A Comparative Analysis of Pregnancy Success Rates between Day 3 and Day 4 for Frozen Human Embryos

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ABSTRACT

Objective: This study aimed to assess the pregnancy success rates following frozen embryo transfers conducted on either day 3 or 4. Embryos cryopreserved on day 3 underwent immediate transfer or were cultured for an additional day before transfer on day 4.

Methods: Retrospectively, the study examined outcomes from 186 frozen embryo transfers. Of these, 93 embryos were thawed and transferred on day 3, while the remaining 93 were thawed on day 3, cultured overnight, and then transferred on day 4.

Results: The current study revealed a higher pregnancy rate in the day 4 transfer group (66.7%) compared to the day 3 group (54.8%), although this difference lacked statistical significance (P=0.098). Furthermore, no significant disparities were observed between the two groups in terms of viable pregnancies (39.8% for day 3 vs. 43% for day 4, P=0.655), multiple pregnancies (15.1% for day 3 vs. 14% for day 4, P=0.835), or chemical pregnancies (21.5% for day 3 vs. 22.6% for day 4, P=0.860).

Conclusion: The findings suggest that transferring day 4 embryos can result in successful implantation and pregnancy rates comparable to those seen with day 3 embryo transfers. However, the statistical analysis indicates no definitive preference for either day.

Keywords: frozen embryos transfer, cryopreservation, thawing, pregnancy rate, transfer day 3, transfer day 4.

INTRODUCTION

One of the significant issues affecting human society is the 15% prevalence of infertility, which presents a threat to human survival.[1] In diverse populations, primary infertility accounts for 79%, while secondary infertility makes up 21%.[2] Globally, both fresh embryo transfer (ET) and frozen-thawed embryo transfer (FET) are commonly practiced. [3] A potential method for avoiding ovarian hyperstimulation syndrome is embryo cryopreservation.[4] Provided an adequate ovarian reserve, embryos at any development stage, from zygote to blastocyst, can be cryopreserved and remain viable for several years. Women utilizing cryopreservation tend to have a more favorable prognosis.[5] During fresh cycles, the endometrium is artificially prepared due to the detrimental effects of high hormone levels caused by controlled ovarian hyperstimulation (COH).

Disappear, the embryos may be cryopreserved and used for the next cycle. Similarly, progesterone and estrogen can be utilized for endometrial priming in frozen-thawed embryo transfers, and COH cycles provide greater control over endometrial development than gonadotropins do.[6] Embryo cryopreservation at the pronuclear, cleavage, and blastocyst stages allows for multiple transfer cycles from a single egg collection. This approach can significantly reduce the overall costs of fertility treatments, as transferring cryopreserved embryos is more cost-effective than initiating a new cycle from scratch.[4] Typically, embryo transfer is performed within three days following egg retrieval and fertilization, as embryos can be successfully transferred at any developmental stage, from zygote to blastocyst.[5] Moreover, one in five infertile couples has infertility, which is a typical difficulty for pairs of procreant ages. FET is now a requirement for obtaining ART.[7] This technique makes it possible to reduce the number of transferred embryos and decreases the likelihood of multiple pregnancies.[8,9] Blastocyst transfers have led to higher pregnancy rates, improved implantation rates, and a

decrease in numerous high-order pregnancies due to the transfer of fewer embryos than those at the cleavage stage.[10] Research indicates that utilizing single embryo transfer (SET) as a method in frozen embryo transfer (FET) can help decrease the likelihood of multiple pregnancies.[11]

Embryo cryopreservation is predominantly conducted using two techniques: vitrification and slow freezing. Vitrification is regarded as more effective and cost-efficient than the slow-freezing method. Due to the high concentration of cryoprotectants in vitrification, the reduced formation of intracellular ice crystals minimizes cellular damage, thereby enhancing the prospects for successful post-thaw reproduction.[12]

Previous research suggests that vitrification may lower the damage caused by freezing and improve the embryo's survival rate.[13] The uterine environment post-controlled ovarian hyperstimulation (COH) may be less conducive to implantation during fresh embryo transfers.[14] Pregnancy rates are generally lower in frozen embryo transfer (FET) cycles than in fresh embryo transfers.[15] Infants born following frozen embryo transfer (FET) tend to have higher birth weights and experience fewer adverse perinatal outcomes compared to those born after fresh embryo transfers.[16] Babies resulting from frozen embryo transfer (FET) display improved birth weights and reduced adverse perinatal outcomes in comparison to those from fresh embryo transfers.[14] The frozen embryo transfer (FET) technique involves transferring a limited number of embryos into the uterus, with surplus embryos being preserved for future use through cryopreservation. This study expected to compare the success rates of frozen embryo transfers on day three versus day four.

METHODS

Research timeframe and participant details

This retrospective cohort study examined all singleton live births from 2017 to 2021 that resulted from in vitro fertilization (IVF) and/or intracytoplasmic sperm injection (ICSI) at the Clinic of the Infertility at the Family Bedaya hospital branch in Beni-Suef.

Cryopreservation was supplied using rapid vitrification media (KITA ZATO), and third-day embryos were allowed to thaw. The primary outcome was to compare the pregnancy rate of frozen embryos thawed on day 3 and transferred the same day (day 3) versus embryos thawed on day 3, cultured the following day, and transferred (day 4). The secondary outcome was the effect of embryo freezing duration on pregnancy success rate.

Positive serum hCG results (≥10 IU/l) 16 days after frozen embryo transfer confirmed pregnancy (viable pregnancy). An early pregnancy loss (biochemical pregnancy) occurred when an ultrasound did not reveal the presence of an embryonic sac, even though the hCG level was positive. A clinical abortion that follows the transfer of embryos from a frozen donor takes place after the pregnancy has been clinically detected but before the 22nd week of gestation. Such deliveries were recorded when the gestational age was more than 22 weeks. A pregnancy rate (positive hCG) was calculated by dividing the total number of embryo transfers from frozen by the number of hCG tests that came back positive. Divide the number of positive hCG tests by the number of early pregnancy losses to get the biochemical pregnancy rate. To find the twin delivery rate, we divided the total number of births by the number of deliveries (Multiple pregnancies).

Ethical Approval

All data expressed in the current article is approved from ethical committee at faculty of medicine, Bnei-Suef University under the ethical approve number: FMBSUREC/03092023/ATWA. The data was obtained from Infertility Clinic of the Family Bedaya hospital branch, Beni-Suef. The data collection period was from 2017 to 2021 by formal acceptance to use data in the study and publish it.

Statistical analysis

Using SPSS 22.0 for Windows, we determined what clinical and embryological variables impacted the success rate of delivering a baby following a frozen embryo transfer. We used the Shapiro-Wilk test to make sure everything was normal. The qualitative data was presented using percentages and frequencies, and any differences between groups were evaluated with Fisher's exact test or chi-square, as the case may be. Group comparisons were done using the Independent T and Mann-Whitney tests, respectively. Customarily distributed quantitative data was reported as mean ± SD, while non-parametric data was given as median and range. A P-value lower than 0.05 was used to determine statistical significance, where $P < 0.001$ is used to indicate vital importance, and $P > 0.05$ is used to indicate no significant difference.

RESULTS

This study was done on 186 patients with thawed embryos on the third day. Half of them were defrosted, and embryo transfers were done on the same day after completely thawing on day 3. In another half (93 cases), the

embryos thawed on day 3 then culture in media and delivered by the end of the fourth day. The following tables show the general characters and results of the two groups.

The elements of the studied group's demographics

Figure 1 provides an overview of the participants' characteristics and the results of the fertilization process. Notably, no significant differences were observed in females' mean age and body mass index (BMI) between the two study groups (P value = 0.434 and 0.248, respectively).

Clinical data among the studied groups

According to Table 1, there is no noteworthy difference in the duration of infertility among the groups (p-value = 0.627), menstrual cycle length (p-value = 0.611), number of unsuccessful IVF cycles (p-value = 0.084), or types of infertility (primary and secondary) (p-value = 0.427).

Variations in clinical features among the examined categories

Table 2 indicate no substantial differences linking the groups regarding the number of embryos transferred (p-value = 0.927) or the time required to freeze embryos (p-value = 0.450).

The pregnancy rate was more significant in the Day 4 group (66.7% vs. 54.8%), as shown in Figure 2, but the difference was not statistically significant (P = 0.098).

Follow-up between the two studied groups.

Table 3 show no significance regarding viable pregnancy in two groups (Day 3 vs day4) (39.8% vs.43% p-value = 0.655) and multiple pregnancies (15.1% vs.14% p-value = 0.835), and chemical pregnancy (21.5% vs.22.6% p-value = 0.860)**.**

Table 1: The study illustrates no statistically significant variances between the groups concerning the duration of the menstrual cycle, the number of unsuccessful IVF attempts, or the categories of infertility.

	Day 3 (n=93)	Day 4 (n=93)	T	P
Infertility duration (years) Mean ± SD	7.41 ± 1.97	7.26 ± 2.23	0.486	0.627
Menstrual cycle length (days) Mean ± SD	30.51 ± 8.33	31.19 ± 9.79	0.510	0.611
Number of failed IVF cycles Mean ± SD	3.45 ± 0.918	3.69 ± 0.964	1.74	0.084
Types of infertility				
Primary infertility	67 (72%)	62 (66.7%)	0.632	0.427
Secondary infertility	26 (28%)	31 (33.3%)		

Table 2: Comparison of Pregnancy Rates between Two Studied Groups

	Day 3 (n=93)	Day 4 (n=93)	T	P
No. of embryos transferred. Mean ± SD	3.04 ± 0.736	3.03 ± 0.853	0.092	0.927
Period frozen Mean ± SD	146.12 ± 189.81	126.15 ± 169.49	0.757	0.450

Table 3: Indicates no statistically significant variance among chemical, multiple, and viable pregnancies.

	Day 3 (n=93)	Day 4 (n=93)	χ^2	P
Viable pregnancy	37 (39.8%)	40 (43%)	0.200	0.655
Multiple pregnancies	14 (15.1%)	13 (14%)	0.043	0.835
Chemical pregnancy	20 (21.5%)	21 (22.6%)	0.031	0.860

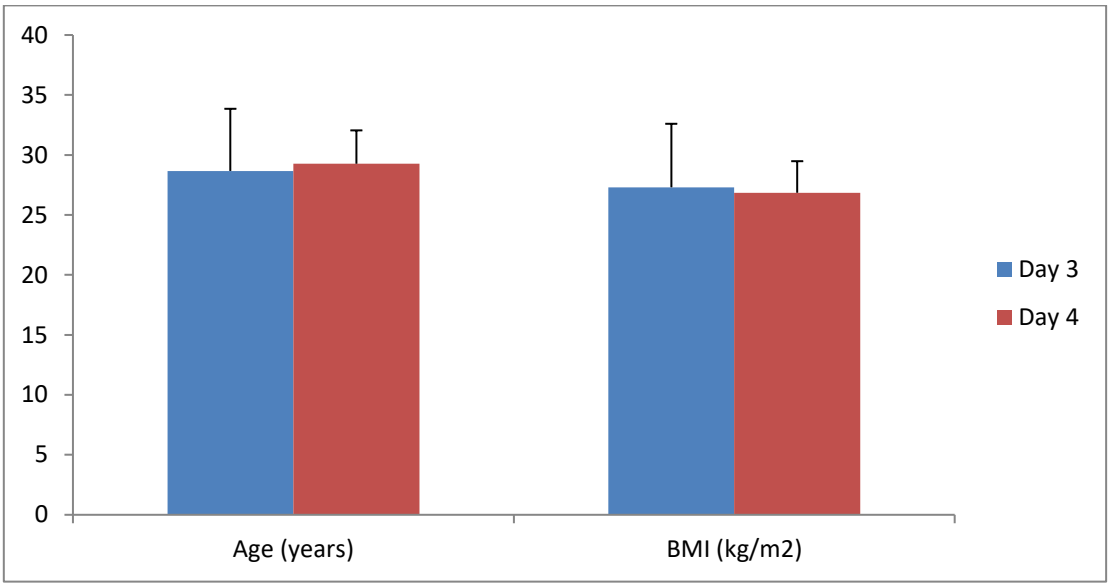

Fig.1: Cumulative histograms of age and body mass index (BMI) of pregnant women at delivery for Day 3 and Day 4 (t test, p>0.43 and 0.24, respectively)

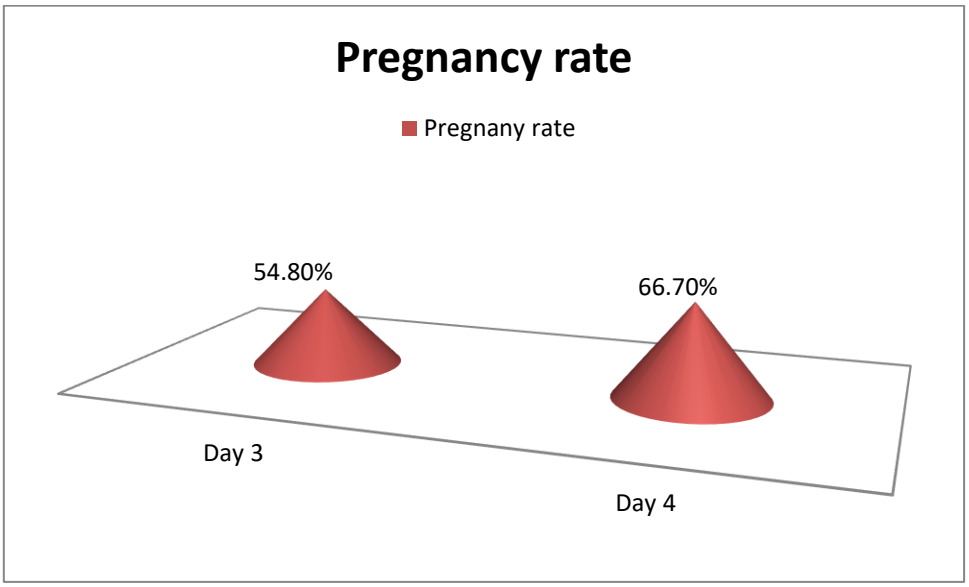

Fig.2: Cumulative histograms of pregnancy rate at delivery for Day 3 and Day 4 (X^2 test, p>0.098)

DISCUSSION

Transferring embryos and doing in vitro fertilization (IVF-ET) may see improved outcomes by transferring multiple embryos, as this approach raises the probability of having a healthy baby. Employing numerous embryo transfers in IVF procedures increases the likelihood of pregnancy. Conversely, this could result in multiple pregnancies and increase the risk of trouble for both the mother and the baby. The optimum method of treatment for an ART term single live birth is suggested to be elective single embryo transfer or ESET. IVF-ET additionally comes with a higher risk of multiple pregnancies, which can result in many significant problems for both the mother and the newborn. Some of the many pregnancy complications include fetal abnormalities, low birth weight, placental abruption, polyhydramnios, amniotic fluid embolism, uterine inertia, postpartum hemorrhage, abnormal fetal positioning and presentation, preterm birth, premature membrane rupture, polyhydramnios, twin transfusion syndrome, and many more.[17]

The most straightforward method to minimize the occurrence of multiple pregnancies of a child is by transferring an embryo. Assuming the number of embryos transferred stays the same, transferring high-quality blastocysts—the product of protracted in vitro embryo cultivation—is linked to better implantation and clinical pregnancy rates than transferring embryos at the cleavage stage.[18]

Reproductive features have used single blastocyst transfer more frequently in recent years. However, throughout the blastocyst culture phase, there is a chance that some or all of the embryos will not successfully blastulate, which might lead to a decline in the embryo pool or even the termination of the transfer cycle. Patients with high aspirations for assisted reproduction cannot accept this situation. Furthermore, in vitro culture embryos differ in terms of their rate of development (potential); a controversy has arisen regarding the relative merits of single blastocyst transfer versus multiple cleavage-stage embryo transfer because viable blastocysts mature on separate days (Days 5/6). There is no worldwide agreement or guideline on the optimal approach for embryo transfer.[19] The study's main goal was to identify the preferred day for embryo transfer, whether the frozen embryo was thawed on day 3 or day 4.

The capacity of embryos to develop in vitro leads to the production of viable blastocysts on varying days (Day 5/6). Currently, there is no consensus or recommended practice for embryo transfer worldwide, with ongoing debate on whether transferring a single blastocyst offers superior clinical results compared to transferring two embryos at the cleavage stage.[20] The study aimed to ascertain the optimal day for embryo transfer, focusing on whether the best outcomes were achieved when the frozen embryo was thawed on day 3 or 4. In Group A, which underwent day 4 embryo transfers, out of 171 women, two failed to respond to controlled ovarian hyperstimulation (COH), and three had no embryos available for transfer. In contrast, Group B, which had day 3 transfers among 172 participants, included 2 women who did not respond to the COH and two more who did not have embryos to transfer. According to Mohammed and colleagues, the study found no considerable difference in the age of females between the 2 groups (P,.09).[21] .the population was split into 2 categories: 1. Group 1 (n = 150) underwent FET (cleaved embryo) after day 3 of embryo freezing. 2. Group 2 (n = 150) had FET (blastocyst) after undergoing embryo freezing on day 5. They demonstrated no discernible difference in age or body mass index (BMI) that separates the two sets of people.

According to the current study, there are no appreciable differences from one set to another according to the duration of the menstrual cycle, the number of unsuccessful IVF rounds, or the forms of infertility.

Our findings agreed with a study by Pantos et al.[20] which found that the two groups' rates of unexplained infertility were similar (P,.35). According to Alfaraj et al.[22], out of the study participants, 293 underwent embryo transfer on day 5. At the same time, 411 had their embryo transfer on day 3. A total of 704 individuals met the inclusion criteria for the study. When examining the causes of infertility—male factor (n=251), female factor (n=248), a combination of factors (n=129), and unexplained (n=70)—there was no statistically significant variation between those in the day 3 and day 5 groups (P=0.362). Furthermore, comparisons regarding primary versus secondary unproductiveness (P=0.17) and the type of fertility treatment, IVF versus ICSI (P=0.42), showed no substantial differences between the 2 embryo transfer groups.

The study by Maxwell et al.[23] conducted by NYUFC from 2003 to 2012, reported 2392 singleton live births developing from IVF/ICSI, contrasting our findings. Patients who had embryo transfers (ET) on days 2, 4, or after day 6 were excluded, leaving 2314 newborns for analysis. Of these, 421 underwent day 3 ET, while 1893 had ET on days 5/6.
No notable variances were observed in the number of prior miscarriages., parity, and gravidity between the two groups. However, women who had day 3 ET compared to those with day 5/6 ET were more likely to have undergone previous ART cycles, had more embryos transferred and had fewer single embryo transfers (SETs). Additionally, these women were more often in ART treatment due to a decreased ovarian reserve.

This study found no statistically significant differences between the FSH, LH, progesterone, E2, and AMH groups. Consistent with the findings of a study by Rao et al.[24] The study documented 609 cycles of frozen embryo transfer (FET), with 353 women undergoing transfers of embryos at the D3-DET stage, 45 women receiving transfers of embryos at the D5-SBT stage, and 43 women receiving transfers of embryos at the D6-SBT stage. Across all groups, the levels of anti-Müllerian hormones were not significantly different.

In the study of Lanlin Yang et al.[25], patients were equally divided into two groups: one underwent single embryo transfer (SET) with blastocyst and cumulus cells selection (D5 + CM), and the other had cleavage-stage SET using a time-lapse hierarchical classification for selection (D3 + TL). Among the per-protocol (PP) population, No notable distinction was observed between the two groups concerning their initial baseline characteristics or the outcomes of their cycles.

Considering grades 1 and 2, we have no discernible difference between the two groups in the study. The 2 groups do not significantly differ regarding the EMPs number or the frozen period. Regarding the quantity of mature oocytes, retrieved oocytes, and fertilized oocytes, the groups were equivalent but did not differ statistically significantly.

Our results indicate no meaningful variance in the number and proportion of good-quality embryos per retrieved oocyte between the two groups. While the Day 4 group exhibited while the Day 3 group had a lower pregnancy rate, the difference between the two groups was not statistically significant. Additionally, there were no prominent findings concerning chemical, multiple, and viable pregnancies.

Currently, most ART procedures involve the transfer of embryos using embryos just before cleavage, also called blastocysts, which are transferred on days two, three, five, or six. It appears that specialists in assisted reproductive technology have not attempted, at least not widely or methodically, to transfer day 4 embryos, despite the potential benefits of doing so using embryos that have progressed through the developmental stages up to the morula stage. Currently, morphologic parameters about the number of blastomeres per embryo, cytoplasmic appearance, and grade of fragmentation are used to choose early-cleavage embryos for transfer; however, these criteria are somewhat arbitrary. A one-day delay from day two to day three is relatively brief to better distinguish the quality of the embryos. Day 4 embryos have initiated genome activation, a process in humans between the 4 and 8-cell stages, with earlier stages experiencing limited gene transcription. So, embryos on day 4 may have less probability of chromosomal abnormalities and more developmental potential. On top of that, day 4 embryos spend less time in vitro cultured than blastocysts.[26]

No studies in the medical literature have investigated the feasibility of embryo transfer on day 4. Huisman et al.[27] compared the outcomes of embryo transfers on days 2, 3, or 4 in retrospective research. The researchers found that while the rates of ongoing pregnancies were similar, cavitating morulas on day 4 had an extremely high implantation rate of 41%. They also found that throughout all embryo transfer days, the development rate into the morula stage was 18.4% when using a single culture medium formulation.[28] Both the clinical pregnancy rate and the live birth rate were found to be similar in a retrospective analysis that was also carried out that year. Day 3 embryo biopsies were replaced with day 4 embryo transfer a few years later.[29] Furthermore, comparable overall implantation rates (14.4%, 14.7%, and 15.5%, respectively) were reported for the three groups in the published results of prospective research that included couples who had transfers on days 3, 4, or 5 with a maximum of two embryos.[30] Additionally Prapas et al.[31] using data collected from transfers on days 3, 4, and 5, researchers in a prospective study comparing two embryo transfer techniques (ultrasound-guided vs. "clinical touch") discovered that the pregnancy rates for ultrasound-guided transfers were 45.9%, 42.3%, and 56.3%, respectively. The rates for "clinical touch" transfers were 37.1%, 27%, and 45.7%, respectively. The use of ultrasound aid significantly improved the results, according to their findings.

A total of 242 transfers on day 4 and 97 transfers on day 3 were included in retrospective research.[32] A grading system for morula/compacted embryos was proposed and implemented by the researchers. The researchers discovered that implantation rates were similar to or even greater with transfers made on day 4, especially when all "good" embryos were transferred. Also, using just one culture medium, they saw that 59.2% of the embryos considered "good" on day 3 were still considered "good" on day 4. Results showed that the clinical pregnancy rate after day 4 transfers was higher (but not substantially) than after day 3 (59.1% vs. 40.1%) and lower (but still not considerably) than after day 5 (59.1% vs. 68.4%). The work was presented at a conference.[33] Embryo transplantation on days 3, 44, and 38 was part of this study.

Additionally, Saadat et al.[34] presented the findings of a retrospective study at a different conference. In in the research, 239 individuals had embryos transferred on day 4, and 286 had them moved on day 3. With 46% and 42%, respectively, the clinical pregnancy rates were similar in the two groups. Nevertheless, the writers saw a significant pattern suggesting an increased pregnancy rate after transfers on day 4 in women under the age of 34.[35] In a prospective randomized trial, researchers conducted 79 embryo transfers on day 3, 76 on day 4, and 79 on day 5. The outcomes following day 4 transfers were comparable to those after day 3 and day 5, except for the implantation rate, which was significantly lower on day 4 (27.6% vs. 41.8%) compared to day 3.

This study used an essential two-scale grading score for embryos on days 3 and 4, because there isn't a commonly acknowledged way to grade compacting or compressed embryos; the only options are customized systems. This method made it easier to compare and understand the results. So, both groups underwent embryo transfers in the same amount of time; however, the group given more time on day 4 did not have a higher rate of multiple pregnancies. Further reduction in the number of transplanted embryos without compromising pregnancy rates may be possible by establishing and implementing strict quality standards for compacting/compacted embryos[32]

The embryos transferred on day 4 have a better chance of survival and hatching because of the large amount of perivitelline space in the compressed precipitated embryo.. Additionally, by delaying the cryopreservation of spare embryos, the competent embryos are chosen for both transference and freezing up. These are some additional potential benefits of day 4 embryo transfers. Nevertheless, it is essential to note that humans and mice have given birth to live children following the transfer of thawed morula or compacted embryos.[36]

Ultimately, a promising alternative to day 3 embryo transfers in assisted reproductive technology is day 4 embryo transfers, which can produce implantation and pregnancy rates that are on par with day 3 transplants.

Conflict of Interests

No conflict or competing interests.

Author contributions

M.N. obtained the clinical data, A.A. and M.A. planned, organized, and executed this investigation. Methods were created, data was evaluated, statistical analysis was carried out, and a manuscript draft was produced by A.A., R.A., M.A., S.O., and M.N. A.D. revised manuscript. Each author reviewed and approved the finished work.

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