Blood Insulin-like growth factor-I (IGF-I) concentrations and some reproductive and physical characteristics of fat-tailed ewes and their litters during the breeding and non-breeding seasons

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SUMMARY

Relationships between peripheral IGF-I concentrations and some reproductive and physical characteristics were investigated in fat-tailed ewes during breeding and non-breeding seasons. Oestrus was synchronised in all ewes using 14 days intravaginal progestagen sponges and after sponge removal, all ewes (n = 146) were treated with pregnant mare serum gonadotrophin and 2 days after, they were exposed to rams for 51 days during non-breeding (n = 75) and breeding (n = 71) seasons. Blood samples were collected on day 19 after sponge removal in order to determine the serum progesterone and IGF-I concentration of ewes using commercial radioimmunoassay and immunoradiometric kits, respectively. The reproductive performance of the ewes in both seasons was assessed by lambing data including litter size, lamb birth weights and lambing rate.

Circulating IGF-I concentrations in ewes were significantly greater in the breeding and non-breeding seasons [26, 31, 39]. In vitro, IGF-I increases blastocyst cell number [7, 34] by promoting development to the blastocyst stage [3, 27, 34], and decreasing the proportion of apoptotic blastomeres [7, 34]. As the pre-implantation embryo expresses the receptor for IGF-I [4, 44], it is admitted that IGF-I directly acts positively on the embryo pre-implantation in cattle [27, 34, 35, 38]. In vivo, the bovine oviduct and uterus express IGF-I [21, 25, 31, 39]. In addition, uptake and utilisation of nutrients by granulosa cells were stimulated by IGF-I in sheep cell cultures [26].

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Mots-clés : Brebis; IGF-I; poids vif; état d’entretien; saison de reproduction; gestation; portée; poids des agneaux; gestation gémellaire

Introduction

Insulin-like growth factor-I (IGF-I) acts as a signal of the energetic status and also modulates mechanisms controlling the reproductive system [23] and plays an essential role in mammalian reproduction [16, 41]. It is demonstrated that IGF-I plays an important role in ovarian physiology in ruminants and in embryonic pre-implantation development and pregnancy [22, 24, 33]. In sheep, an increase in circulating IGF-I concentrations within the physiological range stimulates LH secretion [1]. Proliferation and differentiation
placental and foetal tissues are improved by IGF-I [2, 15]. Consequently, IGF-I influences embryo survival via actions on the ovary, oviduct, and uterus [41, 43].

Several studies reported circulating changes of IGF-I concentrations in ewes [8, 15, 18, 19, 23] and a direct relationship between nutritional status and serum IGF-I concentrations was reported [8]. However, these studies have not explored relationships between peripheral IGF-I concentrations and pregnancy, twinning and physical condition of ewes during breeding and non-breeding seasons. The present study was designed in both breeding and non-breeding seasons to investigate the relationship between peripheral IGF-I concentrations and some reproductive and physical characteristics of fat-tailed ewes, and to compare the circulating IGF-I concentrations between single and twin-bearing fat-tailed ewes in breeding and non-breeding seasons.

### Material and Methods

The present study was conducted during non-breeding (March to July) and breeding (October to February) seasons in Komijan, Markazi province, Iran. Komijan is located at a latitude of 34° 43' N and longitude 49° 19' E. Its altitude is 1750 m above sea level. A total of 146 Mehraban fat tailed ewes (mean body weight: 58.3 kg, mean age: 3.2 years and mean body condition score (BCS): 2.5) were used. All animals were managed under the same conditions on the farm. They were kept under natural field conditions, having access to good quality grasses and water and were maintained in good health. The ewe flock was kept away from the rams and the introduction of rams occurred two days after progestagen removal.

Seventy five and seventy one ewes were used in non-breeding and breeding seasons, respectively. Oestrus was synchronised in all groups using 14 days with intravaginal progestagen sponges (20 mg, Cronolone, Chronogest, Intervet, UK). At the time of sponge removal all ewes were treated with pregnant mare serum gonadotrophin (i.m., 400 IU PMSG-Folligon, Intervet, Holland). Then, two days after progestagen removal, all ewes were exposed to eight rams of proven fertility in the each study, during non-breeding and breeding seasons for 51 days (3 cycles). Blood samples were collected on day 19 after sponge removal using jugular venipuncture to measure serum progesterone and IGF-I concentrations in ewes. Serum was separated by centrifugation at 1 500 g for 15 minutes at room temperature and stored at -21°C until endocrine analysis.

Serum progesterone concentration was determined using a validated commercial radioimmunoassay kit (Immunotech kit, France). The intra- and inter-assay CVs of the assays were 5.8% and 9.0%, respectively. The sensitivity of the test was 0.05 μg/L, and the recovery rate of the assay ranged from 85% to 110%. Serum IGF-I concentration was determined by immunoradiometric assay (IRMA) kit (Immunotech kit, France). The intra- and inter-assay CVs of the assays were 6.3% and 6.8%, respectively. The sensitivity of the test was 2 μg/L, and the recovery rate of the assay ranged from 91% to 103%. The reproductive performance of the ewes in both seasons was assayed by lambing data including litter size, lamb birth weights and lambing rate which were recorded.

Data of five and three ewes in non-breeding and breeding seasons were excluded from analysis, because of different causes such as die and sale. The present study used 78 singleton and 60 twin-bearing ewes. The results were statistically analyzed using the SPSS statistical software (Version 15.0, SPSS Inc, Chicago, Illinois). The correlations of the studied parameters were analysed by non-parametric Spearman's correlation test separately for non-breeding and breeding seasons of sampling as well as pooled data of all samplings. Statistical comparisons for IGF-I and progesterone concentration and birth weight of lambs in non-breeding and breeding seasons were performed between single and twin bearing ewes, and different BCS groups of ewes using the non-parametric Mann-Whitney U-test. Results were expressed as means ± standard deviations. Values of p ≤ 0.05 were considered as significant data.

### Results

Circulating IGF-I concentrations were significantly greater in ewes during the breeding season (210.9 ± 91.6 μg/L) than in the non-breeding season (171.6 ± 70.4 μg/L) (p < 0.01).

As shown in Table I, the IGF-I concentrations determined in 146 ewes during breeding or non breeding seasons positively and significantly correlated with the ewe weights and BCS, the female age, the lamb weight and the litter size and negatively with the progesterone concentrations. However, these associations were more marked when ewes were sampled during the breeding season whereas in ewes sampled during the non breeding season, the circulating IGF-I concentrations were not significantly coupled to female BCS and to the lamb related parameters.

Particularly, the serum IGF-I concentrations were found to be significantly greater in ewes with a BCS above or equal to 2.5 than in females with BCS equal to 2 (p < 0.05) and the differences in the IGF-I concentrations according to the ewe BCS were amplified when females were sampled during the breeding season whereas no statistically significant differences were evidenced in animals sampled during the non breeding season (figure 1).

In the same way, twin bearing ewes exhibited significantly higher IGF-I concentrations than single bearing ones (p < 0.01) and this difference was more marked during the breeding season (Table II). Additionally, it was also noted that body weight and BCS were significantly greater in twin bearing ewes than in single bearing ones (59.6 ± 5.8 kg
vs. 57.6 ± 6.7 kg; p < 0.05 and 2.63 ± 0.36 vs. 2.45 ± 0.38; p < 0.01). No statistical differences in IGF-I concentrations were evidenced in twin bearing ewes according to their BCS whereas significantly higher IGF-I concentrations were found in single bearing ewes with BCS above or equal to 2.5 compared to those with BCS equal to 2 (p < 0.05) (figure 2). In parallel, the birth weights of the litter were dramatically increased in twin bearing ewes (p < 0.01) and the difference was exacerbated during the breeding season (Table II).

** Discussion **

In the present study, ewes had higher IGF-I concentrations in the breeding season compared to those in the non-breeding season. It may due to an increased number of cyclic ewes that exhibit greater IGF-I concentrations than the acyclic ones [23] during the breeding season.
Pregnant cows had higher plasma IGF-I concentrations than non-pregnant ones during the first 3.5 months post-insemination [28]. Circulating IGF-I concentrations increased during the 6 first months after successful insemination then declined during the last 3 months of pregnancy in cattle [14, 30]. IGF-I is known to influence the foetal development especially the foetal crown-rump length, foetal weight, and birth weight [15]. Many factors may be related to the relationship between foetus development and maternal circulating IGF-I concentrations. The IGF-I concentrations in mothers positively correlate with the body weight of foetuses, but they do not positively correlate with the maternal body weight around the 70th day of gestation in cows [11] and even HOSSNER et al. [14] reported negative correlation between growth and weight of bovine foetus and serum maternal IGF-I concentrations. HOLLAND et al. [13] observed that the circulating IGF-I concentrations were lower in cows bearing male foetuses than in females bearing female foetuses as well as in the single bearing cows than in the twin bearing ones during the last trimester of pregnancy. However, another studies reported no effect of the foetal gender and the litter size on the maternal IGF-I concentrations in cattle [10, 14]. Discrepancies between studies may due to differences in voluntary dry matter intake during pregnancy and a dietary supplementation can alter the circulating IGF-I concentrations [9, 20, 30]. In addition, the blood IGF-I concentrations can influence growth of maternal tissues and consequently, it could potentially and indirectly affect the foetal growth [41]. Furthermore, the likelihood of conception in multiparous dairy cattle was increased by increments in IGF-I concentrations [41]. IGF-I is strongly expressed in the basal lamina and also in the apical epithelium of placenta, which are sites of initial trophoblast attachment [36] and this peptide is involved in the remodelling of the uterine epithelium, extracellular matrix and cytoskeleton, and promotes subsequent trophoblast attachment and implantation of embryo [36]. Peripheral IGF-I concentrations may not exactly reflect the IGF-I paracrine secretion, strongly fluctuating particularly in the early gestation [41]. As reported [12, 40], most of the variations in embryonic viability on day 7 could not be strictly related to the peripheral IGF-I concentrations which were not significantly associated to the conceptus development until the 17th day of gestation. It has been reported that a minimum threshold circulation IGF-I concentration has to be reached before reproduction is adversely affected [5, 6].

In ewes, early placental growth may be affected by maternal IGF-I concentration that was positively correlated with the total placentome number in mid gestation [29]. Indeed, ewes with good condition have high circulating maternal IGF-I concentration which may stimulate the placentome formation [15]. Furthermore, maternal IGF-I can improve nutrient availability for transfer across the placenta [2] by increasing placental uptake of substrates [46]. Consequently, high concentration of IGF-I in twinning ewe may result in increasing the litter birth weight and it also can be responsible for positive correlations between IGF-I concentrations and weight and numbers of lambs, as observed in the present study. A high body condition scores is associated with a high adiposity [42]. KENYON et al. [18] have previously reported a higher body condition score in single bearing ewes selected for high IGF-I concentrations than in those with low concentrations in late pregnancy [18]. Higher condition scores were also reported in high IGF-I selected line ewes compared to low line counterparts [17]. It is considered that ewes with a BCS below 2 or above 3 are more susceptibility to metabolic imbalances, whereas BCS equal to 3 appears as ideal [8]. However, CALDEIRA et al. [8] observed highest serum IGF-I concentrations in ewes with a BCS equal to 4, although differences were not statistically significant among ewes with a BCS ranged from 2 to 4. In agreement, ewes with BCS of 2 in the present study exhibited significantly lower IGF-I concentrations than ewes with higher BCS (≥ 2.5) and the circulating IGF-I concentrations were also significantly increased in the group of twin bearing ewes (which have a mean BCS above 2.5) compared to the group of single bearing ones (with a mean BCS of 2.45). Consequently, it appears that high BCS and body weight of the pregnant females were positively associated with high circulating IGF-I concentrations.

Although the circulating IGF-I concentrations does not seem to be directly related to the corpus luteum function [45], they seem to stimulate progesterone production [32]. Nevertheless, an apparent negative relationship between peripheral IGF-I and progesterone concentrations was obtained in the present study; differences in maternal nutrition and in the pregnancy status or not in ewes may partially explained negative correlations between IGF-I and progesterone concentrations. IGF-I concentrations were greater in non-pregnant ewes fed with energy-enriched diets [37]. In the same way, high IGF-I concentrations were found in well nourished pregnant ewes [42]. Furthermore, late gestational under-nutrition of twin-bearing ewes causes a decrease in plasma IGF-I concentrations at parturition as well as during the late gestation [19].

As a conclusion, circulating IGF-I concentrations in ewes were greater in the breeding season than in the non-breeding season and were markedly increased in ewes with a body condition score above 2.5, and particularly in twin bearing ewes. It can be concluded that higher IGF-I concentrations in ewes with good body condition can result in better reproductive performance.

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