



Postnatal phenotypic stability and endocrine homeostasis in Holstein-Friesian heifers conceived via multiple ovulation and embryo transfer vs. artificial insemination

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ABSTRACT

While Multiple Ovulation and Embryo Transfer (MOET) is a cornerstone for accelerating genetic progress in dairy cattle, empirical data directly comparing the postnatal physiological trajectories of MOET-derived calves against conventional Artificial Insemination (AI) remain limited. This study investigated the growth dynamics, somatotrophic axis (GH, IGF-1), metabolic signalling (leptin), and immunological maturation (IgG) of Holstein-Friesian heifers ($n = 20$) from birth through seven months of age. Calves (MOET $n = 10$; AI $n = 10$) born to heifer dams, were reared under standardized environmental, nutritional and colostrum management protocols to isolate the effects of reproductive technology. Beyond birth, no statistically significant differences in body weight were detected and body height did not differ significantly between groups across the monitoring period ($P > 0.05$). Similarly, serum concentrations of leptin, growth hormone, and insulin-like growth factor-1 exhibited no significant divergence between groups. Serum immunoglobulin G levels showed a transient significant difference in one month old calves ($P < 0.05$) before leveling off by month 3. Strong positive correlations were observed between height and weight across all developmental stages ($P < 0.01$). These findings suggest that under controlled management conditions, MOET-derived heifers exhibit postnatal growth, endocrine profiles, and immune maturation comparable to AI-conceived counterparts, supporting the functional reliability of MOET protocols in high-performance dairy breeding.

1. Introduction

The primary objective of Multiple Ovulation Embryo Transfer (MOET) is to accelerate genetic progress in cattle breeding (Amann-DeJarnette, 2012) by harvesting multiple viable embryos from genetically superior donor cows during a single estrous cycle and transferring them to recipient animals (Campolina et al., 2022; McDaniel et al., 1981). This technology facilitates the rapid dissemination of elite genetics, intensifies selection intensity, and significantly enhances milk production and functional traits, particularly in specialized dairy breeds like the Holstein-Friesian (Betteridge, 2003; Hasler, 2014). The widespread global adoption of this technology is highlighted by recent statistics showing that 296,520 embryos were collected

in vivo and 274,334 were transferred worldwide in 2024 (Viana, 2025), with the majority concentrated in North America (39.77%), Europe (37.51%), and South America (10.12%) (Viana, 2025). Consequently, MOET has evolved into a routine management practice on contemporary commercial dairy farms.

Despite their commercial success, the broader biological impacts of various assisted reproductive technologies remain under thorough investigation. Both in vivo and in vitro (IVP) production techniques have been linked to possible long-term physiological consequences. Specifically, elevated late-stage embryonic and fetal losses (Agca et al., 1998), abnormal placental development (Bertolini et al., 2004; Miles et al., 2004, 2005; van Wagtenonk et al., 1998), reduced fetal weight (Bertolini et al., 2004; Miles et al., 2004), and altered birth weights

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(Behboodi et al., 1995; Breukelman et al., 2005; Kruip-Den Haas, 1997; Numabe et al., 2000; van Wagendonk., 1998) have all been reported. Additionally, increased rates of neonatal mortality (Kruip & Den Haas, 1997; Numabe et al., 2000; van Wagendonk et al., 1998), congenital abnormalities (van Wagendonk et al., 1998), stillbirths, and dystocia (Behboodi et al., 1995; van Wagendonk et al., 1998) have been reported, alongside a notable shift in the secondary sex ratio at birth toward male offspring (Block et al., 2003; van Wagendonk et al., 1998). These physiological deviations necessitate rigorous evaluation to safeguard animal welfare and long-term health (Rérat et al., 2005). Furthermore, while studies examining the maternal endocrine environment indicate that progesterone and cortisol levels in recipient cows differ between artificial insemination (AI) and embryo transfer (ET/IVP) groups, these studies primarily address gestational endocrinology rather than the postnatal serum profiles of the offspring (Lopes et al., 2020).

Circulating levels of growth and metabolic hormones, such as growth hormone (GH) and insulin-like growth factor 1 (IGF-1), may differ between individuals derived from in vitro production (IVP) and those produced via AI; however, the long-term clinical relevance of these variations remain negligible (Heras et al., 2025). Furthermore, most studies evaluating biochemical and hematological profiles across embryo origins indicate that IVP-derived offspring do not underperform relative to AI counterparts (Serrano-Albal et al., 2025).

While previous studies have compared calves produced by AI and ET with regarding growth, clinical health, and selected endocrine parameters, comprehensive data specifically comparing in vivo-derived MOET and AI counterparts remain sparse – particularly concerning longitudinal concentrations of leptin, IGF-1, GH, and IgG. Consequently, limited information exists regarding the postnatal endocrine and passive immunity profiles of in vivo-produced MOET calves relative to those generated via AI. To address this gap, the present study systematically evaluated whether AI- and MOET-derived calves display equivalent postnatal developmental trajectories when reared under standardized environmental and management conditions.

We hypothesised that MOET-derived calves would display no significant deviation from their AI-derived counterparts in growth dynamics, endocrine regulations, or passive immune status, thereby supporting the functional and physiological equivalence of these two reproductive technologies.

2. Materials and methods

The experiment was conducted on a herd of Holstein-Friesian dairy cows in Eastern Hungary. Embryo transfer takes place on site, using the donor and recipient heifers. Following similar longitudinal studies (Arfuso et al., 2025; Gholami et al., 2010; Kerr et al., 1991; Ro et al., 2018), 10 newborn calves derived from multiple ovulation embryo transfer (MOET) and 10 newborn calves conceived by artificial insemination (AI) were randomly selected and assigned to two experimental groups. All calves were birthed by heifer dams.

2.1. Multiple ovulation embryo transfer

Estrus synchronization of both donor and recipient animals was achieved using a progesterone-releasing intravaginal device (PRID) (Prid delta 1.55 g Vaginal Delivery System for Cattle, Ceva Animal Health Ltd) in combination with prostaglandin F2 α (PGF2 α) administration prior to embryo transfer. PRID was inserted for 11 days and follicle-stimulating hormone (FSH) administration started on day 6 (Amiridis et al., 2006).

In donor animals, superovulation was induced using FSH (Pluset®, FSHp and 500 IU of LHp, Laboratorios Calier, S.A.) preparation administered in decreasing doses over 4–5 days (Naranjo Chacón et al., 2020), with a total dose ranging between 800 and 1000 IU (Table 1). The applied dosage was adjusted according to individual animal characteristics, including, age, and reproductive status. Lower total doses (~800

Table 1
Superovulation protocol.

Days	ml/heifer/12 h	IU FSH/LH
1.	3.0–3.0	150–150
2.	2.5–2.5	125–125
3.	2.0–2.0	100–100
4.	1.5–1.5	75–75
5.	1.0–1.0	50–50

IU) were typically used in heifers, whereas higher doses (up to 1000 IU) were applied in dairy cows, taking into account parity and production level. All hormonal treatments were applied according to standard commercial MOET protocols routinely used on the farm.

Following the superovulation procedure, donor animals were inseminated with sex-sorted, cryopreserved bull semen.

Seven days post-insemination, embryos were recovered non-surgically, morphologically evaluated, and classified. Superovulation and flushing procedures were performed on approximately 10% of the herd and were selected using commercial genomic evaluation, allowing embryo collection from as early as 11–12 months of age, yielding in average mean $\pm$ SE = 5.08 $\pm$ 0.38 transferred embryos per donor; range: 1–23 recovered embryos. Only fresh, IETS Grade 1 embryos were used for transfer. This term refers to fresh, in vivo-derived bovine embryos at developmental stages 4–7, corresponding to the morula, early blastocyst, blastocyst, and expanded blastocyst stages, respectively, and classified as Grade 1 according to the morphological classification system of the International Embryo Transfer Society (IETS). Grade 1 embryos were considered to be of excellent morphological quality, with minimal or no visible abnormalities and a high proportion of intact, viable cells. The embryo developmental stages and quality grades were assigned according to the criteria described by Stringfellow and Givens (2010). Prostaglandin F2 α (PGF2 α) and a progesterone-releasing intra-vaginal device were used to synchronize recipient heifers, and estrus was tracked appropriately.

Rectal palpation was used to determine the recipients' ovarian status (Yáñez et al., 2023), and only animals with a functional corpus luteum were chosen for embryo transfer. Each recipient received a single embryo into the uterine horn ipsilateral to the corpus luteum through a nonsurgical transcervical procedure.

All calves included in the artificial insemination (AI) group were born to heifer dams. The heifers assigned to the AI group were inseminated at 13 months of age following naturally occurring estrus. All heifers were inseminated with sex-sorted semen. The same sires were used for both groups. Artificial insemination was performed by a trained inseminator using the conventional rectovaginal technique. The thawed semen was loaded into an insemination gun and deposited into the uterine body. The AI procedure was performed according to standard bovine artificial insemination practices (Dahlen et al., 2018; López-Gatius, 2000).

2.2. Technology

Calves were housed individually in Steinmann crates after receiving colostrum via esophageal probe within the first hour of birth. Before being used, all colostrum was pre-pasteurized, quality-evaluated, and frozen; only batches with a Brix greater than 25% were given to guarantee sufficient passive immunity transfer.

After being fed colostrum, calves were fed 2 $\times$ 3.5 L of pasteurized whole milk per day until they were weaned at three months of age. From one week of age onward, calves had free access to fresh drinking water and calf starter (muesli) throughout the milk-feeding period. Animals were switched from starter muesli to a calf-rearing concentrate with 16.5% crude protein between 40 and 50 days of age.

Calves were provided unlimited access to hay, straw, and concentrate feed following weaning until they reached seven months old. The

animals were subsequently kept in deep-litter housing in small groups.

All animals were managed under uniform feeding and housing conditions throughout the study period to minimize environmental variability and allow reliable comparison of developmental outcomes between reproductive groups.

2.3. Measurements

From birth until seven months of age, measurements of body weight and wither height were taken monthly.

A calibrated digital animal scale (LD-Agro TR1000, capacity: 1000 kg) was used to record body weight, and a height gauge (Premiere, 100–180 cm range) and a standardized measuring stick (Animeter, Kerbl GmbH, Germany) were used to measure height at the withers.

To guarantee consistency and reduce measurement variability, the same equipment was used for all measurements during the study period.

Blood sampling was not performed specifically for experimental purposes. Instead, a licensed veterinarian collected all samples as part of the farm's regular herd health monitoring and diagnostic procedures. The farm's established veterinary health screening protocol was prospectively aligned with the timing of sample collection, enabling data acquisition without the need for extra handling or invasive procedures beyond standard practice.

2.4. The determined parameters

From birth to 3 months of age, blood was collected from the jugular vein, while from 4 to 7 months of age, samples were obtained from the coccygeal vein, using 9 mL plain collection tubes.

Following collection, samples were stored under refrigerated conditions until transport to the laboratory. Serum was obtained by centrifugation ($1750 \times g$ for 10 min at 20°C), and aliquots were subsequently stored at -80°C in 2 mL Eppendorf Safe-Lock tubes until further analysis.

Serum IgG levels were determined in newborn and 1-month-old calves. Blood serum growth hormone (GH) and insulin-like growth factor-1 (IGF-1) concentrations were also evaluated in newborn, 1-, 3- and 7-month-old calves. Serum leptin concentrations were measured in newborn, 1-, 3-, 5-, 6- and 7-month-old calves. The serum levels of IgG, GH, IGF-1 and leptin were determined by ELISA. The samples were thawed and then warmed to 37°C to ensure homogeneity. Assays were performed according to the manufacturer's instructions (CSBE06771b, Cusabio, Houston, USA; EIA05862Bo, EIA05856Bo, EIA05719Bo Enlilio, Wuhan, China). All samples and standards were measured in duplicate. Absorbance data were collected using a Synergy HTX Multi-Mode microplate reader (BioTek Instruments Inc., Winooski, USA) set to 450 nm. The IgG, GH, IGF-1 and leptin concentrations of the samples were calculated by interpolating their absorbance values using the linear regression of the standard curves. The intra-assay coefficients of variation (CV) for IgG, GH, IGF-1 and leptin were $<10\%$.

2.5. Statistics

Differences in body weight, height at the withers, and serum concentrations of leptin, GH, IGF-1 and IgG were compared between MOET and AI groups were evaluated using a two-way repeated-measures Analysis of Variance (RM-ANOVA), with group (MOET vs. AI) as the between-subjects factor and time as the within-subjects factor. To evaluate group differences at individual time points, simple main effect post-hoc comparisons were conducted with Bonferroni adjustment for multiple comparisons. Relationships among growth, endocrine, and immunological parameters were examined using Pearson's correlation analysis. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

3. Results

Body weight of both groups increased steadily in both groups over the study period, reflecting normal growth dynamics. Overall, growth trajectories displayed very similar slopes in MOET- and AI-derived heifers, with no statistically significant main effect of reproductive group or group $\times$ time interaction (Fig. 1). However, pairwise comparisons revealed that MOET-derived calves had a significantly higher birth weight compared to AI-derived calves ($P < 0.05$). Beyond birth, no statistically significant differences in body weight were detected between the groups at any subsequent time points ($P > 0.05$). Substantial individual variation was observed at later growth stages in both groups, but this variability was comparable and did not result in systematic differences between reproductive origins.

Body height increased continuously in both MOET and AI groups, reflecting consistent and age-appropriate skeletal development. A two-way repeated-measures ANOVA revealed a significant main effect of time. However, no significant main effect of group or group $\times$ time interaction was detected. Simple main effect post-hoc comparisons with Bonferroni adjustment showed no statistically significant differences in body height between the MOET and AI groups at any of the seven measured time points though from 1 to 5 months of age, MOET-derived heifers exhibited slightly higher mean height values than AI counterparts (Fig. 2). Overall, height trajectories were highly comparable between groups, with no evidence of systematic divergence.

Serum leptin concentrations changed significantly over time in both MOET and AI groups. However, there was no overall main effect of group or group $\times$ time interaction. No significant differences in leptin concentrations were observed between MOET and AI calves at any time point ($P > 0.05$) (Fig. 3). Leptin levels showed a similar temporal pattern in both groups, peaking at 3 months of age, and converged by 7 months of age. Overall, leptin profiles remained stable over time, with no evidence of divergence between reproductive origin.

Serum growth hormone (GH) concentrations changed significantly over time in both MOET and AI calves. However, there was no overall main effect of group or group $\times$ time interaction. Simple main effect post-hoc comparisons with Bonferroni adjustment revealed that GH concentrations exhibited similar patterns in MOET and AI calves at all measured time points (Fig. 4), with no statistically significant observed throughout the study period ($P > 0.05$). Overall, GH profiles remained consistent over time, with no evidence of divergence between reproductive groups.

IGF-1 concentrations showed a highly significant change over time in both MOET and AI calves. However, no main effect of group or group $\times$ time interaction was observed. The simple main effect post-hoc comparisons with Bonferroni adjustment revealed that IGF-1 concentrations were similar between the MOET and AI calves at all measurement points (Fig. 5).

IgG concentrations decreased markedly over time in both MOET and AI calves. A statistically significant main effect of reproductive group was observed, whereas the group $\times$ time interaction was not significant. Simple main effect post-hoc comparisons with Bonferroni adjustment revealed that serum IgG levels did not differ significantly between MOET and AI calves at birth (Table 2). However, at 1 month of age, AI calves maintained a statistically significantly higher serum IgG concentration compared to their MOET counterparts. Correlation analyses were performed on the combined dataset to examine relationships among growth, endocrine, and immunological parameters independent of reproductive origin Table 3.

Body weight and height at the withers were strongly positively correlated at one month of age ($r = 0.75$; $P < 0.05$) (Table 4). GH and IGF-1 concentrations showed a moderately strong positive correlation ($r = 0.61$; $P < 0.05$). Neither hormone, however, was significantly correlated with height or weight. Leptin concentrations were not significantly correlated with growth, endocrine, or immunological parameters at one month of age.

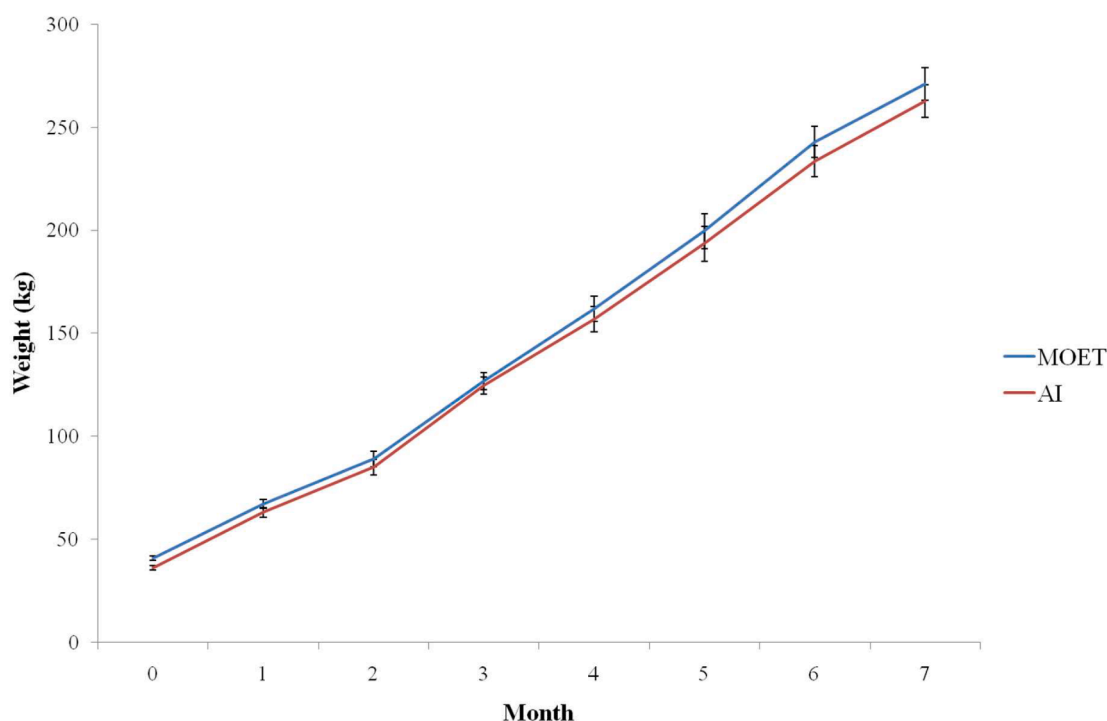


Fig. 1. Calves' weight gain. MOET and AI heifers' weight gain curve from birth to 7 months of age.

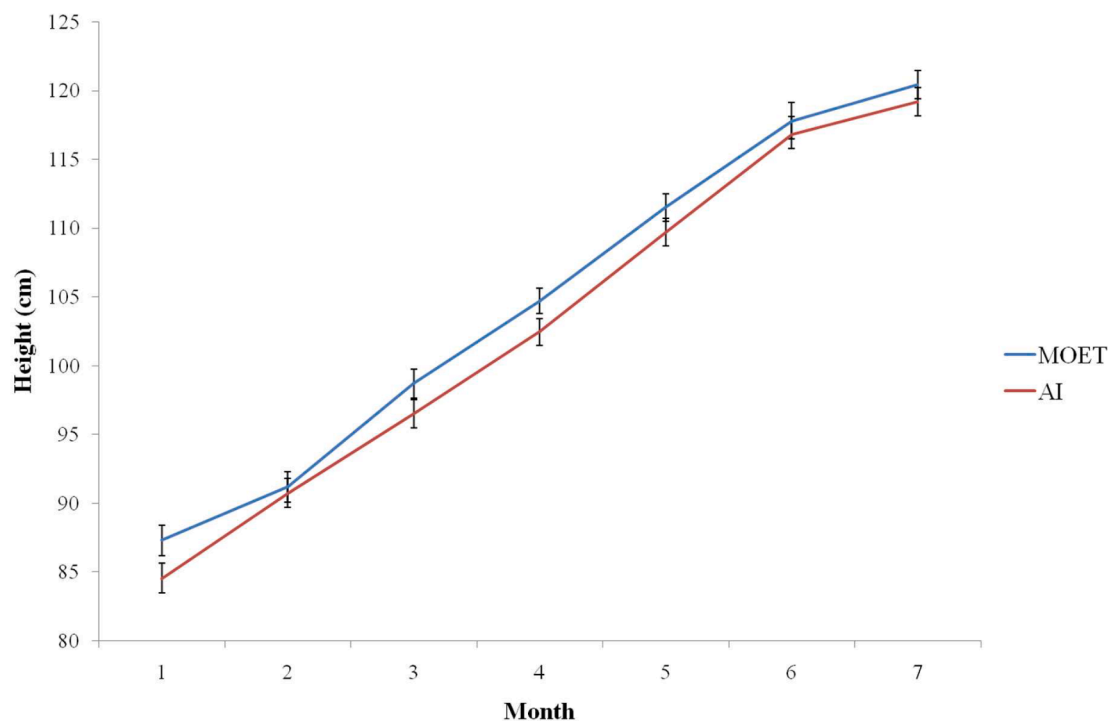


Fig. 2. The growth of calves. MOET and AI heifer height measurements from 1 month to 7 months of age.

Coordinated growth dynamics were maintained at 3 months of age, as indicated by a strong positive correlation between height and body weight ($r = 0.77$; $P < 0.05$) (Table 5), suggesting that larger skeletal size remains closely linked to increased body mass. GH and IGF-1 concentrations did not show statistically significant correlations at this stage; however, a moderately negative relationship ($r = -0.44$; $P > 0.05$) was observed, which may reflect subtle changes in regulatory interactions

within the somatotrophic axis during early postnatal development. Leptin concentrations remained weakly and non-significantly associated with growth or endocrine parameters. The correlation with body weight was weak ($r = 0.45$; $P > 0.05$), and similarly low, non-significant associations were observed with GH and IGF-1 (Table 5).

At 7 months of age, a strong positive correlation between body weight and height persisted ($r = 0.79$; $P < 0.05$), indicating continued

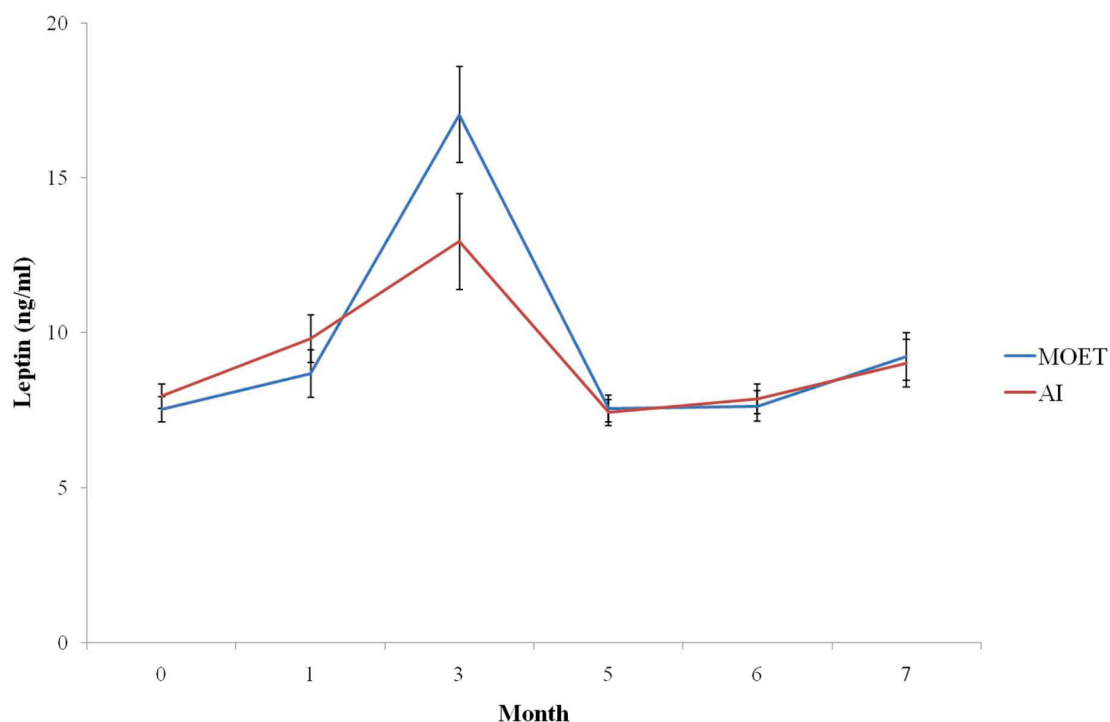


Fig. 3. Leptin levels. MOET and AI heifers' blood serum leptin levels from birth to 7 months of age.

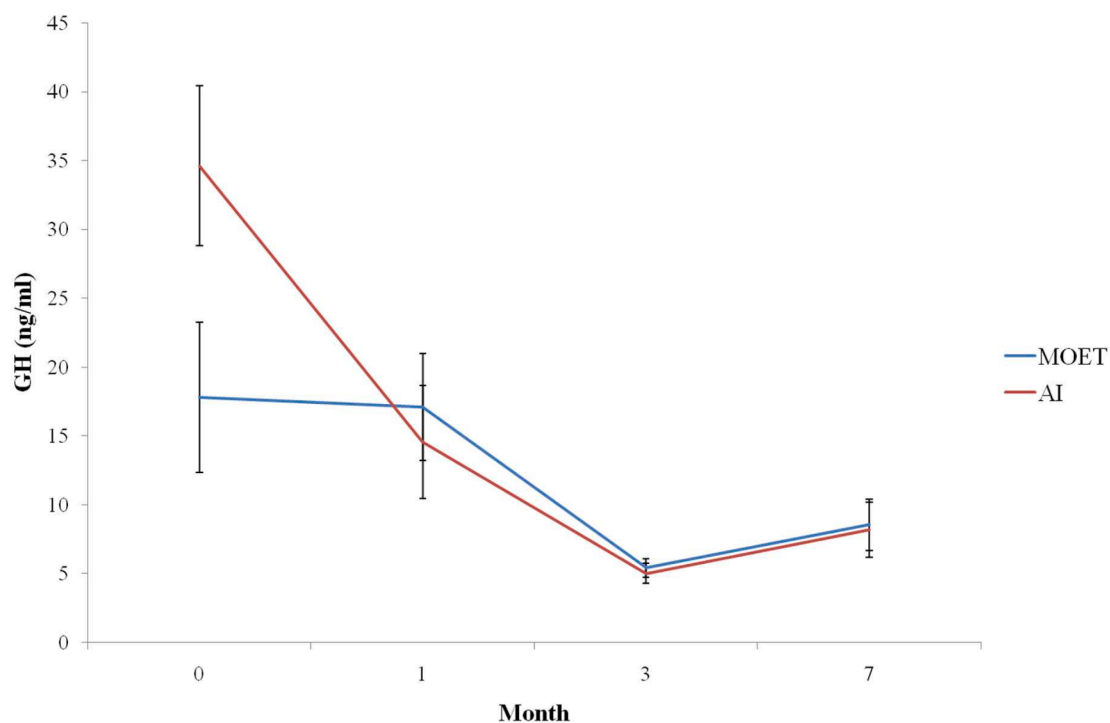


Fig. 4. Growth hormone levels. MOET and AI heifers' blood serum growth hormone (GH) levels measured at birth and at 1, 3, and 7 months of age.

coordinated growth. Among the hormone parameters, a weak to moderate negative trend was observed between GH and leptin concentrations ($r = -0.42$; $P > 0.05$). No significant correlations were found between IGF-1 and growth parameters, including body weight and height. Similarly, the relationship between IGF-1 and GH remained weak and non-significant (Table 6).

At 7 months of age, leptin concentrations exhibited weak and non-significant correlations with growth parameters ($r = -0.13$ to -0.41).

4. Discussion

The current study provides consistent evidence of highly similar postnatal growth, endocrine regulation, and early immunological development in calves derived from MOET vs. AI. The findings indicate that, with the exception of a significantly higher birth weight in MOET calves, there is a lack of statistically significant differences between the two groups, suggesting highly comparable physiological trajectories

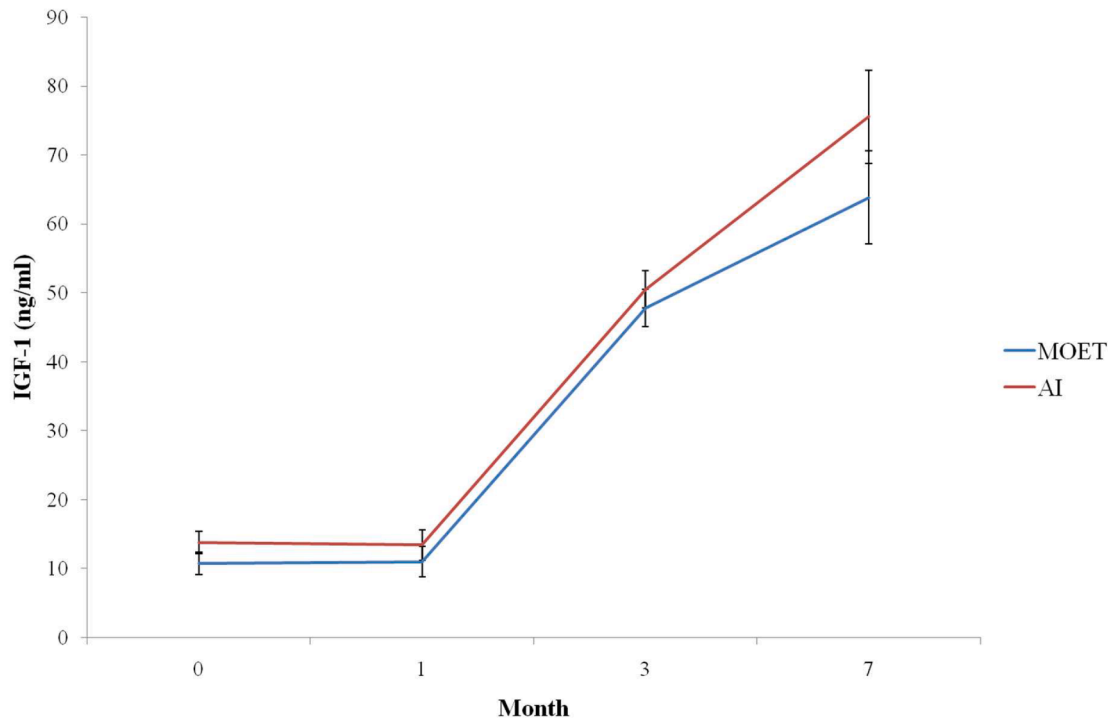


Fig. 5. Insulin-like growth factor-1 levels. MOET and AI heifers' blood serum insulin-like growth factor-1 (IGF-1) levels measured at birth and at 1, 3, and 7 months of age.

Table 2
MOET and AI calves' blood serum immunoglobulin G (IgG) levels tested at birth, repeated at 1 month.

		Monitoring period (month)	
		0	1
IgG (mg/mL)	MOET	28.31±0.75	12.49±0.28 ^a
	AI	29.99±0.75	13.39±0.28 ^b

^{a,b}: the different letters show significant differences between groups within categories ($P < 0.05$).

under the evaluated management conditions. Our results are consistent with reports indicating that, under proper management, assisted

reproductive technologies do not adversely affect calf growth or endocrine maturation negatively (Hasler, 2014).

Body weight and height both showed continuous, age-appropriate growth patterns in both groups. Although a higher birth weight was recorded in the MOET group, subsequent growth trajectories converged, and no statistically significant main effect of reproductive origin was observed. Under the evaluated standardized management conditions, growth dynamics remained highly consistent between MOET- and AI-derived calves.

A similar pattern was observed in skeletal development. Although height values were occasionally higher in MOET-derived heifers, these differences were not statistically significant and did not affect the overall consistency of growth trajectories. The increase in individual variation

Table 3
Correlation between the expression level of leptin, GH, IgG and IGF-1 in cord venous blood and calves birth weights.

	Weight		GH		IGF-1		IgG	
	r	P	r	P	r	P	r	P
Leptin	−0.39	0.09	−0.12	0.62	−0.36	0.12	−0.40	0.09
Weight			0.27	0.26	0.46*	0.04	0.23	0.33
GH					0.63**	0.00	0.44	0.06
IGF-1							0.54*	0.02

*, Correlation is significant at the 0.05 level (2-tailed). **, Correlation is significant at the 0.01 level (2-tailed).

Table 4
Correlation between the expression level of leptin, GH, IGG and IGF-1 in cord venous blood and weights and heights of 1 month old calves.

	Height		Weight		GH		IGF-1		IGG	
	r	P	r	P	r	P	r	P	r	P
Leptin	0.05	0.83	0.07	0.76	−0.24	0.31	−0.25	0.29	0.25	0.30
Height			0.75**	<0.001	−0.29	0.22	−0.23	0.33	0.12	0.62
Weight					−0.17	0.48	−0.11	0.63	0.01	0.96
GH							0.61**	0.01	−0.03	0.91
IGF-1									0.20	0.40

*, Correlation is significant at the 0.05 level (2-tailed). **, Correlation is significant at the 0.01 level (2-tailed).

Table 5

Correlation between the expression level of leptin, GH and IGF-1 in cord venous blood and weights and heights of 3 months old calves.

	Height		Weight		GH		IGF-1	
	r	P	r	P	r	P	r	P
Leptin	0.18	0.48	0.45	0.06	−0.45	0.07	−0.02	0.94
Height			0.77**	<0.01	0.29	0.26	−0.32	0.20
Weight					0.02	0.95	0.05	0.85
GH							−0.44	0.08

*. Correlation is significant at the 0.05 level (2-tailed). **.Correlation is significant at the 0.01 level (2-tailed).

Table 6

Correlation between the expression level of leptin, GH and IGF-1 in cord venous blood and weights and heights of 7 months old heifers.

	Height		Weight		GH		IGF-1	
	r	P	r	P	r	P	r	P
Leptin	−0.41	0.09	−0.13	0.61	−0.42	0.08	0.32	0.20
Height			0.79**	<0.001	0.21	0.41	−0.33	0.18
Weight					0.17	0.51	−0.17	0.50
GH							0.23	0.37

*. Correlation is significant at the 0.05 level (2-tailed). **.Correlation is significant at the 0.01 level (2-tailed).

observed at later stages, particularly at 7 months of age, likely reflects a natural divergence in growth rates over time rather than an effect of reproductive origin. This can be attributed to the growing influence of individual genetic-environmental interactions during postnatal development (Kertz et al., 1998; Owens et al., 1993), rather than effects associated with assisted reproductive technologies.

Physiological stability was further supported by the endocrine profiles. Serum levels of GH and IGF-1 were statistically comparable between the groups throughout the study period, indicating functionally equivalent growth-related endocrine regulation regardless of the conception method (Etherton-Bauman, 1998). Although an increase in individual variability for IGF-1 was observed at 7 months of age, this pattern likely reflects divergence in individual physiological maturation during late development rather than a systematic effect of reproductive origin.

Leptin concentrations also exhibited consistent pattern in both groups, with no evidence of divergence at any sampling point. While a slight numerical variation occurred at three months of age, this difference was not statistically or biologically significant, as serum concentrations reconverged by 7 months of age. The weak and non-significant correlations between leptin and growth or other hormonal factors suggest that leptin plays a limited role in the regulation of early postnatal growth, supporting the view that it is more strongly involved in long-term energy balance and adipose tissue development (Houseknecht et al., 2000).

From an immunological perspective, the transient divergence observed in IgG concentrations at 1 month of age most likely relates to differences in passive immunity transfer and early immune system development, potentially reflecting subtle variations in colostrum intake dynamics (Godden, 2008). Because the synepitheliochorial placenta prevents transplacental immunoglobulin transfer in cattle, initial calf serum IgG is primarily governed by colostrum management rather than dam systemic immunity; therefore, the use of standardized, quality-evaluated colostrum successfully controlled for maternal immunological variation across both groups. Consequently, although a minor difference in IgG was observed at 1 month of age, this effect was strictly transient and IgG concentrations subsequently reconverged, indicating no long-term divergence in immunocompetence or the overall trajectory of immune maturation.

The correlation analyses revealed limited statistically significant associations among the examined parameters, underscoring the independent regulation of these physiological systems during early postnatal development. As expected, a strong and continuous positive correlation was observed between body weight and height throughout the

evaluation period, reflecting coordinated somatic growth and proportional skeletal development. Within the somatotrophic axis, a moderate positive correlation was detected between GH and IGF-1 at 1 month of age, aligning with the active coupling of the axis during early growth. By 3 months of age, this association shifted toward a weak negative numerical trend; however, this trend did not reach statistical significance and remains a purely descriptive observation. Furthermore, circulating leptin showed no statistically significant relationships with GH or any somatic growth measurements at any evaluated time point, indicating that leptin levels were not directly linked to GH secretion or early growth rates under the conditions of this study.

Overall, these findings demonstrate the physiological robustness and stability of growth, endocrine profiles, and immune regulation during early postnatal life in dairy calves, with no evidence of adverse effects associated with assisted reproductive technologies. Under the standardized conditions of this study, the observed trajectories indicate a high degree of physiological similarity between MOET- and AI-derived calves.

Several limitations should be considered when interpreting the present findings. First, the relatively small sample size ($n = 10$ calves per group, $N = 20$ in total) of this longitudinal physiological study may have limited the statistical power to detect subtle, low-magnitude differences, particularly in variable endocrine and immunological parameters. Second, the study was conducted within a single commercial dairy herd, which may restrict the direct generalizability of the findings to other management systems, breeds, or environmental settings. Third, commercial farm conditions inherently limited the degree of experimental control over individual microenvironmental and nutritional variations.

However, the application of uniform management conditions across both groups ensured a strictly comparable environment, thereby minimizing confounding factors and enhancing the practical relevance of the results. Furthermore, while maternal dam immunological status was not directly measured, maternal variation was effectively controlled by standardizing dam parity (all calves were derived from primiparous dams) and administering standardized, quality-evaluated colostrum (Brix > 25%) via an esophageal feeder within one hour of birth, it must be acknowledged that maternal influences extend beyond colostrum passive immunity transfer. Gestational environment, intrauterine nutrient availability, and maternal physiological status during pregnancy were not directly measured in this study and remain potential unmeasured confounders. Consequently, while the longitudinal assessment of multiple physiological systems supports the observed trajectories, potential subtle effects originating from the intrauterine environment or recipient physiology cannot be fully excluded.

5. Conclusions

The results indicate that under standardized management conditions, calves derived from MOET exhibit postnatal growth, somatotrophic axis regulation, leptin dynamics, and immune development highly comparable to those conceived via AI. From a livestock breeding perspective, these findings indicate that MOET can be utilized to accelerate genetic improvement without inducing detectable disruptions in early postnatal development. Furthermore, the observed trajectories highlight that post-birth environmental factors, such as nutrition and colostrum management, likely play a primary role in shaping early physiological traits.

However, given the relatively small sample size ($n = 10$ calves per group, $N = 20$ in total) and single-herd setting, these conclusions should be interpreted within the specific context of this study. While these data indicate that MOET is a practical reproductive approach in dairy systems, larger multi-herd studies are needed to confirm these findings across diverse management environments and broader genetic lines. Additionally, extending physiological and health tracking into the first lactation would provide valuable insights into long-term metabolic health and productivity.

Ethical approval

No separate ethical approval was required for this study because all animal handling, housing, feeding, and sampling procedures were carried out as part of the farm's routine veterinary and herd management practices. Blood samples were collected exclusively during regular health monitoring procedures by a licensed veterinarian, and no additional invasive interventions or experimental manipulations were performed specifically for research purposes. Animal management complied with standard commercial dairy farming practices and applicable national and European Union regulations concerning animal welfare.

Ethics declaration

This study was conducted in accordance with the following guidelines for animal welfare and/or reporting: Decree No. 45/2019 (IX. 25.) of the Ministry of Agriculture (AM). Ethics committee approval was not required. No separate ethical approval was required for this study because all animal handling, housing, feeding, and sampling procedures were carried out as part of the farm's routine veterinary and herd management practices. Blood samples were collected exclusively during regular health monitoring procedures by a licensed veterinarian, and no additional invasive interventions or experimental manipulations were performed specifically for research purposes. Animal management complied with standard commercial dairy farming practices and applicable national and European Union regulations concerning animal welfare.

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CRediT authorship contribution statement

Eszter Tóth: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **János Posta:** Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Gabriella Gulyás:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Levente Czeglédi:** Writing – review & editing. **József Rátky:** Writing – review & editing. **Renáta Knop:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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