

**IMMUNOCYTOCHEMISTRY, ASSISTED BY
COMPUTER IMAGE ANALYSIS, OF
HYPOPHYSEAL PEPTIDE HORMONES OF THE
IMPALA (*AEPYCEROS MELAMPUS*)**

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RESUME

The South African National Defence Force has vast areas of land under its control, often occupied by wildlife, which it uses as training ground for its forces. As the extent to which military activities influences the environment is not commonly known, the SANDF has embarked on research programs to investigate the effects of these activities on the resident wildlife populations in order to manage them optimally.

The measurement of plasma hormone concentrations using radioimmunoassays is commonly used in the evaluation of stress in domestic animals. Such assays are costly, require a degree of sophistication in using specialised techniques in the veld and are difficult to interpret as the stress of restraining wild animals results in acute stress responses. It was thus decided to evaluate the use of immunocytochemistry, a more stable method, combined with computer image analysis, not only to visualise hypophysial cells active for hormones, but also to attempt to determine the magnitude of suspected structural changes. As the combination of these techniques would be utilised for the first time it was decided to first evaluate the technique under known differing physiological circumstances, for example in lactating and in growing animals. If this was successful it would be evaluated to estimate changes in ACTH secretion under different stress circumstances, for example free ranging animals and animals kept in an enclosure for various periods of time.

Impalas (*Aepyceros melampus*) are abundant and thus easily available for the study of their physiology. Animals used in this investigation were culled as part of other projects undertaken in the Kruger National Park.

Lactating impala ewes demonstrated significantly more prolactin immunoreactive images and an increased mean immunoreactive image surface area ($p < 0.05$, 5% level, 2-tail) compared to non-lactating ewes rejecting the null-hypotheses of no difference. However, no significant difference could be demonstrated for the proportion of area stained between the two groups.

Significantly more somatotropin immunoreactive images, an increased mean immunoreactive image surface area ($p < 0.05$, 1% level, 2-tail) as well as a significant larger proportion of total area stained were found in juvenile one year old male impala rams compared to those adult males. This supported the evidence of the prolactin trial, that the combination of these techniques is sensitive and able to distinguish between two physiologically different groups.

Lactating impala, juvenile animals and animals that had been kept in an enclosure for 1 day demonstrated significantly increased mean ACTH immunoreactive image surface area (5% level, Scheffe test) when compared to non-lactating, adult or animals that were kept in an enclosure for 7 and 21 days respectively. The lactating and juvenile animals demonstrated significantly more, and the adult and enclosure (1 day) groups significantly less total ACTH immunoreactive images when compared. The juvenile group also demonstrated a significant larger proportion of total area stained than the lactating, non-lactating, adult and enclosure (day 1) groups, and the lactating group a significant larger proportion of total area stained than the non-lactating, adult and enclosure (day 1) groups.

The combination of immunocytochemistry and computer assisted image analysis proved to be useful in discriminating between physiologically diverse groups. It was, however, not successful in discriminating amongst chronic stressed and free ranging animals.

SAMEVATTING

Die Suid Afrikaanse Nasionale Weermag het groot gebiede grond onder sy beheer waar wild dikwels vrylik op voorkom. Omrede die effek van militêre bedrywighede op die natuur nie alombekend is nie, het die weermag navorsings programme van stapel gestuur om die effekte te probeer bepaal ten einde die terreine optimaal te bestuur.

Die meting van plasma hormoon konsentrasies dmv radioimmuno opnames word algemeen gebruik om stres te evalueer in huisdiere. Sulke opnames is duur, vereis 'n graad van sofistikasie in die gebruik van gevorderde tegnieke in die veld en is moeilik om te interpreteer omrede wilde diere wat gevang moet word akute stres response ondergaan. Om die bogenoemde faktore sover moontlik te beperk is besluit om immunositochemie, 'n meer stabiele metode, in samewerking met rekenaar gesteunde voorwerp analise te gebruik om die hipofiseale selle aktief vir hormone, sowel as die moontlike omvang van die strukturele verandering van die selle, te bestudeer. Omrede die kombinasie van die tegnieke nog nie voorheen gebruik is nie is daar besluit om dit eers op fisiologiese diverse groepe, byvoorbeeld lakterende diere en jong groeiende diere, te toets, en indien suksesvol, dit dan aan te wend om adrenokortikotropien hormoon te evalueer onder verskeie stres toestande, byvoorbeeld vry lewende diere en diere wat in bomas aangehou word vir verskeie tydperke.

Rooibokke (*Aepyceros melampus*) kom algemeen voor en dus geredelik beskikbaar vir studies aangaande hul fisiologie. Diere gebruik in hierdie studie is geskiet as deel van ander navorsingsprojekte in die Nasionale Kruger Wildtuin.

Lakterende rooibok ooie het beduidend meer prolaktien immunoreaktiewe voorwerpe en 'n beduidend groter gemiddelde immunoreaktiewe voorwerp oppervlakte ($p < 0.5$, 5% vlak, 2-tail) vergeleke met nie-lakterende ooie. Dit verwerp dus die nul-hipotese van geen verskil tussen die groepe. Daar kon egter geen beduidende verskil verkry word vir die proporsie van die totale immunoreaktiewe oppervlakte tussen die twee groepe nie.

Jong groeiende rooibok ramme het beduidend meer somatotropien immunoreaktiewe voorwerpe, 'n beduidend groter gemiddelde immunoreaktiewe voorwerp oppervlakte ($p < 0.5$, 1% vlak, 2-tail) sowel as 'n beduidende groter totale immunoreaktiewe oppervlakte vergeleke met volwasse ramme. Die twee evaluasies bevestig dat die kombinasie van die tegnieke die vermoë het en sensitief genoeg is om tussen diverse fisiologiese groepe te kan onderskei.

Lakterende-, jong groeiende-, en rooibokke wat aangehou was vir 1 dag in 'n boma het 'n beduidend groter gemiddelde ACTH immunoreaktiewe voorwerp oppervlakte (5% vlak, Scheffe toets) vergeleke met volwasse-, nie-lakterende-, en rooibokke aangehou in bomas vir 7 en 21 dae respektiewelik. Lakterende- en jong rooibokke het beduidend meer, en volwasse- en rooibokke aangehou vir 1 dag in 'n boma beduidend minder totale ACTH immunoreaktiewe voorwerpe wanneer vergelyk. Die jong groeiende groep het ook 'n beduidende groter totale immunoreaktiewe oppervlakte in vergeleke met die lakterende, nie-lakterende volwasse en die groep wat 1 dag in 'n boma was. Voorts het die lakterende groep weer op hul beurt 'n beduidende groter totale immunoreaktiewe oppervlakte in vergeleke met die nie-lakterende, volwasse en die groep wat 1 dag in die boma was.

Die kombinasie van immunositochemie en rekenaar gesteunde voorwerp analise is bewys as 'n



nuttige metode om diverse groepe aan te dui. Die tegniek was egter nie suksesvol om tussen chroniese stres groepe en vrylewende groepe te onderskei nie.

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ABBREVIATIONS

A	- Adult
ACTH	- Adrenocorticotropic hormone
E	- Enclosure
J	- Juvenile
L	- Lactating
ml	- millilitre
NL	- Non-lactating
PRL	- Prolactin
SANDEF	- South African National Defence Force
SMT	- Somatotropin
MIEM	- Military Integrated Environmental Management