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Sperm Motility of Cryopreserved Buffalo Semen After Thawing at Different Temperatures and Duration

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Abstract:

This study investigates the effect of different thawing temperatures and durations on the motility of spermatozoa in cryopreserved buffalo semen. A total of 40 buffalo semen straws from the Lembang Artificial Insemination Center (batch code AW 1205) were used. The experimental design was a completely randomized design with 10 replications and 4 treatment groups: P0 (37°C for 30 seconds), P1 (28°C for 30 seconds), P2 (28°C for 45 seconds), and P3 (28°C for 60 seconds). Sperm motility was evaluated using a microscope at 400x magnification, and motility was based on the percentage of sperm moving progressively. Statistical analysis was performed using ANOVA followed by Duncan's Multiple Range Test. The results showed that the highest motility (43.50%) was observed in P3 (28°C for 60 seconds), followed by P0 (43%), P2 (35.50%), and P1 (31.59%). These findings suggest that thawing buffalo semen at 28°C for 60 seconds provides the highest sperm motility, which could improve the efficiency of artificial insemination programs.

Keywords **—Buffalo, Sperm Motility, Thawing, Cryopreservation, Artificial Insemination, Temperature, Duration**

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I. INTRODUCTION

Buffalo (Bubalus bubalis) plays a significant role in dairy and meat production in many parts of the world. Cryopreservation of buffalo semen has become an essential tool in animal breeding, particularly for artificial insemination (AI) programs aimed at improving genetic quality and maintaining livestock diversity. However, the thawing process is crucial, as improper thawing can lead to significant damage to spermatozoa, particularly affecting sperm motility, which is a key factor in fertilization success.

Motility is one of the most critical indicators of sperm quality, and different thawing protocols, including temperature and duration, can impact sperm motility. While various thawing protocols have been established for different species, there is limited research on the optimal thawing conditions for buffalo semen. This study aims to evaluate the effects of different thawing temperatures and durations on the motility of buffalo spermatozoa using straws from the Lembang Artificial Insemination Center.

II. MATERIALS AND METHODS

A total of 40 buffalo semen straws from the Lembang Artificial Insemination Center, batch code BIB LEMBANG AW 1205 CAESAR JR 131321 KERBAU, were used for the experiment. The experimental method involved the following treatments:

- **P0**: Thawing at 37°C for 30 seconds
- **P1**: Thawing at 28°C for 30 seconds
- **P2**: Thawing at 28°C for 45 seconds
- **P3**: Thawing at 28°C for 60 seconds

A. Sperm Motility Assessment

To assess motility, a drop of semen is placed on a microscope slide and observed under a microscope with 400x magnification. Motility is evaluated across 5 fields of view. The percentage of

spermatozoa is determined by counting the number of live and progressively moving sperm, with the result expressed as a percentage ranging from 0 to 100%. [1]

B. Statistical Analysis

Data were analyzed using one-way ANOVA to determine the effects of thawing temperature and duration on sperm motility. If significant differences were found, Duncan's Multiple Range Test was used.

III. RESULTS AND DISCUSSION

TABLE I
SPERM MOTILITY AFTER DIFFERENT THAWING TREATMENTS

Treatment	Motility (%)
T0	43,00±2,13^{b}
T1	31,50±0,76^{a}
T2	35,50±1,17^{a}
T3	43,50±1,67^{b}

Different notation shows a significant difference ($P<0.01$)

The results of this analysis clearly demonstrate that both the temperature and duration of the thawing process have a significant effect on the post-thawing motility of buffalo semen ($P<0.01$). The motility percentages observed in each treatment were as follows: P0 (37°C for 30 seconds) exhibited 43% motility, P1 (28°C for 30 seconds) showed 31.59%, P2 (28°C for 45 seconds) displayed 35.50%, and P3 (28°C for 60 seconds) produced the highest motility at 43.50%.

The highest average motility was observed in treatment P3, with a value of 43.50±1.67%, which did not significantly differ from the control treatment P0 (43.00±2.13%). These findings were slightly lower than those reported by [2] who recorded a post-thawing motility of 57.44% when buffalo semen was thawed at 37°C for 30 seconds, and 53.15% using the dry thawing method under the same conditions. However, the results obtained in this study are still promising, particularly when compared to the other findings, who reported significantly lower post-thawing motility rates of 25.40% [3] and 42.51% [4], respectively. This suggests that the thawing protocol applied in the present study, specifically in terms of temperature and duration, may be more effective in preserving sperm motility compared to previously tested methods.

Treatment P0, which followed the Indonesian National Standard thawing at 37°C for 30 seconds, consistently resulted in sperm with progressive motility above 40%. Notably, treatment P3 exhibited results closely resembling the control treatment, indicating that when lower temperatures are used, it is crucial to adjust the thawing time accordingly. Conversely, treatments P1 and P2 yielded less favorable results, with average motility values of 31.50±0.76% and 35.50±1.17%, respectively, both of which fell below the SNI standard. This decline in motility can likely be attributed to incomplete thawing of the sperm cells, as noted by [5] who emphasized that thawing at lower temperatures, such as 28°C, requires a longer duration to ensure complete thawing compared to using warmer water. These findings underscore the importance of optimizing both the temperature and duration of the thawing process in order to achieve the most favorable sperm motility outcomes.

Furthermore, the results emphasize the critical role of temperature in the biochemical processes occurring during thawing. The control treatment (P0), which adhered to the SNI standard, facilitated optimal metabolic activity during thawing, ensuring that sperm cells received adequate energy substrates, thus supporting higher motility [6]. These temperatures are within the physiological range for livestock, supporting optimal enzymatic activity that supplies the chemical energy necessary for sperm movement. Therefore, it is crucial to select the appropriate thawing temperature and duration to optimize sperm motility and enhance the overall quality of buffalo semen

IV. CONCLUSIONS

In conclusion, the optimal thawing protocol for buffalo semen cryopreservation in this study was 28°C for 60 seconds (P3), as it resulted in the highest sperm motility. This finding is significant for improving the efficiency and success rates of artificial insemination programs in buffalo. Although 37°C for 30 seconds (P0) showed similar results in motility, the longer thawing duration at 28°C provides a more favorable environment for

sperm recovery. Further studies should explore the effects of thawing on other semen quality parameters, such as sperm viability and fertilization rates, to enhance the overall success of AI in buffalo breeding.

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