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Production of Live Twins of Single-Bearing Ewes: A Way for Increasing the Sheep Smallholder's Income in the Arabian Gulf Region

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Abstract

Raising sheep and goats in the gulf region is a large entrepreneurial business. This is, of course, derived from the big demands of lamb and goat meats for citizens and expatriates. There is a major population living in the rural desert areas whose main income steps from rising local sheep and goats. This population suffers from the lack of green forages, harsh ambient conditions, and the elevated price of grains and concentrates due to the restrictions of COVID-19.

The low twinning rates in the main two indigenous breeds (i.e. *Najdi and Noemi*) in the region motivated the researcher to pursue an avenue for maximizing the neonatal outcomes per a female. By applying this method, several sheep raisers could retain their business more effectively. The protocol of twin production in this study focuses on the implementation of the Controlled Intravaginal Drug Release (CIDR)-FSH method. This method bases on the insertion of CIDR pessary for 10 days in the ewe vagina, and at days 8, 9, and 10 doses of 60, 60; 40, 40 and 30, 30 IU recombinant human FSH are administered (i.m.) twice daily (AM and PM), respectively. At the fifth FSH dose (i.e. at D 10; AM), an equivalent dose (260 IU) of human Chorionic Gonadotrophin (hCG) is given (i.m.) and the CIDR is removed. Animals exposed to fertile rams 24 h after CIDR removal. Relatively, 90% of the treated ewes express estrous signs within 48 h of the CIDR removal.

Highlights

- Producing viable twins is an alternative approach for increasing the income.
- Implementation of the CIDR-FSH has shown high birth to weaning survival and weaning weight.
- Administration of L-arginine and the date molasses resulted in better lamb yield.
- Implementing the said protocol resulted in $70 profit out of each ewe exposed.

Introduction

Induction of twinning in single-bearing females has recently emerged as a focus of several studies especially in sheep [1-3] and cows [4-6]. Twinning in other species might impose reproductive and health problems for mother and offspring. For example, twinning in mares is undesirable due to the low survival rates, stillbirths, high mortality at birth and the very weak neonates. Only nine percent of the twinned mares gave birth to live twins, this occurs due to the competition for the uterine space [7].

Various Follicle Stimulating Hormone (FSH) preparations and their analogs have been historically tested for their efficiency for ovarian stimulation. Ovulation is a process by which the female expresses her ability for successful reproduction. Breeds differ in their ovulation potency; some of which possess major genes of fecundity. Of these are, *Booroola Merino* and *Inverdale* whose bear fecundity genes (i.e. *Fec*B; *Fec*X). During the latest decades, it has been discovered a growth factor derived of the oocyte, i.e. called Bone Morphogenetic Peptide-15 (BMP-15)

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which is used as a biomarker for prolificacy [8]. FSH is a glycoprotein hormone secreted internally from the adenohypophysis and stimulates the ovarian follicles via adhering to the receptors in granulosa cells, surrounding the egg within its capsule (follicle). Pregnant Mare' Serum Gonadotropin (PMSG), recently named equine Chorionic Gonadotropin (eCG) is an analog for FSH being used commercially for decades with a virtue of using as one dose instead of multiple doses in case of FSH; however, its stimulatory response in ovaries lasted for a long time leading to imbalances of maternal sexual hormones which deteriorate the ova quality. Even though earlier studies [9] implemented the administration of anti-PMSG antibodies subsequent to the hormone injection to mitigate its undesirable action on embryo quality, the results were not encouraging. Additionally, one other alternative for FSH is human Menopausal Gonadotropin (hMG), which possesses FSH and LH activities leading, to not only follicular maturation, but it also causes ovulation and corpus luteum development [10].

By the emerging of the era of genetic engineering, endocrinologists have used monoclonal production for several products. Several number of physiologists, microbiologists, geneticists and biochemists were investigating the mechanisms of molecules produced by this method was more specific and biologically efficient. Recombinant FSH has been recently used for twinning in sheep [11] and dairy cows [5].

On the other hand, a novel idea implemented the immunization against inhibin; a glycoprotein secreted by the Sertoli cells in the testes and granulosa cells within the ovary and suppresses the FSH release. This mechanism would impede the inhibitory effect of inhibin on the internal FSH release, leading to more secretion of FSH, which in turn, leads to more follicular maturation. Immunization against inhibin resulted in twinning in sheep [12,13].

In turn, these results, together promoted the author to implement the human genetically engineered FSH on the CIDR-based estrous synchronized ewes to produce live twins attaining a comparable weight to the single lamb born in the normal circumstance. This approach would be beneficial for the sheep owners, especially of the small enterprises. Zeitoun et al. (2020 a) [11] when applied this protocol, obtained twins weighing 28 kg each at day 90 which were comparable to the single lambs of 28.3 kg produced by the single-bearing control ewes. The author protocol involves the daily oral administration of L-arginine throughout the first 50 days and the daily oral administration of palm date extract throughout the last 50 days of gestation.

Animal production in the Kingdom of Saudi Arabia

The climate challenge facing food availability in the Middle East is a matter of great concern for scientists, entrepreneurs, and policymakers. In the Kingdom of Saudi Arabia, for instance, the country depends mainly on the importation of food in general and animals in particular to meet the demand of the increased population of citizens, expatriates, and religious visitors [14]. The nature of the country's climate (i.e. desert, lack of freshwater resources, sandstorms, extreme high temperature during the summer season) causes severe damages to the productivity and reproductive efficiency of livestock.

According to the latest census [15] of various types of animals in the Kingdom, the number of local sheep population is 9,328,455 heads, and the number of local goats accounts for 3,670,440 heads, which in turn accounts for around 13 million heads of both species. Mostly, the common indigenous sheep breeds are *Noemi* (Figure 1), *Najdi* (Figure 2), and *Harri* (Figure 3). This population represents the local animals; however, the Kingdom imports around 3 million heads annually for religious events. Most of the sheep and goat raisers in the Kingdom inhabit the rural regions; this is due to the larger area that can lodge their animals at less cost. There are major obstacles facing the spread of these small ruminants. Of these are, the lack of green fodders all year round, the lack of freshwater, the high cost of the imported grains and manufactured concentrates, the scarcity of rainfall, the sandstorms, the lack of veterinary services, the low fertility and the high mortality of the newborn, the lack of organizations ruling the market and above all the lack of well-trained labor [16]. The number of slaughtered animals of local and imported origin in the Kingdom of Saudi Arabia in 2018 was about 10 million heads [17]. Around 90% (i.e. ≈ 9 million heads) of these slaughtered animals were sheep and goats. Out of this, about 3 million imported heads and 6 million local heads. Thus, this figure would give a clear idea of the big demand for lamb meat in the country. Indigenous sheep breeds in the Kingdom are mainly: *Najdi*, *Noemi,* and *Harri* whose prolificacy falls between 120-130% [18]. The Saudi's authority pushes on the way to support the animal raisers in the Kingdom for keeping and adopting the latest technologies in the animal farming, and on the other hand, reducing the importation of the red meat [19]. Evidently, this policy would sustain local animal production.

Figure 1: *Noemi* flock owned to a sheep raiser in middle region (Al-Qassim) of Saudi Arabia.

Figure 2: *Najdi* flock owned by a herd man located in the middle region (Al-Qassim) of Saudi Arabia.

Figure 3: *Harri* sheep disseminated in the western region of the Kingdom.

Prolificacy in sheep and goats

Prolificacy is a term that express the lambing rate of a ewe exposed**,** a trait of low heritability (≈ 10%) affected by several factors; i.e. single-gene mutations, season, age of ewe, nutritional status, and hormonal impacts [20]. Crossing a prolific breed (Garole; G) with a non-prolific breed (Malpura; M) in India resulted in a GM cross carrying the *Fec*B gene, producing more twins and higher lamb yield per ewe [21]. In his study, Davis (2004) [22] defined some genes that control multiple fetuses in pregnancy, namely Bone Morphogenetic Peptide Receptor type-1B (BMPR-1B), BMP-15, and Growth Differentiation Factor-9 (GDF-9). Evidently, BMP-15 is of great importance to be used as a biomarker for prolificacy in sheep [23].

Hormones that stimulate ovarian activity (FSH, eCG, hMG, inhibin immunization)

Extensive studies conducted for decades to investigate the effects of various gonadotrophic hormone preparations on the ovarian architecture and function. In humans, different preparations of FSH have been used, of which the urine FSH and the recombinant FSH [24]. One other isomer is the hMG, which is isolated from the urine of post-menopausal women. Starting in 1995, the recombinant DNA-derived FSH was primarily produced and became commercially available [25,26]. This product was highly specific and resulted in better results for ovarian follicular development and oocyte health as compared with the pituitary-derived FSH. Recently, Zeitoun et al. (2020 a) [11] concluded that using 180 IU of a recombinant human FSH in sex descending doses in conjunction with progesterone insert (Controlled Internal Device Release; CIDR) led to the production of live twins in *Noemi* ewes (Figure 4). The ewes that were given saline (Control) gave birth of a single lamb (Figure 5). This experiment used the oral administration of L-arginine during the first trimester (≈ 50 days) and date molasses during the last trimester (≈ 50 days). The 90-days old weaning weight was 28.3 kg for the single lamb born of the control ewes; meanwhile, the same age weaning weight of each twin lamb was 28 kg (Table 1).

Figure 4: *Noemi* ewe delivered twin-lambs in the experimental station of Qassim University after exposed to CIDR-recombinant human FSH treatment of six descending doses.

Figure 5: *Noemi* ewe given saline (Control) delivered a single lamb.

Table 1: Effect of source (human vs. porcine) and number of injections (one vs. six) of FSH on the neonatal traits and cost effectiveness of Noemi ewes induced for twinning (Mean ± SE).

Trait	Control		*P*-FSH		*h*-FSH	
	C1	C6	P1	P6	H1	H6
No. Viable/Total No. lambs	3/3	4/4	2/8	4/11	3/13	8/16
Survival at birth (%)	100	100	25	36	23	50
No. weaned/ No. born	3/3	4/4	2/8	3/11	2/13	7/16
Survival at weaning (%)	100	100	25	27.3	15.4	43.75
Mean birth weight (kg)	4.9 ± 0.1^{a}	5.3 ± 0.15^{a}	3.4 ± 0.2^{b}	5.1 ± 0.12^{a}	2.7 ± 0.15^{c}	3.8 ± 0.5^{b}
Mean weaning weight (kg)	28.3 ± 0.35^{a}	27.2 ± 0.40^{a}	18.0 ± 0.35^{b}	28.2 ± 0.4^{a}	19.5 ± 0.20^{b}	28.3 ± 0.30^{a}

Total number of progeny/ TRT**	3	4	8	11	13	16
Number of weaned lambs/ TRT	3	4	2	3	2	7
Kg lamb meat/ treated ewe	8.5	10.9	3.6	8.5	3.9	19.8
Cost/ treatment ($)	100	100	420	420	420	420
Return/ treatment ($)***	480	640	320	480	320	1120
Profit/ treatment ($)	380	540	- 100	60	-100	700
Profit/ treated ewe ($)	38	54	-10	6	-10	70

[a,b,c] Means in the same row with different superscripts significantly differ ($P< 0.05$); *p*= Porcine; *h*= Human; C1: Control one dose saline; C6: Control six doses saline; P1: Porcine FSH one dose; P6: Porcine FSH six doses; H1: Human FSH one dose; H6: Human FSH six doses; **TRT: Treatment; ***The price of each weaned lamb was $ 160 equivalent to the local currency [11].

However, Zeitoun et al. (2020 a) [11] obtained less viability at birth when they administered either type of FSH (porcine and human) in a collective one dose, attributing these stillbirths and neonatal mortalities to the simultaneous ovulation and fertilization, leaving less time for each individual fetus to have a space in the uterine endometrium. In the same study, they concluded that the best protocol for induction of viable twins is the administration of FSH in a sequence of six descending doses on days 8, 9, and 10 (Figure 6).

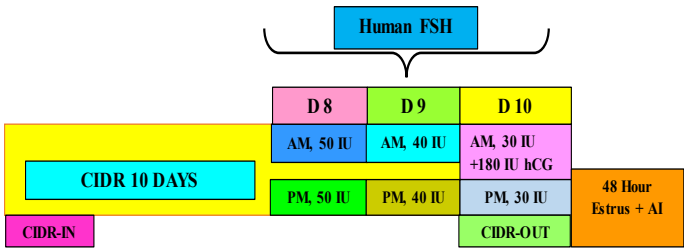

Figure 6: Protocol of production of live twins in local Saudi sheep [11].

Giving the L-arginine concomitant with the twinning protocol for *Najdi* ewes resulted in healthy twin lambs (Figure 7) in a comparable size to the single-born control lamb (Figure 8).

Figure 7: *Najdi* ewe given saline (Control) delivered a single lamb.

Figure 8: *Najdi* ewe delivered twin-lambs exposed to CIDR-recombinant human FSH treatment of six descending doses.

Protocols for estrous synchronization and twinning in cows, sheep and goat

Increasing reproductive efficiency in animal farming is the ultimate goal of any livestock farm. One of the important tools for controlling animal reproduction is the synchronization of estrous/ovulation protocol. Recent estrous/ovulation protocols have been implemented in beef heifers [27] combining heat detection and Timed Artificial Insemination (TAI). In this protocol, all heifers observed in estrus were inseminated 12 hours after standing estrus, however, these heifers not showing estrus signs 72 hours after Prostaglandin (PG) injection, were given each a dose of gonadotrophic Releasing Hormone (GnRH) and timely inseminated. This single injection of GnRH accelerates the estrous expression resulting in better conception rates. Of these protocols, were Select Synch + CIDR®& TAI; Melengestrol Acetate (MGA) ®+ PG &AI or 14–day CIDR® - PG & TAI. Furthermore, Fesseha and Degu (2020) [28] proposed various synchronization protocols on heifers and cows raised under hot climates in Africa. These researchers recommended the use of PG injection beyond day 5 of the estrus cycle in the pubertal heifers or cows, while they found it better to use GnRH in parous cows since heifers are not responsive yet for this GnRH and to the hypothalamic-pituitary-ovarian axis.

Twinning in cattle was a motivating approach for investigators recently [5,6], whereas in his most recent review López-Gatius (2020) [29] did not promote the twin or multiple pregnancies in dairy cows because it compromised the animal welfare and productive longevity. Opposite to what has been found in cows, sheep and goats were a promised species for twinning/ multiple births, as they are, relatively owned by the poor sector

of the society. Naturally delivering twins is of great importance to the sheep raisers, which depends on the available breeds. Under the arid and semi-arid climate of Namibia, Kandiwa et al. (2020) [30] investigated the effects of season and breed on the lambing/kidding rates, growth performance, and viability of neonates and weaning traits of sheep (*Damara, Dorper*, and *Swakara*) and goats (*Boer* goat and *Kalahari Red*). They found breed effect on the litter size, with twinning rates more frequent in *Dorper* sheep and *Kalahari Red* and *Boer* goats than in the *Damara* and *Swakara* sheep.

Consequences of twinning on the maternal and neonatal level

Earlier in 2008, Rumball [31] and his colleagues found metabolic differences between ewes carrying twins and these carrying singles. The high glucose requirements during late gestation in twin-bearing ewes led to lower birth weights, in the case of the regular feeding diets. One other important factor for neonatal survival is the strong bonding between the mother and her lambs [32]. Maternal metabolism during twin-pregnancy indicated increases in glucose [33] and cholesterol [34]. Generally, the birth weight of the individual lamb of twin offspring is less than that of the single born lamb. In addition, percent of survival at birth decreases in multiple than single births [31]. Apparently, the injection of FSH in one dose, not only reduced the pregnancy rate (Table 2), but also compromised the survival at birth and weaning (Table 1) compared with the serial FSH injections. The best protocol resulting in the highest lamb yield at weaning was the conjunction of 10 days-CIDR with the descending dose of FSH [40, 40 (D8), 30, 30 (D9) and 20, 20 IU FSH (D10)], with an equivalent dose (180 IU) of hCG (Pregnyl®, Organon, The Netherland) given with the fifth dose of FSH.

Table 2: Effect of source (porcine *vs.* human) and number of injections (one vs. six) of FSH on the maternal reproductive performance of *Noemi* ewes induced for twinning (Mean ± SE).

	Control		*P*-FSH		*h*-FSH	
Trait	C1	C6	P1	P6	H1	H6
No. Ewes	10	10	10	10	10	10
% Estrus	40	60	100	80	100	90
No. Estrous ewes/Total ewes	(4/10)	(6/10)	(10/10)	(8/10)	(10/10)	(9/10)
At time of estrus						
No. Ovarian dominant follicles/ewe	1.0 ± 0.12^{a}	1.0 ± 0.15^{a}	3.5 ± 2.18^{b}	3.5 ± 2.15^{b}	5.0 ± 1.35^{b}	4.5 ± 1.51^{b}
At 40 days post-mating						
No. Corpora Lutea	1.0 ± 0.00^{a}	1.0 ± 0.00^{a}	2.0 ± 0.50^{b}	3.0 ± 0.50^{b}	3.0 ± 0.50^{b}	3.0 ± 0.50^{b}
Mean diameter of dominant follicle at estrus (mm).	4.77 ± 0.1^{b}	4.67 ± 0.15^{b}	6.10 ± 0.7^{ab}	5.80 ± 0.2^{ab}	5.37 ± 0.1^{b}	6.32 ± 0.3^{a}
Time CIDR-Estrus (hrs.)	64.5 ± 2.0^{a}	61.5 ± 2.0^{a}	47.7 ± 2.4^{b}	28.2 ± 2.5^{c}	26.6 ± 2.3^{c}	25.3 ± 1.6^{c}
% Pregnancy rate (No.)	75 (3)	66.7 (4)	50 (5)	75 (6)	70 (7)	88.9 (8)
Single-lambing ewes (%)	100	100	20	33.3	28.6	12.5
(No. ewes giving single/Total ewes)	(3/3)	(4/4)	(1/5)	(2/6)	(2/7)	(1/8)
% Multiple-lambing ewes*	0 (0/3)	0 (0/4)	80 (4/5)	66.7 (4/6)	71.4 (5/7)	87.5 (7/8)

^{a,b,c} Means in the same row with different superscripts significantly differ ($P< 0.05$); *p*: Porcine; *h* : Human; C1: Control one dose saline; C6: Control six doses saline; P1: Porcine FSH one dose; P6: porcine FSH six doses; H1: Human FSH one dose; H6: Human FSH six doses. *No. ewes giving multiple births/Total No. ewes giving birth [11].

Nutritional requirements during twin-gestation in sheep

The body condition score of the female bearing twin or multiple fetuses must be higher than that bearing singles. Increased plane of nutrition in pre-lambing and post-lambing in Bengal sheep raised birth and weaning weights [35]. Lately, Knight et al. (2020) [36] concluded that reducing nutrition during mid- and late- pregnancy in ewes not only lowered the birth weights, but also reduced the lean-to -carcass ratio. Furthermore, in a recent study to investigate the effects of increased energy level during late pregnancy in triplet-bearing ewes, there obtained better maternal body condition score, leading to better lamb birth weight, weaning weight, and lamb growth rate [37]. Additionally, Zeitoun (2017) [38] investigated the effects of oral administration of L-arginine at 37.5 or 75 mg/kg BW/d versus polypropylene glycol or their combination during the last eight weeks of gestation in Saudi local sheep ewes. He obtained the highest survival at birth, maternal progesterone concentration and birth weight when the ewes were given the low dosage of L-arginine combined with propylene glycol during late gestation. Also, using L-arginine at early gestation and date molasses during late pregnancy for a duration of 50 days each resulted in heavier twins and high survival in CIDR (CIDR® device; inter-Ag, New Zealand) –FSH (rh-FSH; GONAL-F, Merck Sharp and Dohme)-treated ewes (Table 1; [11]).

Economic feasibility of the twinning programs in sheep and its impact on smallholders

In a recent scientific report released by Cornell College of Agriculture and Life Science, Cornell's small farms program [39], the author suggested that one of the main goals of raising sheep is to obtain twins instead of singles out of each ewe in the sheep farm. Table 1 exhibits the data of economic feasibility of twinning induction in the *Noemi* ewes exposed to a program of estrous synchronization combined with an optimum dosage

of human recombinant FSH. The net profit for each treated ewe approached 70 USD if the sheep owner sold these offspring at weaning. After that, keeping the feedstuff and other overhead costs in mind, if these twins sold beyond weaning it would exaggerate the profit.

Conclusion

Raising sheep by a big sector of Bedouins inhabiting the desert of the Arabian Gulf region necessitates out-of the-box thoughts for motivating these people to sustain their business. One promising approach to achieve that goal is the induction of live twins out of the single-bearing ewes. Coincidently, there must take into account the nutrition requirements of the ewe during early and late pregnancy. The implementation of the CIDR-human FSH (180 IU) on the primiparous or multiparous ewes resulted in live twins attaining weaning weight comparable to the single born lambs. This approach might help to solve a socioeconomic problem facing the sheep enterprises in the gulf region and keep their business running.

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