farm. Two breeds of wool sheep, 15 Corriedale and seven Bond sheep, were used as donors. All ewes were determined to be in oestrous after CIDR-G withdrawal. There was no difference between breeds in timing of oestrus. Ovarian responses, embryo production and pregnancy rates for each breed are shown in Table 2. All ewes had positive ovarian responses. The number of recovered good (Grades 1 and 2) embryos per superovulated donor was 4.8 ± 5.0 and 4.0 ± 2.5 in Corridale and Bond ewes, respectively. Following embryo transfer, 15 of 22 recipients with transferred Corriedale embryos established pregnancy, whereas 13 of 14 recipients became pregnant after transfer of Bond embryos. In addition, 28 surplus Corriedale embryos were cryopreserved.

Table 2. Ovarian responses, embryo production and pregnancy rates in Corriedale and Bond donor ewes after superovulatory stimulation with a split-single follicle stimulating hormone administration protocol

Breed	Corriedale (n = 15)	Bond (n = 7)
No. CL at flushing	9.9 ± 4.9	7.8 ± 1.0
No. ova/embryos per ewe	9.0 ± 5.1	6.5 ± 1.7
No. fertilised embryos per ewe	5.0 ± 5.2	4.0 ± 2.5
No. grades 1 and 2 embryos	4.8 ± 5.0	4.0 ± 2.5
No. recipients	22	14
Pregnancy rate (%)	15/22 (68.2%)	13/14 (92.8%)
No. lambs born	20	20
No. embryos frozen	28	0

DISCUSSION

In the present study, we validated the efficacy of a simplified superovulation protocol (a split-single dose of HA-FSH) for embryo production compared to traditional multiple FSH injections. Embryos produced by this procedure were competent to generate pregnancy, thus validating a simplified superovulation protocol when using FSH, which traditionally has been given 6–8 times every 12 h to effectively stimulate superovulation (Cognie 1999). Such a traditional procedure can cause animal stress and is also time-consuming and labour-intensive.

The use of a single injection of FSH diluted in various degradable agents such as propylene glycol (PG), polyvinylpyrrolidone, aluminum hydroxide gel or hyaluronan (HA) was previously tested in cattle and ewes (Lopez-Sebastian et al. 1993; Dattena et al. 1994; Yamamoto et al. 1994; Takedomi et al. 1995; D'Alessandro et al. 2001; Kimura et al. 2007; Tribulo et al. 2012). Although these agents were effective at eliciting a slow release of FSH to induce a superovulatory response, in humans, PG and polyvinylpyrrolidone have caused allergic reactions (Catanzaro and Smith 1991; Gonzalo et al. 1999; Yoshida et al. 2007; Marques et al. 2011), while aluminum hydroxide induced neuropathological reactions and macrophagic myofascitis (Gherardi et al. 2001; Shaw and Petrik 2009). In contrast, there have been no reports of side effects from HA administered in mammals. HA is a natural polysaccharide compound, which, in animals, is found in the extracellular matrix, especially of connective tissues. It has been shown to be well suited to medical applications such as tissue engineering (Radice et al. 2000). Similar to the present study, there was no allergic reaction at the injection site and no systemic effects in any of the ewes.

The mechanism underlying the controlled slow release of hormone/drug in HA has been examined in several studies (Kim and Park 2002; Lehr and Haas 2002; Cho et al. 2003; Lee et al. 2010; Oh et al. 2010; Upadhyay et al. 2010). The basis of the interaction between HA and the deliverable agent was a multivalent anionic charge, resulting in the diffusion of hormones in each layer of the

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HA polymer surface. At the site of the combined HA-agent injection, body temperature slowly broke the HA structure in each layer and, thus, caused its release into blood circulation (Cascone et al. 1994; Luo et al. 2000). In cattle, HA was successfully used for superovulation by single injection with slow FSH release (Tribulo et al. 2011). However, in this preliminary study, in which we used a single FSH injection for superovulation, the ovarian response was poorer than that of a traditional FSH multiple injection protocol. Similarly, in other reports (Alvarez et al. 2010; Tribulo et al. 2012), an additional injection of a lower dose of FSH 48 h after single administration in cows resulted in more ovulations (mean number of CL) than a single injection. Thus, a superovulation protocol in ewes using a split-single administration of HA-FSH was tested in this study. We conclude that a split-single *i.m.* injection of FSH dissolved in 10 mg/ml of HA was effective in inducing ovarian superstimulation in a similar way to a traditional multiple FSH injection protocol, based on numbers of ovulations, fertilisation and good-quality embryos.

This study was carried out to validate and verify the efficiency of a split-single HA-FSH injection on superovulatory responses and *in vivo* embryo production in ewes. The onset of oestrus was shorter ($P < 0.05$) in the S group than in the M group. Similarly, in a preliminary study (unpublished), a split-single injection of HA-FSH in ewes had a shorter interval of progesterone removal to oestrus (26.9 ± 3.5 h) compared to other groups (multiple FSH injection: 27.8 ± 6.2 h; single HA-FSH injection: 28.0 ± 4.2 h). This may be an additional advantage of the split-single HA-FSH protocol that could be used as a simplified preparation of donor ewes for fixed-time insemination. The mechanism of this phenomenon is still unclear, but is likely to be mediated at the level of sex steroids. The split-single HA-FSH-treated ewes had higher peak oestradiol 17-β concentrations than the control group. A threshold level of oestrogen is required to trigger oestrus and LH surge (Evans and Robinson 1980).

The efficiency of producing good-quality embryos was better in the S group compared to the M group. Continual release of FSH in HA may constantly stimulate small follicles to develop in an optimised environment. Then, good quality and mature oocytes in these follicles should continue to ovulate and be ready for fertilisation (Thomas et al. 2003). Moreover, the number of CL in all donors in the S group ranged between five and 16, indicating an effective superovulation protocol.

Consistent with ovulation rate, plasma progesterone concentrations in the S group were higher than those in the M group on day 8 ($P < 0.01$). Goto et al. (1988) reported that progesterone concentrations were significantly correlated with the quality and quantity of collected embryos in superovulated cattle. In agreement with Sharma et al. (1993), they reported a positive correlation between superovulatory responses (ovulation rate, recovery rate and number of embryos) and progesterone concentrations during superovulation in ewes. Furthermore, plasma progesterone profiles of all ewes injected with split-single HA-FSH were similar, indicating consistent efficacy. Furthermore, pregnancy rates were higher in recipients that received embryos from the S group compared to those that received them from the M group.

In conclusion, our superovulatory protocol including laparoscopic artificial insemination and ET was validated for use in the sheep industry in Thailand, in particular, with sheep breeds of high genetic merit. We conclude that the simplified superovulation protocol with split-single FSH injection dissolved in 10 mg/ml 750 kDa HA can be safely used for successful superovulatory stimulation of sheep with less animal handling and stress, and is appropriate for the Thai sheep industry. In trials conducted on two commercial farms, the simplified superovulation protocol successfully stimulated ovaries to ovulate and produce embryos in both Corriedale and Bond ewes. However, further investigation is needed to fully meet the biotechnological challenge of importing purebred sheep under tropical conditions, particularly in Thailand. In addition, with validation and perhaps some modifications, superovulation procedures developed for domestic ruminants may also be useful for nondomestic caprinae species and other goat-antelopes such as gorals, ibexes, Barbary sheep or deer.

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REFERENCES

Alvarez R, Martinez A, Pires R (2010): Superovulatory response of zebu cows treated with pFSH in a single subcutaneous injection followed by an additional intramuscular sub-dose 48 h later. Reproduction in Domestic Animals 45, 421–424.

Anakkul N, Suwimonteerabutr J, Tharasanit T, Panyaboriban S, Khunmanee S, Thanomsuksinchai N, Techakumphu M (2013): Production of black goat using laparoscopic artificial insemination and embryo transfer. The Thai Journal of Veterinary Medicine 43, 259–263.

Bo G, Hockley D, Nasser L, Mapletoft R (1994): Superovulatory response to a single subcutaneous injection of Folltropin-V in beef cattle. Theriogenology 42, 963–975.

Cascone M, Di Silvio L, Sim B, Downes S (1994): Collagen and hyaluronic acid based polymeric blends as drug delivery systems for the release of physiological concentrations of growth hormone. Journal of Materials Science: Materials in Medicine 5, 770–774.

Catanzaro JM, Smith JG (1991): Propylene glycol dermatitis. Journal of the American Academy of Dermatology 24, 90–95.

Chasombat J, Sakhong D, Nagai T, Parnpai R, Vongpralub T (2013): Superstimulation of follicular growth in Thai native heifers by a single administration of follicle stimulating hormone dissolved in Polyvinylpyrrolidone. The Journal of Reproduction and Development 59, 214–218.

Cho K, Chung T, Kim B, Kim M, Lee J, Wee W, Cho C (2003): Release of ciprofloxacin from poloxamer-graft-hyaluronic acid hydrogels in vitro. International journal of pharmaceutics 260, 83–91.

Cognie Y (1999): State of the art in sheep-goat embryo transfer. Theriogenology 51, 105–116.

D'Alessandro A, Martemucci G, Colonna M, Borghese A, Terzano M, Bellitti A (2001): Superovulation in ewes by a single injection of pFSH dissolved in polyvinylpyrrolidone (PVP): effects of PVP molecular weight, concentration and schedule of treatment. Animal Reproduction Science 65, 255–264.

Dattena M, Vespignani S, Branca A, Gallus M, Ledda S, Naitana S, Cappai P (1994): Superovulatory response and quality of embryos recovered from anestrus ewes after a single injection of porcine FSH dissolved in polyvinylpyrrolidone. Theriogenology 42, 235–239.

Evans G, Robinson T (1980): The control of fertility in sheep: endocrine and ovarian responses to progestagen-PMSG treatment in the breeding season and in anoestrus. The Journal of Agricultural Science 94, 69–88.

Ganaie BA, Khan MZ, Islam R, Makhdoomi DM, Qureshi S, Wani GM (2009): Evaluation of different techniques for pregnancy diagnosis in sheep. Small Ruminant Research 85, 135–141.

Gherardi R, Coquet M, Cherin P, Belec L, Moretto P, Dreyfus P, Pellissier JF, Chariot P, Authier FJ (2001): Macrophagic myofasciitis lesions assess long-term persistence of vaccine-derived aluminium hydroxide in muscle. Brain 124, 1821–1831.

Gonzalo M, De Argila D, Garcia J, Alvarado M (1999): Allergic contact dermatitis to propylene glycol. Allergy 54, 82–83.

Goto K, Ohkutsu S, Nakanishi Y, Ogawa K, Tasaki M, Ohta H, Inohae S, Tateyama S, Kawabata T, Ishii S (1988): Endocrine profiles and embryo quality in superovulated Japanese Black Cattle. Theriogenology 29, 615–629.

Kamonpatana M, Luvira Y, Bodhipaksha P, Kunawongkrit A (1976): Serum progesterone, 17a-hydroxyprogesterone and 17-b oestradiol during oestrous cycle in swamp buffalo in thailand: A preliminary report. Proceedings of the International Symposium on Nuclear Techniques in Animal Production and Health as Related to the Soil Plant System International Atomic Energy Agency. 569–578.

Kim MR, Park TG (2002): Temperature-responsive and degradable hyaluronic acid/Pluronic composite hydrogels for controlled release of human growth hormone. Journal of Controlled Release 80, 69–77.

Kimura K, Hirako M, Iwata H, Aoki M, Kawaguchi M, Seki M (2007): Successful superovulation of cattle by a single administration of FSH in aluminum hydroxide gel. Theriogenology 68, 633–639.

Lee Y, Chung HJ, Yeo S, Ahn CH, Lee H, Messersmith PB, Park TG (2010): Thermo-sensitive, injectable, and tissue adhesive sol-gel transition hyaluronic acid/pluronic composite hydrogels prepared from bio-inspired catechol-thiol reaction. Soft Matter 6, 977–983.

Lehr CM, Haas J (2002): Developments in the area of bioadhesive drug delivery systems. Expert Opinion on Biological Therapy 2, 287–298.

Lopez-Sebastian A, Gomez-Brunet A, Lishman A, Johnson S, Inskeep E (1993): Modification by propylene glycol of ovulation rate in ewes in response to a single injection of FSH. Journal of Reproduction and Fertility 99, 437–442.

Luo Y, Kirker KR, Prestwich GD (2000): Cross-linked hyaluronic acid hydrogel films: new biomaterials for drug delivery. Journal of Controlled Release 69, 169–184.

Marques B, Denizarde I, Pinheiro KF, de Freitas do Carmo LP, Costa MC, Abensur H (2011): Anaphylactic reaction induced by a polysulfone/polyvinylpyrrolidone membrane in the 10th session of hemodialysis with the same dialyzer. Hemodialysis International 15, 399–403.

Mayorga I, Mara L, Sanna D, Stelletta C, Morgante M, Casu S, Dattena M (2011): Good quality sheep embryos pro-

https://doi.org/10.17221/52/2016-VETMED

duced by superovulation treatment without the use of progesterone devices. Theriogenology 75, 1661–1668.

Oh EJ, Park K, Kim KS, Kim J, Yang JA, Kong JH, Lee MY, Hoffman AS, Hahn SK (2010): Target specific and long-acting delivery of protein, peptide, and nucleotide therapeutics using hyaluronic acid derivatives. Journal of Controlled Release 141, 2–12.

Panyaboriban S, Suwimonteerabutr J, Phutikanit N, Swangchan-Uthai T, Tharasanit T, Techakumphu M (2015): Effect of various combinations of sugar supplementation in the extender on frozen-thawed ram semen quality and fertility. The Thai Journal of Veterinary Medicine 45, 229.

Radice M, Brun P, Cortivo R, Scapinelli R, Battaliard C, Abatangelo G (2000): Hyaluronan-based biopolymers as delivery vehicles for bone-marrow-derived mesenchymal progenitors. Journal of Biomedical Materials Research 50, 101–109.

Satoh H, Numabe T, Takada T, Oikawa T, Kifune A, Watanabe G, Taya K (1996): Superovulation in Japanese beef cows using polyvinylpyrolidone (PVP) as the vehicle for porcine FSH (pFSH). Theriogenology 45, 332.

Sharma V, Gupta R, Khar S, Khurana N (1993): Plasma progesterone profiles, ovarian response and embryo recovery in crossbred ewes superovulated during breeding and non-breeding seasons. Animal Reproduction Science 34, 119–126.

Shaw CA, Petrik MS (2009): Aluminum hydroxide injections lead to motor deficits and motor neuron degeneration. Journal of Inorganic Biochemistry 103, 1555–1562.

Shin ST, Jang SK, Yang HS, Lee OK, Shim YH, Choi WI, Lee DS, Lee GS, Cho JK, Lee YW (2008): Laparoscopy vs. laparotomy for embryo transfer to produce transgenic goats (Capra hircus). Journal of Veterinary Science 9, 103–107.

Sugano M, Shinogi T (1999): Superovulation induction in Japanese black cattle by a single intramuscular injection of hMG or FSH dissolved in polyvinylpyrrolidone. Animal Reproduction Science 55, 175–181.

Takedomi T, Aoyagi Y, Konishi M, Kishi H, Taya K, Watanabe G, Sasamoto S (1995): Superovulation of Holstein heifers by a single subcutaneous injection of FSH dissolved in polyvinylpyrrolidone. Theriogenology 43, 1259–1268.

Thomas FH, Walters KA, Telfer EE (2003): How to make a good oocyte: an update on in-vitro models to study follicle regulation. Human Reproduction Update 9, 541–555.

Tribulo A, Rogan D, Tribulo H, Tribulo R, Alasino RV, Beltramo D, Bianco I, Mapletoft RJ, Bo GA (2011): Superstimulation of ovarian follicular development in beef cattle with a single intramuscular injection of Folltropin-V. Animal Reproduction Science 129, 7–13.

Tribulo A, Rogan D, Tribulo H, Tribulo R, Mapletoft RJ, Bo GA (2012): Superovulation of beef cattle with a split-single intramuscular administration of Folltropin-V in two concentrations of hyaluronan. Theriogenology 77, 1679–1685.

Upadhyay KK, Bhatt AN, Mishra AK, Dwarakanath BS, Jain S, Schatz C, Le Meins JF, Farooque A, Chandraiah G, Jain AK (2010): The intracellular drug delivery and anti tumor activity of doxorubicin loaded poly (γ-benzyl l-glutamate)-b-hyaluronan polymersomes. Biomaterials 31, 2882–2892.

Wright J (1998): Photographic illustrations of embryo developmental stage and quality codes. Manual of the International Embryo Transfer Society 3, 167–170.

Yamamoto M, Ooe M, Kawaguchi M, Suzuki T (1994): Superovulation in the cow with a single intramuscular injection of FSH dissolved in polyvinylpyrrolidone. Theriogenology 41, 747–755.

Yamamoto M, Ooe M, Kawaguchi M, Suzuki T (1995): Dose response to a single intramuscular injection of FSH dissolved in polyvinylpyrrolidone for superovulation in cows. Journal of Reproduction and Development 41, 93–96.

Yoshida K, Sakurai Y, Kawahara S, Takeda T, Ishikawa T, Murakami T, Yoshioka A (2007): Anaphylaxis to polyvinylpyrrolidone in povidone-iodine for impetigo contagiosum in a boy with atopic dermatitis. International Archives of Allergy and Immunology 146, 169–173.

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