Hormones for Superovulation in Sheep

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Superovulation is typically achieved using gonadotropic hormone preparations that promote the development of subordinate follicles in order to ovulate, or through inhibin immunization to eliminate the inhibitory mechanism of the dominant follicle. Various gonadotropic preparations (FSH and eCG) are widely applied to induce superovulation in sheep. Other hormones, such as horse anterior pituitary (HAP) extracts, human menopausal gonadotropin (hMG), gonadotropin-releasing hormone (GnRH), and human chorionic gonadotropin (hCG) have also been applied, although less frequently, during sheep superovulation.

Keywords: hormonal protocols

1. FSH and Superovulatory Response

FSH is the most commonly used hormone for inducing superovulation in sheep and other livestock [1][2]. Generally, it is administered via multiple injections (6–10) at 12 h intervals over a period of 3–5 days during the follicular phase [3][4][5]. Variation in ovarian response to this procedure has been observed in sheep (**Table 1**). Collectively, maximum rates of ovulation (15.9) and embryo recovery (10.7) were observed using the conventional FSH protocol [6]. The major disadvantage of FSH is its short half-life, necessitating daily administration [7][8][9]. In addition, multiple injections are time consuming and stressful for animals, which may be detrimental to the female reproductive performance [6]. Furthermore, the potential for premature luteal regression, substantial individual variability in ovarian responses, and the low quality of recovered embryos, are also limiting factors of FSH [2][10].

Table 1. Comparative ovarian responses of simplified and traditional superovulation protocols.

Breed	Superovulation Protocol	OR [1]	ER [2]	FR [3] (%)	TRR [4]	References
Awassi breed	FSH decreasing doses	8.75 ± 0.4 [ac]	4.83 ±0.6	-	-	[11]
	eCG 1200 IU, single dose	5.66 ± 0.4 [bd]	4.66 ± 0.6 [a]	-	-	
Corriedale and Bond	In simplified protocol, FSH 180 mg	10.2 ± 3.4	9.9 ± 3.6	52.5	5.1 ± 4.9	[7]
	6 FSH administered twice daily	10.8 ± 4.7	10.5 ± 5.2	37.1	2.9 ± 2.9	
Corriedale	oFSH + eCG, single injection	13.8 ± 1.9 [a]	8.4 ± 1.4 [a]	64.2 [b]	-	[1]
	oFSH dissolved in saline, divided into 4 equal doses	6.2 ± 1.1 [b]	3.1 ± 1.1 [b]	45.9 [b]	-	
	oFSH dissolved in 30% polyvinylpyrrolidone, single dose	4.7 ± 1.0 [b]	3.2 ± 1.1 [b]	89.7 [a]	-	
	oFSH 72 h before and 12 h after sponge removal, 8 decreasing doses	10.7 ± 0.9 [a]	5.5 ± 0.8	93.9	-	

Breed	Superovulation Protocol	OR [1]	ER [2]	FR [3] (%)	TRR [4]	References
Fine wool Merino	FSH,7 decreasing doses 48 h before sponge removal during breeding and non-breeding season	13.9 ±0.8 a / 11.3 ±1.8 a	6.0 ± 0.5 a / 3.5 ± 1.0 b	-	-	[12]
	FSH 70 mg + eCG, single dose 48 h before sponge removal during breeding and non-breeding season	3.2 ± 1.2 b / 6.0 ± 1.1 b	1.2 ± 0.6 b / 1.6 ± 0.5 b	-	-	
Merino breed	eCG + 11.5 mg pFSH, 6 decreasing doses	14.2±1.2 a	5.2±1.9 a	58.3 a	-	[13]
	eCG, 1200 IU	6.2 ± 0.8 b	1.0 ± 0.5 b	26.3	-	
	eCG, 1600 IU	11.0 ± 3.0 ab	1.2± 0.6 b	19.2	-	
Ojalada	280 IU pFSH, 6 decreasing doses	15.9 ± 2.0 a	10.7±1.7 a	86 a	-	[6]
	210IU pFSH + 500 IU eCG, single dose	14.5 ± 2.1 a	11.3 ± 1.8	76 a	-	
Sarda	250 IU pFSH, 4 decreasing doses	11.8 ± 4.0 a	8.80 c	81.7	-	[14]
	125 IU pFSH + 600 IU eCG	8.05 ± 3.8 b	4.82 d	82		
Suffolk	eCG, 750–1000 IU, single injection	7.7 ± 1.4	3.5 ± 1.6	-	-	[15]
	FSH, 20–24 mg, multiple injections	8.4 ± 0.4	5.3 ± 0.5	-	-	
Xinji fine wool	FSH at the rate of 60, 50, and 30 IU per injection on days 1, 2, and 3	9.67 ± 1.93	7.85 ± 2.4	-	4.52 ± 2.5 b	[16]
	150 mg Folltropin-V, twice daily at 35, 25, and 15 mg per injection on days 1, 2, and 3	12.47 ± 1.5	9.27 ± 1.8	-	7.86 ± 1.75 a	

[1] OR, ovulation rate; [2] ER, embryos recovered; [3] FR(%), fertilization rate; [4] TRR, transferrable embryos. Values with different superscripts within the column show a significant difference at $p < 0.05$.

To cope with these shortcomings, various researchers have attempted to replace the classical FSH multiple injection protocol with either a single FSH injection [17][18][19][20] or a single injection of FSH in combination with eCG [1][6][21][22][23][24]. However, no significant difference between single and multiple injections has been found for ovulation rate (10.2 vs. 10.8), embryonic recovery (9.9 vs. 10.5), or fertilized embryos (5.2 vs. 3.9); however, the proportion of good-quality embryos (5.1 vs. 2.9) was reported to be comparatively higher in the simplified than the conventional superovulation protocol (**Table 1**) [7].

Furthermore, a comparable ovulation rate (14.5 vs. 15.9, $p > 0.05$) and embryonic recovery (11.3 vs. 10.7, $p > 0.05$) was obtained for the FSH plus eCG single injection and FSH multiple doses, respectively [6]. FSH plus eCG in a single injection not only resulted in a greater proportion of viable embryos, but also the advanced onset of estrus and LH peak [1]. A few successful simplified attempts have also been made in cattle [25][26][27][28][29] and goats [30].

These findings indicate that FSH in combination with eCG in a single injection could produce an acceptable ovarian response. However, due to the lack of studies on this simplified superovulation protocol in sheep, the endocrinological basis of this protocol remains poorly understood.

2. eCG and Superovulation Response

Equine chorionic gonadotropin (eCG) is extensively used for superstimulation in sheep. This hormone is typically injected via a single dose for 1–2 days before estrus synchronization [13]. Although eCG is cost-effective and can easily be applied to an open flock with minimal bodily stress [31], dose-dependent responses may lead to an increased number of persistent large follicles [31] and premature corpus luteum regression [32]. Furthermore, it also influences the pattern of steroidal hormone synthesis, thus disrupting sperm and gamete transport, as well as preimplantation embryo development [33]. To potentiate the ovarian response, the combination of eCG and FSH has been applied, and increased ovulation rate (14.2 vs. 6.2; $p < 0.05$) and embryonic recovery (5.2 vs. 1.0; $p < 0.05$) was observed [13]. A significantly higher ovulation rate (13.8 vs. 6.2) and embryo recovery (8.4 vs. 1.0) has been reported for FSH/eCG combined administration compared to FSH dissolved in saline divided into four equal doses [1]. No significant difference in the mean number of corpora lutea (CL; 8.7 vs. 9.4), transferable rate (85.3% vs. 88.8%),or degenerated embryos (5.4% vs. 4.8%) was observed; however, the average number of transferable embryos (5.5 and 6.6; $p < 0.001$) was significantly higher in the eCG plus FSH group than with eCG alone [34]. Similarly, eCG at a dose rate of 800 IU with 12 or 16 mg FSH (multiple injections) obtained a higher ovarian response than with eCG alone (14.8 vs. 19.1 vs. 3.5; $p < 0.05$) [35]. These results demonstrate that the administration of eCG combined with FSH in a simplified form can provide a high superovulatory response as compared to the single injection of eCG, injected 1–2 days before sponge removal.

3. Other Rarely Used Hormones

Some investigators also tested alternative gonadotropin preparations for superovulation in sheep, such as HAP, hMG, GnRH, and hCG. For example, HAP, obtained from the pituitaries of slaughter material, has been reported to induce a comparable response to p-FSH conventional protocols in sheep [36][37][38]. This hormone reduced the excess of large, non-ovulatory follicles, and also improved the quality of transferrable embryos [33].

In addition, hMG has been shown to produce a comparable ovulation rate (10 vs. 9.9; $p > 0.05$), rate of transferable embryos (84% vs. 80%), and fertilization rate (86% vs. 95%; ($p > 0.05$) to that of FSH [39]. Similar comparable responses of hMG with FSH have also been obtained [40], and in one case, hMG produced higher ovulation rates and improved embryo quality than obtained through FSH [33]. However, this hormone has not been extensively utilized for superstimulation due to its high cost [41].

Several researchers injected GnRH after sponge removal in order to improve the synchronization of the ovulation of follicles when injecting FSH or eCG. It has been reported that the association of longer progesterone exposure with GnRH administration is an alternative method to improve oocyte fertilization rates, particularly during fixed-time insemination [42]. Similarly, GnRH administration 24h after sponge withdrawal increased ewes ovulation rate when treated with eCG. The number of recovered embryos from ewes treated with eCG plus GnRH (4.3 vs. 1.06; $p < 0.05$) was higher than in ewes treated with eCG alone [43]. Similarly, in Santa Inês ewes, donors were synchronized with an insertion of a progesterone controlled-internal drug release (CIDR) device for 14 days, exchanging with a new CIDR on day 7, and administered $PGF_{2\alpha}$. On day 12, the superovulatory treatment was initiated using 133 mg of pFSH (Folltropin) at eight decreasing doses twice daily. On day 14, at the time of CIDR removal, ewes were divided into the following three groups: control group (progesterone device withdrawn at day 14); 12h P4 group (progesterone device maintained for an additional 12 h, i.e., until day 14.5); 12h P4 GnRH group (progesterone maintained for an additional 12 h, i.e., until day 14.5, plus a GnRH agonist). The results showed that a higher fertilization rate (77% vs. 34% vs. 41%; $p > 0.05$) was found in the group of ewes with progesterone devices maintained for 14.5 days plus a GnRH agonist during the last FSH injection, than the other two groups [42]. In addition, GnRH administration at the end of superovulation using FSH resulted in greater synchronization between the first and last ovulation events [42]. Similarly, at the end of the superovulation protocol, the administration of GnRH increased the fertilization rate, which ultimately enhanced the proportion of viable embryos [44].

A short-term protocol has been described, consisting of intravaginal sponges inserted for six days in Santa Inês ewes. On day 5, 300 IU of eCG and 37.5 µgd-cloprostenol were injected. The GnRH agonist at 24 h showed no benefits; however, using a GnRH agonist at 36 h more efficiently synchronized ovulation and promoted the desired environment, with the absence of dominant follicles after ovulation. Therefore, combining a GnRH agonist at 36 h after sponge removal, as in the short-term protocol, with the start of superovulatory treatment at 80 h, may be recommended in ewes. The GnRH agonist at 24 h compared to 36 h after sponge removal showed no estrus response (0.0% vs. 78.0%) [45]. The GnRH at 36 h after sponge removal efficiently synchronizes ovulation in ewes treated with FSH due to the improved synchrony in ovulation, promoting the absence of the dominant follicles, which improves fertilization rate and increases the production of viable embryos [44].

hCG is structurally similar to LH, and has been shown to increase total CL weight in ewes [46]. One explanation for this could be that hCG administration during the early luteal stage encouraged supplementary CL formation [47]. Moreover, administering hCG and vaginal sponges has been shown to have positive effects on lambing rates [48].

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