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Original Article

Hematological characteristics and endocrine profiles of cloned dromedary camels (*Camelus dromedaries*)

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ABSTRACT

Background: Somatic cell nuclear transfer (SCNT) is a prominent technology that can preserve superior genetic traits of animals and expand the population in a short time. Hematological characters and endocrine profiles are important elements that demonstrate the stability of the physiological state of cloned animals. To date, several studies regarding cloned camels with superior genes have been conducted. However, detailed hemato-physiological assessments to prove that cloned camels are physiologically normal are limited. In this study, We evaluated the hemato-physiological characteristics of cloned male and female dromedary camels (*Camelus dromedaries*).

Methods: Therefore, we analyzed variations in hematological characteristics and endocrine profiles between cloned and non-cloned age-matched male and female dromedary camels (*Camelus dromedaries*). Two groups each of male and female cloned and non-cloned camels were monitored to investigate the differences in hemato-physiological characteristics.

Results: All the animals were evaluated by performing complete blood count (CBC), serum chemistry, and endocrinological tests. We found no significant difference between the cloned and non-cloned camels. Furthermore, the blood chemistry and endocrine profiles in male and female camels before maturity were similar.

Conclusions: These results suggest that cloned and non-cloned camels have similar hematological characteristics and endocrine parameters.

Keywords: biochemical parameters, *Camelus dromedaries*, cloned animal, endocrine profile, hematological characteristics

INTRODUCTION

Since the birth of the first cloned sheep, "Dolly," numerous cloned offspring of various species have been produced using somatic cell nuclear transfer (SCNT) (Campbell et al., 1996; Lee et al., 2005; Ouyang et al.,

2021) SCNT is an important technology that enables the conservation of genetic resources of endangered species and breeding of animals with superior genetic traits (Galli et al., 2012; Selokar et al., 2019; Son et al., 2021). Additionally, animal cloning is rapidly attracting the interest of researchers because of its potential applications in animal disease models and production of useful proteins for humans (Whitelaw et al., 2016; Wang et al., 2017; Kashim et al., 2021).

Most animal cloning studies have been conducted to enhance cloning and offspring production efficiency using SCNT. Simultaneously, concerns regarding the physiological and genetic stability of cloned animals have been raised. Accordingly, broad studies investigating the growth pattern and physiological and epigenetic status of cloned animals have been conducted. For instance, the physiological status and DNA methylation patterns of cloned cows have been reported (Lanza et al., 2001; Cibelli et al., 2002; Fairburn et al., 2002; Lin et al., 2008). Previous studies showed that the anatomy, physiology, neurology, and growth patterns of cloned dogs were found to be similar to those of non-cloned dogs (Park et al., 2010; Kim et al., 2018). Additionally, in cloned pigs, the expression of hematological and biochemical markers was similar to that of those in non-cloned pigs after sexual maturity (Gu et al., 2019). Nevertheless, most physiological status studies involve cloned cows and dogs; moreover, they are limited to evaluation of abnormal gene expression profiles and DNA methylation differences.

Since the production of the first cloned dromedary camels (*Camelus dromedaries*) in 2010, another group has also reported the successful large-scale production of cloned camels (Wani et al., 2010; Olsson et al., 2021). Camels are livestock that can provide milk and meat even in extreme environments. Moreover, some camels are important commercially and culturally in the racing sector of the Arabian Peninsula (Elitok and Cirak, 2018). Therefore, the production of camels with superior performance using SCNT has recently attracted tremendous attention.

Camels required for racing competitions should be selected from among young ones considering that they usually begin training at the age of 2-3 years. Furthermore, abnormalities that cause frequent extension of gestation periods and survival rates of cloned animals can cause blood-physiological disorders, and early validation indicators are essential. However, studies regarding the overall hematological and endocrinological parameters of cloned young camels (< 1 year old) are lacking.

We conducted this study to investigate the hematological characteristics and endocrine profiles of cloned camels. We also evaluated the differences in sex-dependent hematological characters and endocrine profiles between age- and sex-matched cloned and non-cloned camels.

MATERIALS AND METHODS

Chemicals

All the chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise noted.

Animals

This study was conducted from November 2020 to December 2022. A total of 81 young camels (34 female cloned camels, 16 female non-cloned camels, 7 male cloned camels, and 24 male cloned camels; age, < 1 year) without any disease and having normal health status were included. Four donor cell lines were used to produce the cloned camels (two female and two male camels). All the camels were bred on the same farm.

The present study was conducted according to the animal study guidelines approved by the ethics committee of the Management of Scientific Centers and Presidential Camels (accession No: PC4.1.5). All animal experiments complied with the ARRIVE guidelines and were conducted in accordance with the UK Animals (Scientific Procedure) Act, 1986 and associated guidelines described by the EU Directive 2010/63/EU.

Blood sample collection

Venous blood samples of the camels in each group were collected via jugular venipuncture three times at 1-month intervals. They were transported in ice packs to the laboratory. We collected blood samples at the same time of day (11:00 am). Blood samples were centrifuged for 10 min at 3,000 rpm and the separated serum samples were used for the experiment within 6 hours.

Hematological and endocrinal analysis

The Procyte DX hematology analyzer (SYSTEM XN-1000, Vet Sysmex, Japan) was used to conduct complete blood count (CBC) analysis comprising the following 9 parameters: red blood cell (RBC), hemoglobin (HGB), packed

cell volume (PCV), white blood cell (WBC), neutrophils, lymphocytes, monocytes, eosinophils, and nucleated red blood cell (NRBC).

A benchtop dry chemistry analyzer (Cobas 8000, Roche, Japan) was used for blood chemistry analysis comprising the following parameters: glucose, blood urea nitrogen (BUN), creatine, total protein (TP), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), calcium, phosphate, albumin, iron (Fe), alanine aminotransferase (ALT), and copper (Cu).

Endocrinological parameters, namely triiodothyronine (T3), thyroxine (T4), and insulin-like growth factor-1 (IGF-1), were assessed using the Cobas analyzer (Cobas e411, Roche, Germany).

Statistical analysis

All the data were analyzed using the Student's *t*-test of variance with IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY, USA). The graphs were prepared using GraphPad Prism version 4.0 for Windows (GraphPad Software, www.graphpad.com). Statistical significance among mean values was estimated using the Tukey's post hoc test for multiple comparisons. The data were represented as means ± standard error (SE), and the differences were considered significant when $p < 0.05$.

RESULTS

Analysis of hematological values

Blood samples were analyzed based on the CBC results of each group for determining the related ratios pertain-

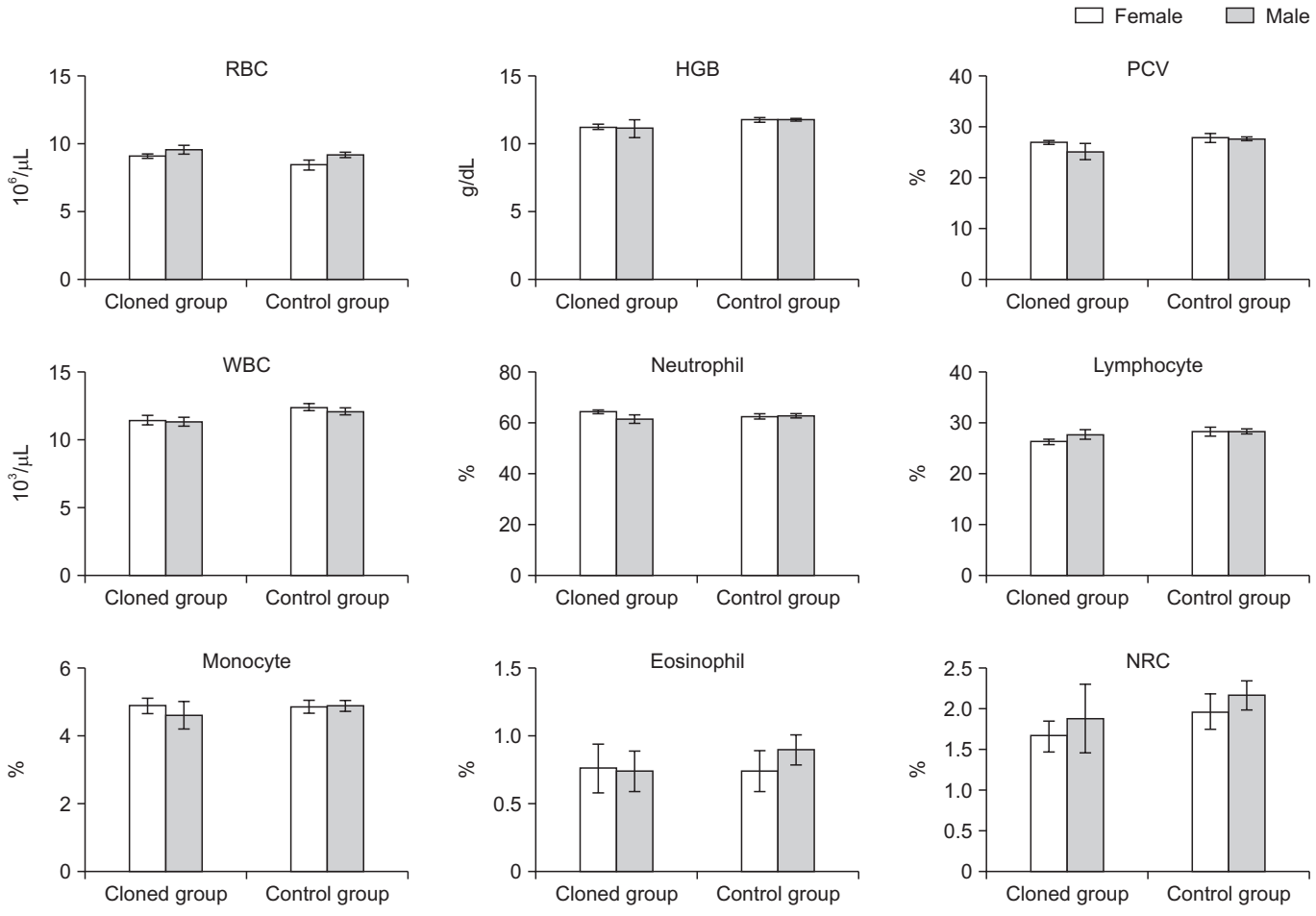

Fig. 1. Evaluation of complete blood count (CBC) of cloned and control age- and sex-matched camels comprising the following parameters: red blood cell (RBC), hemoglobin (HGB), packed cell volume (PCV), white blood cell (WBC), neutrophils, lymphocytes, monocytes, eosinophils, and nucleated red blood cell (NRBC). There are no significant differences in these values between the cloned and control groups. Data are presented as mean ± SE (standard error) of three independent experiments.

ing to the physiological conditions of the cloned and non-cloned camels (Fig. 1). The hematological parameters demonstrated no significant differences between cloned and non-cloned age-matched male and female camels. All the results were within the reference range presented in a previous study (Elitok and Cirak, 2018).

Analysis of biochemistry and endocrine profiles

Before maturity, the cloned and non-cloned camels revealed similar values of serum chemistry parameters (Fig. 2). The tests were repeated at three-time points for 3 months, and no significant differences were observed between the cloned and non-cloned age-matched male and female camels. The observed results were within the reference range for domestic camels [14]. Additionally, the

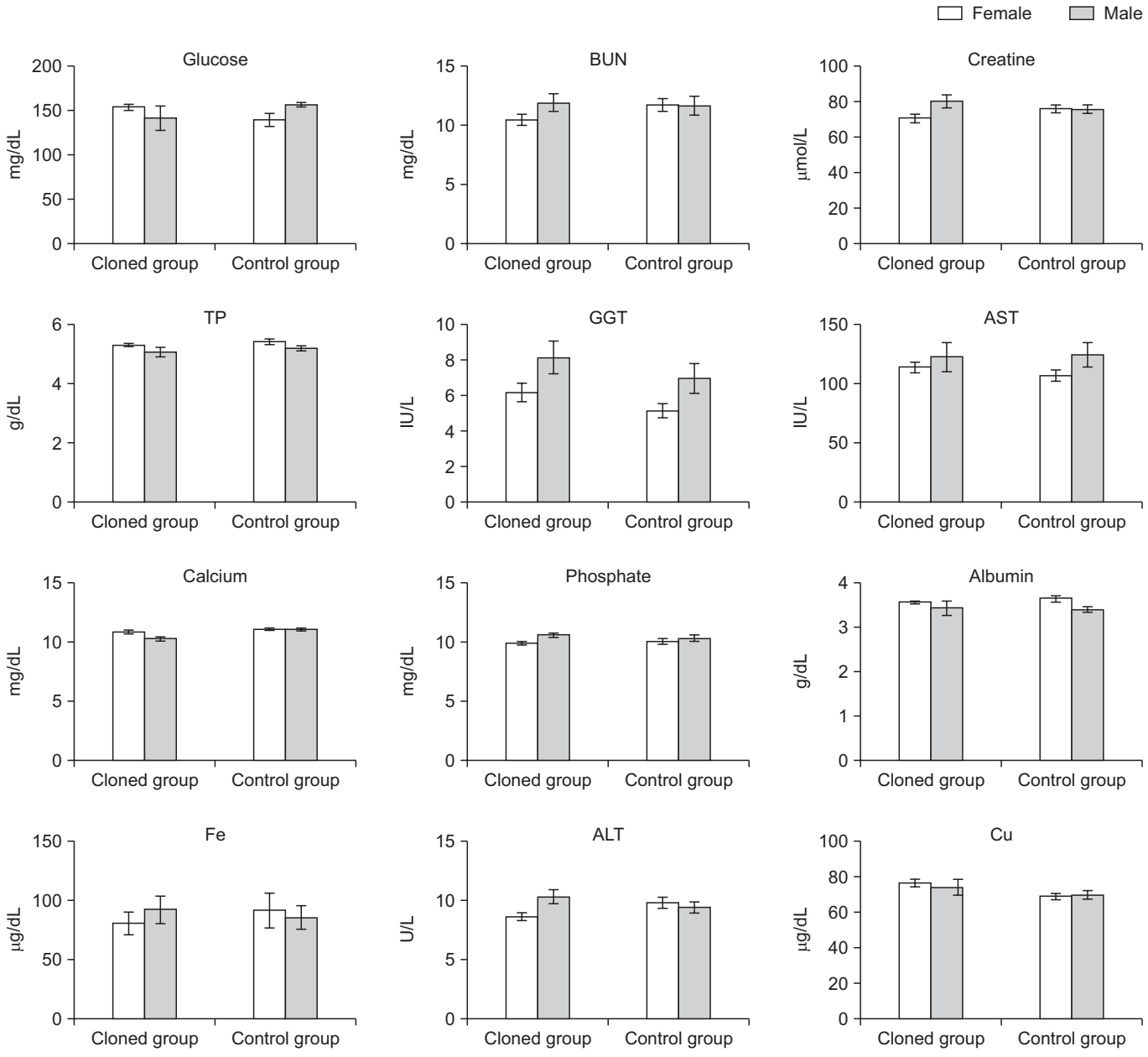

Fig. 2. Evaluation of blood chemistry of cloned and control age- and sex-matched camels comprising the following parameters: glucose, blood urea nitrogen (BUN), creatine, total protein (TP), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), calcium, phosphate, albumin, iron (Fe), alanine aminotransferase (ALT), and copper (Cu). There are no significant differences in these values between the cloned and control groups. Data are presented as mean ± standard error (SE) of three independent experiments.

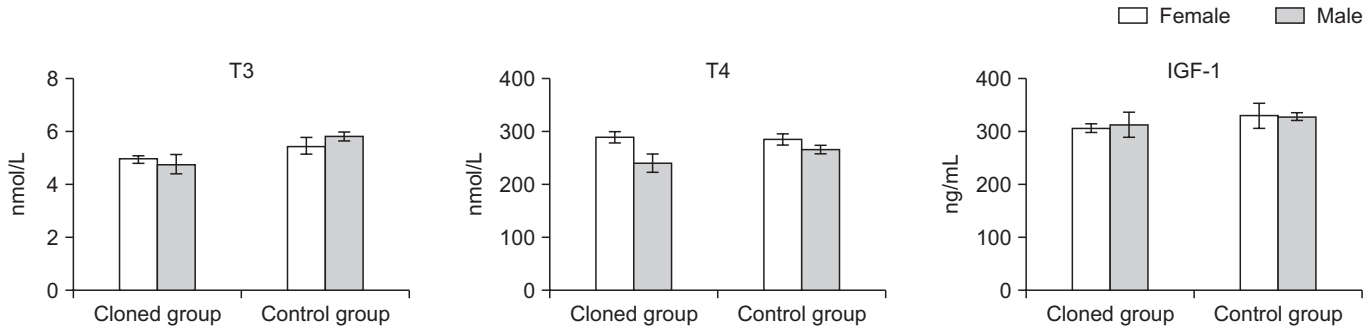

Fig. 3. Evaluation of endocrinological parameters (triiodothyronine [T3], thyroxine [T4], and insulin-like growth factor-1 [IGF-1]) of cloned and control age- and sex-matched camels. There are no significant differences in these values between the cloned and control groups. Data are presented as mean ± standard error (SE) of three independent experiments.

endocrinological test values were similar between cloned and non-cloned age-matched male and female camels (Fig. 3).

DISCUSSION

The camel is an animal of great interest in the milk and meat supply industry as well as the camel racing business in the Arabian Peninsula. Breeding camels is challenging because of their long gestation period, short breeding season, and relatively late age of sexual maturity (Singh et al., 2019). Hence, cloning technology using SCNT is being actively researched. Systematic studies concerning cloned camels have important applications in various fields. Moreover, they help maintain the genetic stability of superior traits in cloned camels. However, unlike other species, the physiological status and endocrinological parameters of cloned camels have not been reported to date.

Previously, epigenetic alterations have been reported in cloned animals that exhibit abnormal characteristics when compared with those of non-cloned animals. Minipigs cloned via SCNT showed increased teratogenicity and mortality during the perinatal period (Schmidt et al., 2015). Furthermore, reductions in overall DNA methylation, histone methylation, and acetylation have been reported (Jin et al., 2017; Hirose et al., 2018; Silveira et al., 2018). Hematological and biochemical profiles provide important information regarding the sex and health status of animals (Lee, 2014). In this study, hematological characteristics and endocrine profiles were analyzed to evaluate the physiological stability of young cloned camels.

Several researchers have reported that hematologic and serum biochemical parameters are influenced by age and sex (Harper et al., 2003; Swanson et al., 2004; Mundim et al., 2007; Lee, 2014). Previous studies have shown that the variation of hematological and biochemistry values observed in cloned pigs and goats are similar to those of the controls (Landry et al., 2005; Lee, 2014). Therefore, the cloned animals maintain a physiologically normal state. Similarly, our study showed no significant difference in more than 20 CBC, biochemistry, and endocrine parameters between cloned and non-cloned camels. Furthermore, our data confirmed that the CBC and biochemistry results of the dromedary camels were within the normal range (Elitok and Cirak, 2018).

Analysis of endocrinological parameters is an important factor in understanding the physiological state of cloned animals. The endocrine profiles, including IGF, T3, and T4 values, of cloned male and female minipigs have been reported to be similar (Lee, 2014). Moreover, previous studies showed that IGF-1, T3, and T4 levels of cloned and control goats are similar (Hashizume et al., 2000; Landry et al., 2005). We also found that IGF-1, T3, and T4 levels in the cloned camels were not significantly different in both males and females when compared with those of the control group.

CONCLUSION

The present study evaluates the hematological characteristics and endocrine profiles of age- and sex-matched cloned camels. We found that both male and female cloned camels show similar CBC and serum biochemistry values when compared with those of the non-cloned group. Moreover, these values were within the normal

range. Additionally, no significant differences were observed in T3, T4, and IGF-1 levels between the cloned and non-cloned groups. While further studies are required to assess the overall physiological and developmental status, our study suggests that non-cloned and young cloned camels have similar hematological and physiological parameters.

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Ethical Approval: All animal experiments were conducted according to the animal study guidelines, which were approved by the ethics committee of the UAE Biotech Research Center (Approval No.: UAEBRC-B01). These guidelines comply with the ARRIVE guidelines, the UK Animals (Scientific Procedure) Act, 1986, and EU Directive 2010/63/EU.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

Availability of Data and Materials: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: No potential conflict of interest relevant to this article was reported.

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